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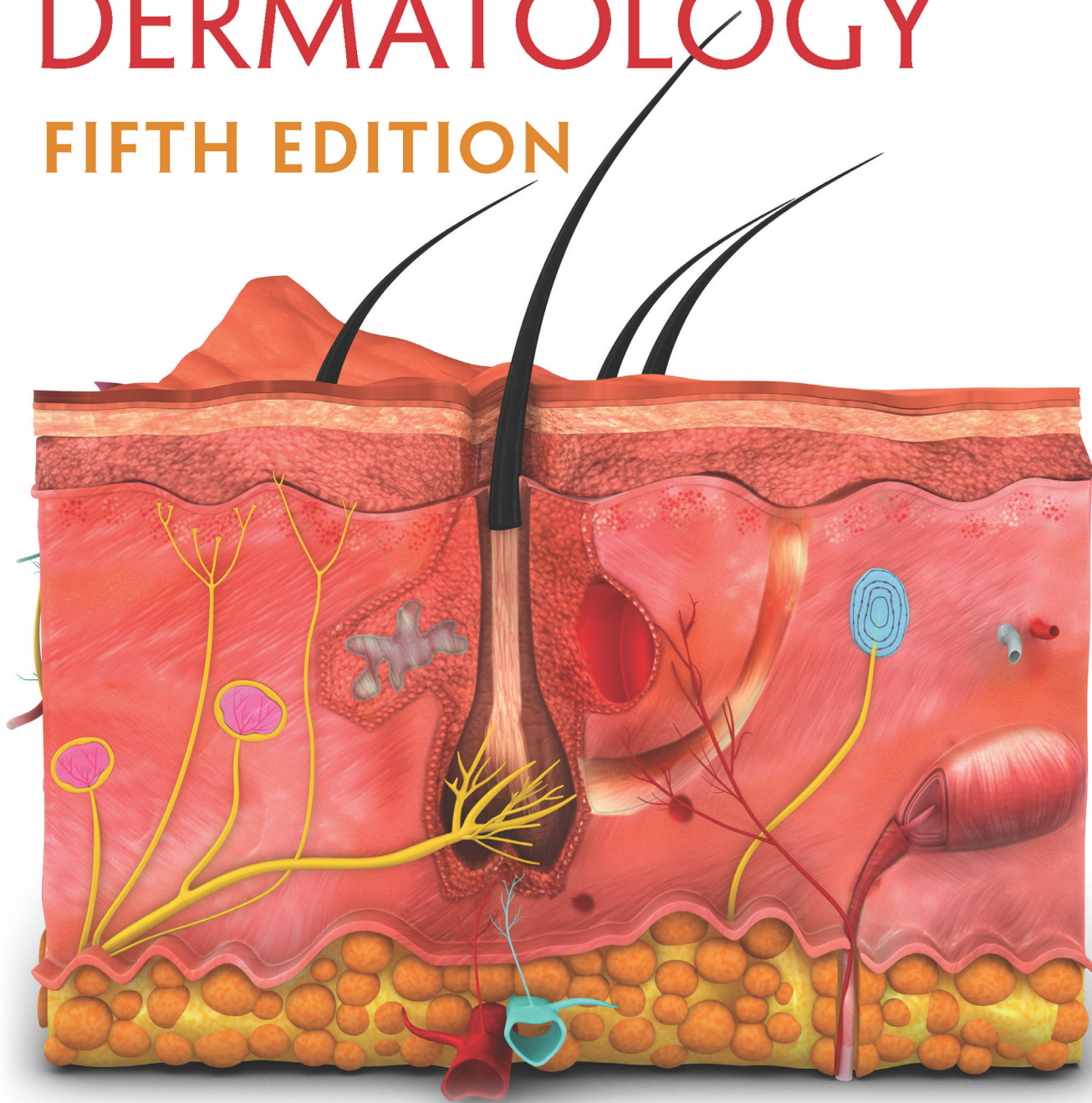
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# TEXTBOOK OF COSMETIC DERMATOLOGY

## FIFTH EDITION



SERIES IN  
COSMETIC AND  
LASER THERAPY



EDITED BY  
**ROBERT BARAN**  
**HOWARD I. MAIBACH**



CRC Press  
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# Textbook of Cosmetic Dermatology



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# Textbook of Cosmetic Dermatology

## Fifth Edition

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# Contents

Contributors .....	ix
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## Section I: Skin Science and Parameters

1. Skin Physiology and Gender .....	3
<i>Ethel Tur</i>	
2. Climatic Influence on Cosmetic Skin Parameters .....	16
<i>Mathias Rohr and Andreas Schrader</i>	
3. Transepidermal Water Loss .....	28
<i>Jan Kottner and Annika Vogt</i>	
4. Nail Penetration .....	32
<i>Rania Elkeeb, Xiaoying Hui, and Howard I. Maibach</i>	

## Section II: Pharmacology of Cosmetic Products and Ingredients

5. Sensitive Skin: New Findings Yield New Insights .....	45
<i>Miranda A. Farage and Howard I. Maibach</i>	
6. Organic Acids with Novel Functions: Hydroxy, Bionic, N-acetylamino Acids and N-acylpeptide Derivatives .....	56
<i>Ruey J. Yu and Eugene J. Van Scott</i>	
7. Retinyl Propionate and Related Retinoids .....	71
<i>John E. Oblong</i>	
8. Idebenone (Hydroxydecyl Ubiquinone) .....	76
<i>Birgit A. Neudecker, Falko Diedrich, and Howard I. Maibach</i>	
9. Antioxidants .....	80
<i>Frank Dreher</i>	
10. Topical Retinol: An Efficacious Solution for Improvement of Main Photodamage Signs .....	88
<i>Christiane Bertin and Thierry Oddos</i>	
11. Applications of Non-Denatured Soy in Skin Care .....	93
<i>Jue-Chen Liu, Jeff Wu, and Miri Seiberg</i>	
12. Kinetin .....	113
<i>Stanley B. Levy</i>	
13. Urokinase and Plasmin in Dry Skin and Skin Aging .....	117
<i>Yuji Katsuta</i>	
14. Ceramides and the Skin .....	123
<i>David J. Moore, Clive R. Harding, and Anthony V. Rawlings</i>	

<b>15. 4-Hexyl-1,3-Phenylenediol, an NF-kB Inhibitor, Improving Clinical Signs of Aging</b> .....	143
<i>Cécilia Brun, Simarna Kaur, Michael D Southall, Christiane Bertin, and Thierry Oddos</i>	
<b>16. Perfumes</b> .....	148
<i>Jeanne Duus Johansen</i>	
<b>17. Alternative and Natural Treatments in Dermatology</b> .....	153
<i>Daniel Oxman and Cheryl Levin</i>	
<b>Section III: Non-Pathological Skin Treatments</b>	
<b>18. Skin Care Products for Normal, Dry, and Greasy Skin</b> .....	167
<i>Christine Lafforgue, Céline Try, Laurence Nicod, and Philippe Humbert</i>	
<b>19. Self-Tanning Products</b> .....	174
<i>Stanley B. Levy</i>	
<b>20. Astringents, Masks, and Ancillary Skin Care Products</b> .....	178
<i>Zoe Diana Draelos</i>	
<b>21. Regulatory Overview of Cosmeceuticals</b> .....	182
<i>Lauren A. Hassoun, Howard I. Maibach, and Raja K. Sivamani</i>	
<b>22. Photodamage: Protection</b> .....	185
<i>Laurent Meunier</i>	
<b>23. Photodamage and Skin Cancer: How Successful Are Sunscreens as a Means of Prevention?</b> .....	193
<i>Xinyi Du and Douglas Maslin</i>	
<b>24. Photodamage: Protection and Reversal with Topical Antioxidants</b> .....	199
<i>Karen E. Burke</i>	
<b>25. Actinic Keratosis</b> .....	214
<i>Brigitte Dréno</i>	
<b>26. Safety of UV Nail Lamps as Used in Professional Nail Salons</b> .....	220
<i>Douglas Schoon</i>	
<b>Section IV: Specific Locations and Conditions</b>	
<b>27. Hair Care</b> .....	227
<i>John Gray</i>	
<b>28. Dandruff and Seborrheic Dermatitis</b> .....	248
<i>James R. Schwartz and Thomas L. Dawson, Jr.</i>	
<b>29. The Periorbital Wrinkle</b> .....	259
<i>Martin R. Green</i>	
<b>30. Cosmetology for Normal Nails</b> .....	264
<i>Robert Baran and Douglas Schoon</i>	
<b>31. Cosmetics for Abnormal and Pathological Nails</b> .....	276
<i>Douglas Schoon and Robert Baran</i>	
<b>32. Evaluating Hand and Body Lotions</b> .....	287
<i>F. Anthony Simion</i>	
<b>33. Anticellulite Products and Therapies</b> .....	308
<i>Enzo Berardesca</i>	



<b>34. Therapy of Telangiectasia and Varicose Veins and Their Complications . . .</b>	<b>312</b>
<i>Christian R. Halvorson, Robert A. Weiss, and Margaret A. Weiss</i>	
<b>35. Management of Hirsutism and Hypertrichosis . . . . .</b>	<b>321</b>
<i>Ralph M. Trüeb and Daisy Kopera</i>	
<b>36. Pigmentation: Dyschromia . . . . .</b>	<b>330</b>
<i>Thierry Passeron and Jean-Paul Ortonne</i>	
<b>37. Treatment of Keloids. . . . .</b>	<b>349</b>
<i>Joshua E. Lane</i>	
<b>38. Keratolytic Treatment of Acne . . . . .</b>	<b>360</b>
<i>Brigitte Dréno</i>	
<b>39. Hidradenitis Suppurativa . . . . .</b>	<b>369</b>
<i>Emil Knudsen List and Gregor B.E. Jemec</i>	
<b>Section V: Specific Groups</b>	
<b>40. Age-Related Changes in Male Skin . . . . .</b>	<b>377</b>
<i>Stefanie Lübbberding and Nils Krüger</i>	
<b>41. Ethnic Cosmetics . . . . .</b>	<b>384</b>
<i>Enzo Berardesca</i>	
<b>42. Ethnic Variation in Hair. . . . .</b>	<b>390</b>
<i>Nina Otberg</i>	
<b>43. Ethnic Differences in Skin Properties . . . . .</b>	<b>398</b>
<i>Rishu Gupta and Howard I. Maibach</i>	
<b>44. Changes in Female Hair with Aging: New Understanding and Measures . .</b>	<b>413</b>
<i>Paradi Mirmirani, R. Scott Youngquist, and Thomas L. Dawson, Jr.</i>	
<b>45. Menopause, Skin, and Cosmetology. . . . .</b>	<b>424</b>
<i>Michel Faure and Evelyne Drapier-Faure</i>	
<b>Section VI: Cosmetological Treatments</b>	
<b>46. Mesotherapy . . . . .</b>	<b>431</b>
<i>Maria Pia De Padova, Gabriella Fabbrocini, Sara Cacciapuoti, and Antonella Tosti</i>	
<b>47. Microneedles and Cosmetics. . . . .</b>	<b>436</b>
<i>Raja K. Sivamani and Howard I. Maibach</i>	
<b>48. Photodynamic Therapy in Dermatology . . . . .</b>	<b>442</b>
<i>Jacques Savary</i>	
<b>49. Cosmetic Cryotherapy . . . . .</b>	<b>450</b>
<i>Eshini Perera, Poorna Weerasinghe, and Rodney Sinclair</i>	
<b>50. Botulinum Toxins . . . . .</b>	<b>459</b>
<i>Doris Hexsel</i>	
<b>51. Soft Tissue Augmentation. . . . .</b>	<b>473</b>
<i>Kathleen Sikora Viscusi and C. William Hanke</i>	
<b>52. Bioelectricity and Its Application in Cosmetic Dermatology . . . . .</b>	<b>481</b>
<i>Ying Sun and Jue-Chen Liu</i>	
<b>53. Chemical Peels . . . . .</b>	<b>498</b>
<i>Philippe Deprez</i>	

<b>54. Lasers and Light Sources for Vascular and Pigmented Components of Photoaging</b> .....	<b>510</b>
<i>Anne Marie Mahoney and Robert A. Weiss</i>	
<b>55. Nonablative Laser Rejuvenation</b> .....	<b>519</b>
<i>Christian R. Halvorson, Karen L. Beasley, and Robert A. Weiss</i>	
<b>56. Cryolipolysis for Non-Surgical Fat Reduction</b> .....	<b>535</b>
<i>Christine C. Dierickx</i>	
<b>Section VII: Assessment Techniques</b>	
<b>57. Using the Behind-the-Knee Test to Evaluate Lotion Transfer from Products to Skin</b> .....	<b>551</b>
<i>Miranda A. Farage</i>	
<b>58. Assessing the Efficacy of Moisturizers</b> .....	<b>561</b>
<i>Whitney Hannon</i>	
<i>Index</i> .....	<i>585</i>





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# Section I

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**Skin Science and Parameters**



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# Skin Physiology and Gender

Ethel Tur

## INTRODUCTION

Many characteristics of the body are reflected in the skin, gender being a prominent one. Genetic and hormonal differences affect skin structure and function, resulting in variations between women and men and causing these gender variations to change with age. In addition, exogenous factors differ according to differences in lifestyle between the sexes.

During the last few decades, methodologies used in dermatological research have improved substantially, providing means of objective evaluation of skin function and characteristics. The number of studies addressing various aspects of differences between women and men has increased in the last few years along with the growing interest in studying gender-related differences of physiological and disease processes (1,2). However, the subject has not yet been systematically studied, so much of the data are by-products of studies with a different focus. This chapter outlines the various aspects of physiological differences between the skin of women and men, based on the available data.

## STRUCTURAL AND ANATOMICAL CHARACTERISTICS (TABLE 1.1)

The skin of female frogs is thicker than that of males in all body regions (3) (the opposite is true for rat skin[4]). In humans, skin thickness (epidermis and dermis) is greater in men than in women (5), up to 1.428 times (6), whereas the subcutaneous fat thickness is greater in women (7). The skin of men is thicker across the entire age range of 5–90 years (8). Hormonal influence on skin thickness was demonstrated when conjugated estrogens were given to postmenopausal women (9). Following 12 months' therapy, the dermis was significantly thicker, and histologic improvement in the previously atrophic epidermis was noted. Epidermal thickness alone, as measured by optical coherence tomography, does not differ between men and women, except for the forehead epidermis which is thinner in women (10).

Skin collagen and collagen density were measured in addition to dermal thickness (11). The skin of men demonstrated a gradual thinning with advancing age (12–93 years), whereas the thickness of women's skin remained constant up until the fifth decade, after which it decreased with age. The male forearm skin contained more collagen at all ages in the range 15–93 years. In both sexes there was a linear decrease in skin collagen with age. Collagen density calculated as the ratio of skin collagen to thickness was lower in women at all ages. The rate of collagen loss was similar in both sexes. Women start with lower collagen content; therefore they seem to age earlier than men. Collagen density, representing the packing of fibrils in the dermis, is lower in women than in men. This may be due to androgen, since skin collagen density is increased in patients with virilism.

Forearm skinfold thickness, as measured by a caliper, decreases starting at age 35 for women and 45 for men. Starting at age 35, it is thinner in women than in men (12). In younger subjects 17–24 years, forearm, thigh, and calf skinfold thickness in women is lower than in men (13).

Heel pad thickness, an indicator of soft tissue thickness in the body, was thicker in Ethiopian men than in women (14). Skinfold compressibility in Japanese students was greater in women than in men at the pectoral site, and smaller at nuchal, submental, biceps, thigh, suprapatellar, and medial calf sites (7). The changes in the distribution of fat between the ages of 6 to 18 years were studied in 2300 subjects (15). Up to 12 years of age, there was no difference between the two sexes: the mass of the subcutaneous fat increased more than threefold, while that of the internal mass increased less than twice. After the age of 12, the relative mass of the subcutaneous fat continued to increase in girls but not in boys.

The distribution of fat over the body is different in men and women (16). In men, an increase in fat tends to accumulate in the abdominal region and upper parts of the body, whereas in women it is located in the lower body, particularly in the gluteal and femoral regions. In addition, the proportion of body fat is higher in non-obese women than in non-obese men. The characteristic difference in body fat distribution between the sexes exists both in non-obese and obese subjects. Lipoprotein lipase activity and mRNA levels were higher in women in both the gluteal and abdominal regions. In women, higher enzyme activity was found in the gluteus than in the abdomen, whereas in men it was higher in the abdomen. These regional and sex differences in lipoprotein lipase activity might underlie the difference in fat distribution and total fat content. Variation is at both the mRNA level and post-translational level.

## BIOCHEMICAL COMPOSITION (TABLE 1.2)

Significant age-related differences in the stratum corneum sphingolipid composition were found in women, but not in men (17). From prepubertal age to adulthood there was a significant increase in ceramide 1 and 2 accompanied by a decrease in ceramide 3 and 6. After maturity there was a decrease in ceramide 2 and an increase in ceramide 3. These findings indicate an influence of female hormones on the composition of stratum corneum sphingolipids. These lipids play an important role in the water permeability barrier function of the human epidermis, and thus endocrinological factors may influence this barrier.

Human tissue kallikreins are a family of 15 trypsin or chymotrypsin-like secreted serine proteases (hK1-hK15). hK5, hK6, hK7, hK8, and hK13 have been identified in the stratum

**Table 1.1** Structural and anatomical characteristics

Ref.	Finding	Obtained by	Subjects	Conclusions
<b>(a) Significant differences</b>				
10	Forehead epidermis thinner in women Other sites: Epidermal thickness does not differ between men and women	Optical coherence tomography	83 Caucasians; Young: 20–40 y Old: 60–80 y	
5	Skin thickness in humans greater in men than in women, except for lower back in young subjects	Echographic evaluation	24 women; 24 men; half 27–31 y half 60–90 y	
8	Men's skin thicker than women's across the entire age range of 5–90 y	Ultrasonic echography; forearm	69 women; 54 men; 5–90 y	
6	Men's skin thicker than women's, up to 1.438 times	12.0-MHz- in B-mode	112 healthy; 43 women; 69 men; 19–28 years; 24 sites	
9	Thickening of dermis following 12 months estrogen therapy	Conjugated estrogen therapy; ultrasound measurement	28 estrogen; 26 placebo; women: 51–71 y	Estrogens affect skin thickness
11	Men: Gradual thinning of skin with advancing age Women: Thickness constant up to 5th decade, then decreasing with age	Skin collagen, skin thickness and collagen density, measured chemically and histologically	Collagen: 80 women; 79 men; 15–93 y Thickness: 107 women; 90 men; 12–93 y Density: 26 women; 27 men; 15–93 y	Rate of collagen loss same in men and women, although total skin collagen content is less in women than men at all ages
12	Forearm skinfold thickness decreases starting at age 35 for women and 45 for men Starting at age 35 it is thinner in women than in men	Caliper; forearm	145 women and men; 8–89 y	
13	Skinfold thickness lower in women	Caliper; forearm, thigh, and calf	42 women; 37 men; 17–24 y	
7	Subcutaneous fat thickness greater in women	Caliper and ultrasound	45 women; 41 men; Japanese; 18–22 y	
14	Heel pad thickness thicker in men than in women; correlation with body weight	Ankle x-ray	113 women; 125 men; Ethiopian; 10–70 y	
7	Skinfold compression in women is greater in the trunk and lower in the limbs	Caliper and ultrasound	45 women; 41 men; Japanese; 18–22 y	
15	Up to 12 years of age no difference between the sexes Subcutaneous fat increases more than threefold, while internal fat mass increases less than twice After 12 y, the relative mass of the subcutaneous fat increased in girls but not in boys	Caliper	1292 women; 1008 men; ages 6, 8, 10, 18	
16	Lipoprotein lipase activity higher in women Women: Higher values in gluteus than abdomen Men: Higher in abdomen	Lipoprotein lipase activity and mRNA levels measured; hybridization, Northern blot	8 women; 11 men; 37 ± 4 y	Regional and sex differences in lipoprotein lipase activity might underlie the difference in fat distribution and total fat content Variation is both at mRNA and post-translational levels
<b>(b) No significant differences</b>				
15	Up to 12 y: The mass of the subcutaneous fat increases more than threefold, while that of the internal mass increases less than twice in both sexes	Caliper	1292 women; 1008 men; ages 6, 8, 10, 18	

**Table 1.2** Biochemical composition

Ref.	Finding	Obtained by	Subjects	Conclusions
<b>Significant differences</b>				
17	Stratum corneum sphingolipid composition differs with age in women but not in men	Ethanollic extracts; biochemical methods of lipid identification	27 women; 26 men; 10–79 y	Female hormones influence the composition of stratum corneum sphingolipids
19	Women: Higher concentrations of metals in hair Concentrations of copper did not differ with age in men, whereas in women they increased with age	Liquid chromatography; trace metal determination	60 women; 72 men; 6–40 y	

corneum (SC), stratum granulosum, and skin appendages. HK6 and hK14 were significantly lower in women between 20 and 59 y (18).

Differences in the metal content of human hair were found between men and women: higher concentrations of metals were noted in women. Concentrations of copper did not differ with age in men, whereas an increase with increased age was noted in women (19).

### MECHANICAL PROPERTIES (TABLE 1.3)

Clinical assessment, as well as objective measurements of stratum corneum hydration, and grading of scaling (by adhesive tape strippings followed by densitometry readings) showed no differences between men and women (20). A positive effect of estrogens on stratum corneum hydration and wrinkles was demonstrated when estriol or estradiol cream was applied on the face of perimenopausal women (21).

The degree of facial wrinkling is affected by gender. In men, forehead wrinkles were increased in all age groups as compared with women. However, no gender-dependent differences were found in upper eyelid wrinkles. Other facial wrinkles were greater in men than in women in all except the oldest group (65–75 years), in which wrinkles in women were greater than or equal to those in men (22).

Photographs and dermal elasticity measurement by cutometer showed that the morphology, areas of sagging, and elasticity in male faces are similar to those in females in the cheek, but sagging at the lower eyelid is more severe in males after middle age (23).

Epidermal hydration affects the friction between the skin and textiles. Friction of women showed higher moisture sensitivity than men, when measured at different hydration states, when forearm skin was rubbed with dry to completely wet textile. Higher skin hydration caused gender-specific changes in its mechanical properties and surface properties, leading to softening and increased contact area (24).

Other studies showed no difference of frictional properties of the skin, as well as stratum corneum hydration, between men and women, in both young and old subjects (25,26,27). In addition, transepidermal water loss showed no difference between the two sexes. In contrast, another study (28) found lower basal transepidermal water loss values in women compared with men aged 18–39 years.

The adhesion of the stratum corneum, measured in vitro in skin biopsy samples, did not differ between men and women in several body regions (29). But age (and probably hormonal) related differences were demonstrated in vivo by

measuring the speed of dermal–epidermal separation utilizing the time required for blisters to form by controlled suction (30). From 15 up to 69 years of age, women exhibited longer blistering times than men in both antecubital and abdominal sites. The difference was more pronounced in the age range 15–39 years than 40–69 years, and disappeared in older ages.

Skin elasticity did not differ between the sexes, as measured utilizing two suction cup methods (24,31). Similarly, torsional extensibility of the skin, as measured by a twistometer, did not differ between the sexes (8).

Cutaneous extensibility was identical in men and women, but after hydration it increased only in women (32). Hydration changes the properties of the stratum corneum, softening it, thus allowing the difference in dermal thickness to express itself as a difference in extensibility. Since the dermis is thinner in women, elimination of the stratum corneum factor allows a rapid extensibility of the skin in women.

Plasticity was found to be greater in women than in men in three sites of the foot in one study (33).

### FUNCTIONAL DIFFERENCES (TABLE 1.4)

Following pilocarpine iontophoresis, sweat secretion rates were higher in men than in women in both healthy and chronic renal failure subjects (26).

Body sweat distribution over the upper body in nine clothed male and female runners of equal fitness while running at 65% and subsequent 15-min rest in a moderate climate (25° C, 53% rh) was investigated using technical absorbent materials to collect the sweat produced. Local sweat rates were higher in men for the mid-front, sides, and mid lateral back as compared to women. Both sexes showed similar sweat distribution patterns over the upper body with some exceptions. Men showed higher relative (local to overall) sweat rates than women for the mid lateral back, while it was lower for the upper arm, lateral lower back, and upper central back. Sweating in both sexes was highest along the spine, and higher on the back as a whole than the chest as a whole. Upper arm sweat rate was lowest. Men showed a higher ratio of highest to lowest local sweat rates (34).

Increases in sweating as a function of increasing concentration of acetylcholine significantly differed between males and females. Maximum values were lower in females in response to acetylcholine (35).

The fatty acid composition of sebum is affected by androgens in both sexes (36).

**Table 1.3** Mechanical properties

Ref.	Finding	Obtained by	Subjects	Conclusions
<b>(a) Significant differences</b>				
30	From 15 y to 69 y women exhibited longer blistering times than men. The difference was more pronounced in the age range 15–39 y than 40–69 y, and disappeared in older ages.	Measuring the speed of dermal–epidermal separation utilizing the time required for blisters to form by controlled suction; antecubital and abdominal sites	178 women, 15–101 y 209 men, 16–96 y	
24	Friction of women showed higher moisture sensitivity than men	Corneometry Forearm skin Rubbing with various hydration states, dry to wet textile	11 women 11 men	Higher skin hydration causes gender specific changes in its mechanical properties, leading to softening and increased contact area
22	Men: Increased forehead wrinkles compared with women; no differences in upper eyelid wrinkles. Other facial wrinkles were greater in men than in women in all except the oldest group (age, 65–75 y), in which wrinkles in women were greater than or equal to those in men.	Photographs: Replicas from five facial sites used to measure surface roughness	173 Japanese men and women	Men tend to have more severe wrinkles than women. This tendency disappeared or was reversed in some regions of the face and in individuals more than 60 y old.
23	Sagging in male faces: Similar to females in the cheek, but sagging at the lower eyelid is more severe in males after middle age.	Photograph-based grading, cutometer	98 Japanese men, 108 women 20–60 y	Dermal elasticity of male facial skin decreased with age similar to that of females, except for the lower eyelids.
<b>(b) No significant differences</b>				
20	Stratum corneum hydration, and grading of scaling showed no differences between men and women.	Clinical assessment and bioengineering measurement	50 women; 22 men; 21–61 y	
21	A positive effect of estrogens on facial skin: Moisture increased, wrinkles decreased.	Stratum corneum hydration and wrinkles—profilometry of skin replicas	18 women (8 applied estriol, 10 estradiol) 46–66 y	Topical treatment with estrogen seems promising.
25	No difference between men and women in friction, moisture, transepidermal water loss.	Bioengineering measurement	7 women, 25 y (mean) 7 men, 29 y; 7 women, 75 y; 8 men, 74 y	
26	No difference in moisture.	Bioengineering; healthy and chronic renal failure subjects	Healthy: 24 women, 21 men Patients: 30 women, 50 men	
31	Skin elasticity did not differ between the sexes, as measured by suction devices.	In vivo suction device (bioengineering)	Young: 8 women (26 y); 8 men (28 y) Old: 9 women (75 y); 8 men (75 y)	
24	Skin viscoelasticity comparable for women and men.	Suction chamber; forearm skin; rubbing with various hydration states, dry to wet textile	11 women, 11 men	
8	Torsional extensibility did not differ between men and women.	Twistometer	69 women; 54 men 5–90 y	
29	The adhesion of the stratum corneum did not differ between men and women.	Biopsy; in vitro measurement of the force needed to separate cells	9–34 women and men (number varied with site studied) 20–40 y	



**Table 1.4** Functional differences

Ref.	Finding	Obtained by	Subjects	Conclusions
<b>Significant differences</b>				
26	Men sweat more than women	Pilocarpine iontophoresis – healthy and chronic renal failure subjects	Healthy: 24 women; 21 men CRF patients: 30 women; 50 men; 18–75 y	
34	Local sweat rates higher in men for the mid-front, sides, and mid lateral back Men showed higher relative (local to overall) sweat rates than women for the mid lateral back, while it was lower for the upper arm, lateral lower back, and upper central back	Technical absorbent materials to collect the sweat produced in a moderate climate (25 degrees C, 53% rh)	9 clothed male and female runners while running at 65% and subsequent 15-min rest	
32	Cutaneous extensibility increased only in women after hydration	Bioengineering methods	15 women; 14 men 23–49 y and 60–93 y	Hydration allows the effect of thinner dermis in women to be reflected in extensibility
35	Increases in sweating with increasing concentration of acetylcholine significantly differed between men and women Maximum values were lower in women in response to acetylcholine	Intradermal microdialysis	12 women, 12 men	Peripheral modulation of sudomotor activity in females

Sex-related differences in the metabolism in the skin of topically applied compounds were found in guinea pig skin (37).

### DIFFERENCES IN RESPONSE TO IRRITANTS (TABLE 1.5)

The incidence of irritant dermatitis is higher in women than in men, but experimental irritant dermatitis does not differ between men and women (38,39). Occupational factors leading to a greater exposure to irritants by women may provide an explanation of this discrepancy. In a study of skin irritability by sodium lauryl sulfate, women showed lower baseline transepidermal water loss compared with men, but after irritation both sexes gave similar transepidermal water loss values (28). The importance of interpretation of the results, and the lack of a standardized way of analyzing them, is illustrated in the latter study. The authors define an irritation index as the ratio of the difference between the values for irritated and non irritated skin to the value for non irritated skin. Although the value for irritated skin did not differ between men and women, this index was higher in women, since the value for non irritated skin was lower in men, and so the authors conclude that women's skin is more irritable. A review article considering the absolute values following irritation interpreted the same results as indicating no sex-related differences in sodium lauryl sulfate irritation.<sup>38</sup> Until a universal way of interpreting the results is established, contradictory conclusions may be reached by different analyses of the same set of data. In another study, baseline transepidermal water loss did not differ between men and women (40). This study found no significant differences between men and women in developing cumulative irritant dermatitis when visual scoring, transepidermal water loss, skin blood flow, and dielectric water content were assessed. Changes during the menstrual cycle, however, were demonstrated by measuring baseline transepidermal water loss (41).

### CUTANEOUS MICROVASCULATURE (TABLE 1.6)

Hormonal factors affect the skin blood flow: differences between men and women were found during the reproductive years, and differences were found within different phases of the menstrual cycle (42). Moreover, vasospastic diseases, such as Raynaud's phenomenon, are more common in women, more prevalent in the reproductive years, and improve during pregnancy, suggesting an influence of female sex hormones (43). Skin circulation varied during the menstrual cycle. There might be a direct influence of sex hormones on the blood vessel wall or an indirect systemic hormonal action causing a cyclic pattern in women. Estrogens influence the sympathetic nervous system, inducing an upregulation of (vasoconstrictive)  $\alpha_2$ -adrenoceptors. Thus blood flow measurements utilizing laser Doppler flowmetry revealed a reduction of basal cutaneous blood flow in women compared with men (43,44,45), but these differences existed only in young women and not in women over 50 years (46). This reduction was due to a basal increase in sympathetic tone rather than to a local structural or functional difference in the cutaneous circulation.

The vasodilatation induced by local heating occurred at a lower skin temperature in women (47). However, the maximum skin blood flow following heating of the skin was not different between men and women, and neither was the post-occlusive reactive hyperemia response in a study including a group of women aged 20–59 years (43). In contrast, in a study that divided women according to age, the reactive hyperemia response was lower in young women compared both with women over 50 years and with young men (46). The latter study also measured the response to cooling, which was prolonged in young women compared with the other two groups.

Skin microvascular response to vasodilators was evaluated by laser Doppler perfusion imager, an instrument that maps the skin blood perfusion. The substances used were acetylcholine, an endothelium-dependent vasodilator, and nitroprusside and isoprenaline—two endothelium-independent vasodilators with different modes of action. The substances

Table 1.5 Irritants

Ref.	Finding	Obtained by	Subjects	Conclusions
<b>(a) Significant differences</b>				
38	Incidence of irritant dermatitis higher in women than in men			Occupational factors
28	Lower baseline transepidermal water loss in women compared with men, but after irritation similar values in both sexes	Sodium lauryl sulfate irritation; evaporimeter	15 women; 23 men; 18–39 y	Comparing the irritation index (the difference between irritated and unirritated values over unirritated): female skin more irritable
41	Higher on the day of minimal estrogen/progesterone secretion compared with the day of maximal secretion Also higher on the day of maximal progesterone secretion compared with the day of maximal estrogen secretion	Back and forearm sites; baseline transepidermal water loss; evaporimeter	9 women; 19–46 y (mean 32)	Barrier function is less complete just prior to the onset of menses compared with the days just prior to ovulation
<b>(b) No significant differences</b>				
39	No significant differences between men and women with or without hand eczema	Irritation tested for 11 irritants at several concentrations	21 women; 21 men with hand eczema; 21 women; 21 men without hand eczema; 20–60 y	No tendency to stronger reactions in either sex Speculation: Women's occupations lead to a greater exposure to irritants
40	No significant differences between men and women in developing cumulative irritant dermatitis	Repeated once-daily application of 3 concentrations of irritant (SLS), 5 days, followed by a patch test; upper back; bioengineering measurements	7 women; 7 men; 16–65 y	No sex-related susceptibility to develop cumulative irritant dermatitis. Speculation: Women's occupational and domestic duties lead to a greater exposure to irritants

were iontophORIZED into the skin. The response to nitroprusside, and to a lesser extent to acetylcholine, was higher in women before menopause than after (48), reflecting functional and structural changes in skin vasculature with aging.

The cutaneous blood flow response to topical and intradermal administration of histamine was comparable in men and women at three anatomical sites: the back, the volar side of the forearm, and the ankle (49). These observations indicate that there are no functional differences between men and women in the skin microvascular response to histamine. However, histamine administered by iontophoresis produced bigger wheals in women, as measured by laser Doppler flowmetry (44). The bigger wheals were attributed to differences in the stratum corneum layer, which is the main obstacle to penetration.

*Transcutaneous oxygen pressure* is a method that measures changes in oxygen pressure at the skin surface that are mainly determined by changes in skin blood flow. During skin surface measurement, significantly higher values of transcutaneous oxygen pressure were noted in women (50,51). The difference might be explained by the thinner epidermis of women. Age-related sex differences were noted in measuring transcutaneous oxygen pressure during postocclusive reactive hyperemia. Greater values were found in adult women than in men, but no differences were found between boys and girls (52).

The contribution of endothelin-B receptors to resting cutaneous vascular tone differs between men and women. In men, endothelin-B receptors mediate vasoconstriction, whereas in women, endothelin-B receptors mediate vasodilation. Blockade of endothelin-B receptors by a competitive antagonist (BQ-788) in men caused skin vasodilation consistent

with removal of a tonic vasoconstrictor effect of endothelin-B. In women, it caused a vasoconstriction, demonstrating release of tonic vasodilator activity (53).

## SENSORY FUNCTIONS (TABLE 1.7)

### Thermoregulatory Response

Studies of human thermoregulation were conducted by exposing subjects to various thermal environments. The importance of taking into account all the possible variables is demonstrated in studies of the physiological responses to heat stress (54): data showed differences between women and men. But when taking into consideration the differences in the percentage of fat in the body and the ratio between the body surface and mass, the effect of gender disappeared.

In contrast to these results of heat stress, the response of Japanese young subjects to cold stress differed with gender, although body surface area-to-mass ratios were similar (55). Subjects were exposed to cold (12°C) for 1 hour at rest in summer and in winter. In winter, women's tolerance to cold was superior to men's, whereas no significant differences between the sexes were found in the summer. The differences in cold tolerance may be caused by differences in the distribution of fat over the body, even though body surface area-to-mass ratios were similar in the two sexes.

The thermal sensitivity distribution (topographical mapping) over the glabrous skin of the hand in men and in women was assessed by measuring warm and cold thresholds in 25 healthy volunteers (12 women, 13 men), applying a multi-site test of 23 locations on the volar part of the hand. The palm



**Table 1.6** Cutaneous microcirculation

Ref.	Finding	Obtained by	Subjects	Conclusions
<b>(a1) Significant differences</b>				
43	Reduction in basal skin blood flow in women	Bioengineering measurement	56 women; 44 men; 20–59 y	Sympathetic tone is increased, not a structural or functional difference in the cutaneous circulation
45	Reduction in facial basal skin blood flow in women	Laser Doppler	5 women; 5 men; 25–52 y	
44	Reduction in basal skin blood flow in women	Bioengineering measurement; cooling and warming to change sympathetic tone	26 women; 23 men; 23–38 y	
42	Skin circulation varied during menstrual cycle: Basal flow lowest in the luteal phase, highest in the pre-ovulatory phase Greatest cold-induced constriction and lowest recovery in the luteal phase	Bioengineering measurements at 4 times during the menstrual cycle	31 women; 15–45 y	
46	Reactive hyperemia response lower in young women as compared to both women over 50 y or young men Response to cooling prolonged in young women compared with the other two groups	Bioengineering measurement; postocclusive reactive hyperemia and direct and indirect cooling	12 women, 19–39 y 13 women, 51–67 y 13 men, 22–47 y	Hormonal factors might explain the differences Different dressing habits may also contribute
47	Vasodilatation induced by local heating occurs at a lower skin temperature in women	Bioengineering measurement	9 women; 6 men; age not specified	
48	Response to nitroprusside higher in women before menopause than after	Laser Doppler perfusion imager; iontophoresis	21 women; 13 men; 18–80 y	Indicating functional and structural changes in skin vasculature of women with aging
4	Histamine produced bigger wheals in women	Histamine administered by iontophoresis	33 women; 38 men; 15–52 y	Differences in the stratum corneum layer
53	Endothelin-B receptors mediate vasoconstriction in men and vasodilatation in women	Laser Doppler, microdialysis	11 women; 11 men; 33± 3 women; 30± 3 men	Resting tone is different in women and men
<b>(a2) Significant differences: Transcutaneous oxygen pressure</b>				
50	Significantly higher values of transcutaneous oxygen pressure in women	Bioengineering; anterior chest, forearm	18 women; 42 men; 22–88 y	Might be explained by women's thinner epidermis
51	Significantly higher values of transcutaneous oxygen pressure in women	Bioengineering; 23 sites on face, extremities, and trunk	7 women; 12 men; 21–63 y	
52	Transcutaneous oxygen pressure during postocclusive reactive hyperemia greater in adult women than in men, but did not differ between boys and girls	Bioengineering measurement; forearm; postocclusive reactive hyperemia, 35–37°C	Adults: 30 women; 37 men; 22–60 y Children before puberty: 34	Hormonal influence is indicated
<b>(b) No significant differences</b>				
49	No difference in cutaneous blood flow response to histamine	Topical and intradermal administration; bioengineering methods	10 women; 10 men; 24–34 y	No difference in postocclusive reactive hyperemia and maximum skin blood flow following heating
43	No difference in postocclusive reactive hyperemia and maximum skin blood flow following heating	Bioengineering methods	56 women; 44 men; 20–59 y	

area was more sensitive than the fingers to both warm and cold stimuli. On the palm itself, the proximal part was the most sensitive. Women were more sensitive than men to both warm and cold sensations (56).

Cold-induced vasomotor response was measured by laser Doppler flowmetry in 12 healthy men and 12 healthy women. Both direct response (at the site of cooling) and indirect response (at a site remote from the cooling site) were measured (57). The

women were tested twice, once in the follicular and once in the luteal phase of the menstrual cycle. Blood flow was measured before and during local cooling of one hand at 15°C. Local cooling evoked a significantly greater decrease in cutaneous blood flow in women than in men in direct as well as in indirect response conditions. Direct response to local cooling was significantly greater in the luteal phase than in the follicular phase. In contrast, there was no menstrual-cycle-dependent difference in

**Table 1.7** Sensory function

Ref.	Finding	Obtained by	Subjects	Conclusions
<b>(a) Significant differences</b>				
61	Women more sensitive to small temperature changes and to pain caused by either heat or cold	Marstock method—quantitative	67 women; 83 men; 10–73 y	
62	Lower threshold values in women than in men	Pricking pain sensation to heat; threshold determination, volar forearm	93 women; 165 men; 18–28 y 132 women; 135 men; 50–90 y	
63	Women more sensitive than men: Palm and sole, but not on the forearm	Pressure threshold measurement; palm, sole, forearm	68 women; 68 men; 17–30 y	
64	Neonate girls: Significantly higher conductance than boys	Skin conductance (autonomic function)	20 women; 20 men; neonates: 60–110 h	These differences may represent differences in maturation Very young: No effect yet of training and different behavior accorded the sexes
55	Women's tolerance to cold superior to men's in winter	Exposed to cold (12°C) for 1 h at rest in summer and in winter; skin and body temperature	7 women; 8 men; Japanese; 18–26 y	Differences in fat distribution over the body, even though body surface area-to-mass ratios were similar in the two sexes, might have contributed to the differences in cold tolerance
59	Greater decrease in women in finger temperature as a response to musical stimulus	Auditory stimulation, music; skin temperature, index finger	60 women; 60 men; young students	Possible explanation: Difference in vascular autonomic sensitivity to music
60	Men: More asymmetry between hands, larger skin conductance responses on the left hand Women: Less asymmetry, larger skin conductance responses on right hand	Auditory stimulus Magnitude and frequency of skin conductance responses	15 women; 15 men; 19–27 y; right-handed	Possible hemispheric differences in response to auditory stimuli
65	Acute muscle or skin pain: Skin blood flow increased in women, whereas in men it decreased	Skin sympathetic nerve activity Hypertonic saline injected into tibialis anterior muscle or into skin Skin blood flow measurements	Awake human subjects	
<b>(b) No significant differences</b>				
54	Physiological responses to heat stress differ with gender, but depend on fat content and body surface area	Heat stress; ergometer; oxygen uptake; body and skin temperature; sweat rate	12 women; 12 men; 20–28 y	Differences between women and men disappeared when differences in the percentage of fat in the body and the ratio between body surface and mass were taken into account

the indirect response to cold. Thus, sympathetic neural reactivity, as assessed by way of an indirect response to a cold stimulus, significantly contributes to gender differences in the response to local cooling. In contrast, the variation in microvascular responsiveness to cold exposure due to the menstrual cycle is most probably caused by local vascular mechanisms rather than by variation in sympathetic neural reactivity to local cooling.

Sex-related differences in thermoregulatory responses while wearing protective clothing were found (58). Women were at a thermoregulatory disadvantage compared with men when wearing protective clothing and exercising in a hot environment. This disadvantage can be attributed to the lower specific heat of adipose versus non-adipose tissue and higher percentage body fatness.

### Thermal Response to Stimulation

The decrease in finger temperature as a response to musical stimulus was greater in women (59). This may be due to differences between men and women in vascular autonomic sensitivity to music, or to differences in sensitivity or density of peripheral vascular adrenergic receptors.

*Electrodermal responses:* electrodermal asymmetry has been considered as an index of hemispheric specialization. A study recorded the magnitude and frequency of the skin conductance responses when subjects listened to tones (60). Subjects were right-handed in order to control the effects of handedness. Men displayed more asymmetry between hands, with larger skin conductance responses on the left hand. In women, asymmetry was less marked, and larger skin

conductance responses were found on the right hand. These results indicate a possible hemispheric difference in response to auditory stimuli.

### Thermal and Pain Sensation, Pressure Sensitivity

Sensation in the skin can be studied in relation to pain. Pain can be induced mechanically, electrically, by chemical stimulus or by thermal stimulus. Pain sensation is best determined by the threshold at which pain begins, and the stimulus required to produce it can be quantified. Thermal and pain sensations are mediated by cutaneous receptors and travel through myelinated (A $\delta$ ) and unmyelinated (C) nerve fibers. Women were more sensitive to small temperature changes and to pain caused by either heat or cold (61). Another study measured the threshold of the pricking sensation provoked by heat projected to the skin from a lamp (62). The pricking pain threshold increased with age in both sexes. In addition, the threshold of women was lower at all ages in the range 18–90 years. Possible explanations to the difference between the sexes are:

- Anatomical differences in skin thickness
- Differences in blood flow and blood vessels that absorb part of the heat transmitted to the skin
- Differences in nervous structure or function

Unlike the forearm lower pricking pain sensation threshold in women, pressure threshold was lower in women than men on the palm and on the sole, but not on the forearm (63).

### Autonomic Function

Skin conductance measures one aspect of the autonomic function. Neonate girls manifested a significantly higher conductance than boys (64). These differences may represent differences in maturation.

Both acute muscle and skin pain evoked a measurable sympathetic activity in human subjects who were awake. Sweat release was increased to the same level in men and in women, but dissimilar changes in skin blood flow were recorded: skin blood flow increased in women, whereas in men it decreased (65).

### SKIN COLOR (TABLE 1.8)

An article by Tegner (66) gives several examples of artists depicting their female models as lighter skinned than males. Such differences were indeed found utilizing spectrophotometric measurements, in various ethnic populations. A lighter skin in women was demonstrated in studies from Iran (67), India (68), and Australia (69). In addition to hormonal influences, differences in melanin, hemoglobin, and carotene might be involved, as well as differences in sun exposure. Skin reflectance spectroscopy was measured in 10 anatomical sites in 20 healthy Caucasian babies (mean age 5 months, range 1 to 10 months). The level of skin pigmentation was the same in all the 10 measured sites and there were no gender differences in pigmentation for any site (70). In general, both sexes darken as age increases (69). But the changes are more intricate (68): from the end of infancy to the onset of puberty there is a progressive skin darkening in both sexes. During adolescence they both lighten, but women lighten more. Simple hormonal effects cannot explain this difference, since both testosterone and estrogen provoke darkening rather than lightening of the skin. These changes might be partly attributed to differences in exposure to sunlight, since UV irradiation increases

the number of melanocytes in both exposed and unexposed skin. Another study assessed skin color in adolescents (71). The forehead (sun-exposed) pigmentation of boys was darker than that of girls. But the medial upper arm (less sun exposure) pigmentation varied among the different phases of adolescence: girls were darker than boys during early adolescence, during middle adolescence the pigmentation was similar in the two sexes, and during late adolescence girls were significantly lighter than boys.

The lighter skin color of women was attributed to differences in melanin, hemoglobin (variations in vascularity) and carotene (72). Natural selection might give an explanation of the overall visual effect of lighter skin. In addition, women are more homogenous in color than men, since regional variations in reflectance spectrophotometry were smaller in women than in men (72). Colorimetric measurements revealed a darker and redder skin in elderly men (65–88 years) compared with elderly women, but such differences were not found in young subjects (18–26 years) (73). Another study of 461 women and 346 men aged 20–69 years found that both sexes darken with age (69). Yet another study did not find differences between men and women in epidermal melanocyte counts (74).

### HORMONAL INFLUENCE (TABLE 1.9)

Any of the above differences between women and men might be related to hormonal effects. Some evidence for hormonal influence on the skin has already been mentioned above, like the increase of skin thickness following conjugated estrogens treatment of postmenopausal women (9), or the positive effect of estrogens on stratum corneum hydration and wrinkles of the face of perimenopausal women (21), or the changes during the menstrual cycle demonstrated by measuring baseline transepidermal water loss (41) and skin blood flow (42). Hormone replacement therapy for menopause had an effect on skin extensibility (75): in untreated women a steep increase in skin extensibility was evidenced during the menopause. Hormone replacement treatment limited this age-related increase in skin extensibility, thus having a preventive effect on skin slackness. Other parameters of skin viscoelasticity were not affected. After menopause the skin becomes thinner, associated with loss in skin collagen content. Collagen content increased with hormone replacement therapy by 48% compared with non-treated subjects (76). Moreover, the ratio of type III to type I collagen in the skin is reduced with age. Postmenopausal women receiving hormone replacement therapy showed an increased proportion of type III collagen in the skin (77). In the future, further hormonal manipulation might change the skin of both men and women in ways we cannot yet predict.

### PILOSEBACEOUS UNIT (TABLE 1.10)

The sebaceous glands are hormone-dependent. The increase in their activity during puberty can be stimulated by the administration of the appropriate hormone. Androgenic steroids, of either gonadal or adrenal origin, have a direct stimulatory effect on sebaceous gland activity. Most of the hormones (TSH, ACTH, FSH, LH) act indirectly by stimulating their respective endocrine tissues. In other cases the hormones (for instance GH) act synergistically with another hormone to which the sebaceous gland is sensitive. Average values for sebum secretion were significantly higher in men than in women for age ranges 20 to over 69, but not for 15–19 years (78). This difference in sebaceous gland activity becomes more apparent in the

**Table 1.8** Skin color

Ref.	Finding	Obtained by	Subjects	Conclusions
<b>(a) Significant differences</b>				
19	Women's skin lighter	Spectrophotometry	Review article	Not a simple hormonal effect Differences in melanin, hemoglobin and carotene
67	Women's skin lighter	Spectrophotometry	33 women; 68 men; 8–24 y	Differential tanning; vascularity variations
68	Women's skin lighter	Spectrophotometry; upper inner arm	566 women; 578 men; 1–50 y	During puberty, males darken, females lighten Different levels of MSH Hereditary and environmental factors
71	Forehead: Boys darker than girls. Medial upper arm: Girls darker than boys during early adolescence, not different from boys during middle adolescence, and during late adolescence girls lighter than boys	Skin color, measured by reflectance of forehead and medial upper arm, in adolescents	105 women, 10–16 y; 105 men, 12–18 y	Physiologic changes during adolescence may cause these sex differences
69	Women's skin lighter Both sexes darken with age	Spectrophotometry; inner upper arms, lateral forearms, back of hands	461 women; 346 men; 20–69 y	Different levels of MSH Difference in sun exposure (tanning and thickening of skin)
73	In the elderly: Skin of men darker and redder compared with women, but not in the young	Colorimetric measurements of forehead (sun-exposed) and forearm (protected)	8 women, 5 men; 65–88 y 9 women, 4 men; 18–26 y	
<b>(b) No significant differences</b>				
74	No difference between men and women in epidermal melanocytes counts	5 mm paraffin embedded sections	38 skin samples of men and women of different ages DOPA reagent.	
73	In Caucasian babies: Pigmentation same for men and women	Colorimetric measurements of 10 sites	10 women, 10 men; 1–10 mo	

**Table 1.9** Hormonal influence

Ref.	Finding	Obtained by	Subjects	Conclusions
<b>Significant differences</b>				
75	Hormone replacement treatment limited the age-related increase in skin extensibility Other parameters of skin viscoelasticity were not affected	Computerized suction device measuring skin deformability and viscoelasticity; inner forearm	Women: 43 nonmenopausal (19–50 y) 25 menopausal not treated (46–76 y) 46 on hormone replacement therapy since onset of menopause (38–73 y)	Hormone replacement therapy has a preventive effect on skin slackness
76	Collagen content increased by 48% with hormone replacement therapy compared with nontreated subjects	Hydroxyproline and collagen content; biopsies of right thigh below the greater trochanter	Postmenopausal women (35–62 y) 29 untreated; 26 estradiol+ testosterone	Estrogen or testosterone, or both, prevent the decrease in skin collagen content that occurs with aging
77	Increased proportion of type III collagen in the skin of postmenopausal women receiving hormone replacement therapy	Analysis of collagen types; biopsies of lateral thigh	Postmenopausal women (41–66 y) 14 untreated; 11 estradiol + testosterone	The clinical improvement in the skin following hormone replacement therapy is due not only to increase in total collagen but also to changes in the ratio of type III to type I



**Table 1.10** Pilosebaceous unit

Ref.	Finding	Obtained by	Subjects	Conclusions
<b>Significant differences</b>				
79	During January women's hair was denser and the percentage of telogen hair lower compared with men	Phototrichogram; hair count after washing	7 women, 29–49 y; 7 men, 25–47 y	
78	Higher sebum secretion in men than in women for age ranges 20 to over 69, but not for the 15–19 age range In the 50–70 age range the secretion in men remains unaltered, whereas in women there is a significant decrease in sebum output, probably as a result of decreased ovarian activity	Sebum production	330 women; 458 men; 15 y to over 69 y	
78	No correlation between sebum production and plasma testosterone	Sebum production and plasma androgen levels	8 women; 28 men	

50–70 age range, when the secretion in men remains unaltered whereas in women there is a significant decrease in sebum output, probably a result of decreased ovarian activity.

Beginning in young adulthood there is an age-related decline in wax ester secretion—thus hormones also affect the composition of sebum.

The distribution of hair over the body differs between men and women. The hair follicles possess individual mechanisms controlling the evolution and triggering of successive phases, but systemic factors like hormones and external factors also play a significant part. The season of the year has an effect on hair growth and hair shedding. From data given in a study concerning this seasonal effect (79), we calculated sex differences, which were not discussed in the study. The data referred to the month of January. Women's hair was denser and the percentage of telogen hair lower compared with men.

The diversity of male and female hair patterns is determined by a difference in the transformation of vellus to terminal hair, stimulated by androgens, but also by racial and genetic factors. In Koreans, women had a significantly higher number of terminal hairs than men (80).

The effect of androgens on hair growth varies according to body site, and may be opposite, like transforming vellus hair on the face to terminal beard hair at puberty and the reverse on the scalp. The face, scalp, beard, axilla, and pubic hair follicles are targets for androgens. Androgen affects different cells in the dermal papilla, which is also affected by melanocyte-stimulating hormone (MSH), prolactin, thyroid hormones, pregnancy, and nutritional state (81). In addition to higher serum levels of testosterone, female facial hirsutism correlated with obesity and age (82).

Despite exposure to the same circulatory hormones, the activity of hair follicles depends on the body site, varying from no effect on the eyelashes to stimulation in many other areas. High levels of testosterone inhibit the hair papilla cells and outer root sheath keratinocytes and have a lesser effect on fibroblasts and interfollicular keratinocytes, while low levels of testosterone have no effect. The opposite was found with estrogen and cyproterone (83).

The effect of estrogens (17-beta-estradiol, E2) on estrogen receptor (ER) expression and gene regulation of human scalp

hair follicles was studied in vitro. The distribution pattern of ERbeta and TGF-beta2-immunoreactivity differed between male and female hair follicles after 48 h culture. Of 1300 genes tested, several genes were regulated differently as relates to gender. Thus, substantial sex-dependent differences were found in the response of frontotemporal human scalp hair follicles to E2 (84).

## CONCLUSIONS

Maintaining skin health is an intricate orchestration of many variables. The need for hard data is paramount, not only for gaining knowledge about the anatomy and biology of human skin, but also for the assessment of pathophysiological processes and for clinical management of skin diseases. New and improved instrumentation will allow for more studies, leading to a detailed description of physiological differences between men and women.

We hope that this chapter will trigger further investigations of the subject.

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# Climatic Influence on Cosmetic Skin Parameters

Mathias Rohr and Andreas Schrader

## INTRODUCTION

In addition to good compatibility, which should be a matter of course for cosmetic products, the physiologic effectiveness, in particular moisture and smoothing effects on the skin, is the main interest for cosmetic products. Techniques such as fast optical in vivo topometry of human skin (FOITS) (1,2) and corneometry are used to investigate their effectiveness. A high degree of standardization is required to quantify the effects of cosmetics (3,4). To obtain reproducible and statistically significant results, experimental conditions, such as test panel-controlled climatic conditions and a test design including a positive and a negative standard, are the basic starting tools. Nevertheless, as the following discussion will show, it is not only the normal standardization procedures, such as acclimatization of volunteers in special air-conditioned laboratories, which have to be taken into consideration when interpreting objective and subjective cosmetic parameters, but also the effect of the actual climate during the application phase and especially during the days of measurement. The influence of the indoor climate in the laboratory as well as the outdoor climate will be analyzed. What will happen to the level of skin moisture during the preconditioning phase or what will happen at different seasons of the year? Will it be influenced by the level of relative room humidity and/or the actual climate conditions? Will the influence vary for different kinds of products? Will the influence on skin moisture and skin structure be comparable? Will the influence change for different types of volunteers? What is the best time for preconditioning? Could the regeneration of the stratum corneum be influenced by the climate? Will effects felt subjectively (washing the bend of the elbow) be equally dependent on climatic conditions as objectively rated parameters?

A summary of individual results and averages of thousands of volunteers will be given. Both a positive standard (in the sense of increasing moisture and smoothness) and a negative standard (in the sense of increasing dehydration, roughness or side effects) are used to present the effect of climatic conditions on skin physiology tests.

## MATERIALS AND METHODS

### Climatic Data

To be able to correlate climate data with skin physiology parameters, the relative humidity and outside temperature are measured continuously at a station by a computer (CAN system, Lufft Company, Fellbach, Germany). Capturing the data by computer ensures that the climate is recorded day and night. Let us take climatic changes in Holzminden (longitude 9.27 east and latitude 51.49 north; Middle Germany) over a year as an example. As Figure 2.1 shows, temperature fluctuates

between values of about  $-10$  and  $25^{\circ}\text{C}$  in a year. Relative humidity is about 50% in summer and 90% in winter.

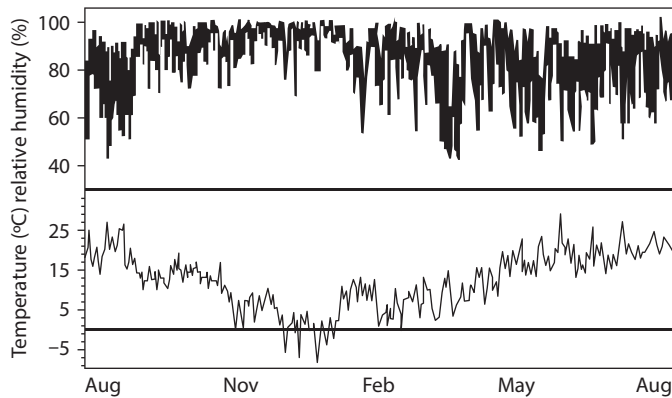
## Positive and Negative Standards

Tests have been carried out with the same products repeatedly over a period of several years, and these will serve to demonstrate the effect of climatic conditions on skin physiology. The positive standard is a well-accepted former brand product that is currently unavailable on the European market. However, we have been making it at a constant quality level for years using the known formulation. This product, referred to hereafter as "standard L" (Table 2.1), is tolerated very well by the skin and demonstrates a moisture-retaining and skin-smoothing effect that can be easily classified in terms of physiologic effectiveness. This makes it an ideal standard, because other products can be classified as better or worse with respect to their effectiveness. Another aspect of demonstrating the effectiveness of products on skin physiology relates to negative effects that, for instance, can be induced by aggressive surfactants. Here, too, we have been using the same standard product for years. This is sodium dodecyl sulfate (SDS), which is referred to as the "negative standard" from now on.

## Laser Profilometry

The laser profilometry technique is used to investigate the anti-wrinkle effect. Skin replicas are taken from the test areas on the volar forearms by means of a white pigmented silicone substance (two components, Optosil, Bayer, Inc., Germany), before the first application and 12 hours after the last application. A round impression having a diameter of 18 mm is made using a label especially designed for this purpose. While the impressions are being made the volunteers are seated on chairs with adjustable armrests so that the angle between the upper arm and the forearm can be adjusted to  $90^{\circ}$ . Fixing the forearms in this way ensures that no factitious smoothing or roughening effects, due to stretching of the arms when the impressions are taken after application, are evaluated and included in the documentation.

An automated laser scanner with an optical autofocus sensor is used for contactless scanning of the skin replicas (UBM, optical measuring system Microfocus, UBM RC14, Karlsruhe, Germany) (5). The measuring range of the laser scanner is  $\pm 500$  mm at a resolution less than 0.01% of the measuring range. The measuring spot (focus of the laser diode) has a diameter of about 1 mm. The z resolution is increased to  $\pm 25$  mm by an additional shift of the z-axis if necessary. The resolution in the x- and y-directions is identical to be independent of any predominant direction of wrinkles. The skin replica taken from the volar forearm of a volunteer is scanned over an area of  $8\text{ mm} \times 8\text{ mm}$  in the x- and y-directions at a



**Figure 2.1** Climatic outdoor conditions at Holzminden, Germany, from August 2001 to 2002.

**Table 2.1** Declaration of Positive “Standard L” According to the International Nomenclature of Cosmetic Ingredients

Ingredients
Water
Liquid paraffin
Caprylic/capric triglyceride
Hydrogenated coco-glycerides
Glycerine
Myristyl alcohol
Isohexadecane
Glyceryl stearate
Cetyl alcohol
Proprietary composition
4-Methylbenzylidene camphor
Tocopheryl acetate
Butyl methoxydibenzoylmethane
Aloe barbadensis
Isopropyl myristate
Methylparaben
Polyaminopropyl biguanide
Bisabolol
Soluble collagen
Simethicone
Sodium hydroxide
Ethylenediaminetetraacetic acid

resolution of 25 points/mm. Thus 40,000 individual measurements are available, permitting an exact three-dimensional reconstruction of the skin surface (5,6).

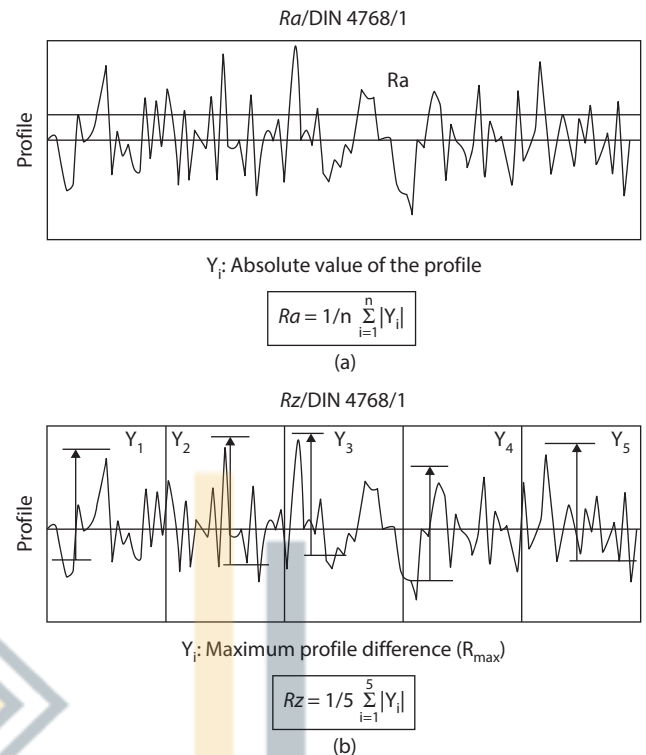
### Ra Parameter

The Deutsche Industrie Norm (DIN) parameter Ra represents the mean roughness index according to DIN 4768. Ra indicates the arithmetic mean of the absolute values of the skin profile's deviations from the center line over the total distance.

If the overall structure of the profile remains unchanged (Rz constant) but the fine structure of the profile changes, then the Ra parameter will indicate smoothing or roughening by a reduced or increased value, respectively (7,8).

### Rz Parameter

The Rz parameter represents a mean peak-to-valley height according to DIN 4768/1. If, in the two-dimensional case, a profile line is divided into five equal parts and the Rmax parameter is calculated for each part, Rz will be the arithmetic mean



**Figure 2.2** Definition of DIN parameters Ra (a) and Rz (b) according to DIN 4768/1.

of these five individual values. The Rz parameter will indicate roughening of the skin profile by a significantly increased value if the profile is changed by the influence of a product (Figure 2.2).

## FAST OPTICAL IN VIVO TOPOMETRY OF HUMAN SKIN

After a successful validation phase, the new FOITS technology was introduced in 1997 (1). In comparison to the replica-driven technique during the previous decade, the touch-free technique of fringe projection became state-of-the-art to investigate skin surface (2,9–11). Because of many technical advancements (for example, improved camera resolution, the use of blue LED lighting systems, or laser-supported and computer-optimized overlaying procedures), an easy-to-operate system has been realized recently. As there has always been a great deal of scientific interest on the mechanisms of wrinkle evaluation, the technical developments led to a tool of high scientific standard (12–15).

FOITS is a touch-free optical technique with a history of more than a decade of investigating skin surface structures in a direct three-dimensional measurement by fringe projection (16). The fringe-projection technique used is a combination of gray-code and phase-shift technique (7). In less than a few hundred milliseconds, the absolute space coordinates of all object points in the selected image area are measured with great precision. The FOITS measurement system consists of a projection unit and a CCD camera. Both are fixed under the triangulation angle. In the gray-code method, grids with a rectangular brightness distribution by different numbers of lines are projected. The number of

lines is doubled at each new projection. This gives a clearly defined hierarchy of lines for each image point. In the phase-shift technique, only one grid with a sinus-like intensity distribution is projected several times with different phase positions. The FOITS technique is able to realize a depth sharpness area of  $\pm 10$  mm on an inspection area of 30 mm  $\times$  40 mm. The resolution in the vertical z-direction with 0.2% of the measured area leads to an effective resolution of 4 mm in the z-direction. A CCD camera with horizontal and vertical resolution in x- and y-directions of about 30 mm is used. The resolution in the z-direction is not limited by 256 gray steps

of the CCD camera. The high resolution in the vertical direction is achieved by analysis of the intensity and phase displacement of the projected grids. The surface structure of the analyzed area causes a deviation of the intensity and phase information of the projected grid structures from the theoretical model structure of a plane surface. With corresponding mathematical algorithms, the absolute three-dimensional coordinates of the inspected area can be calculated of these deviations. A synopsis of the most important experimental side parameters is shown in Figure 2.3, from the first experiments up to the current time (Figure 2.4).

FOITS	1995	1998	2003	2006
Technique	Gray-code and phase-shift technique			
	Contact free direct skin measurement in vivo			
	Halogen light		Blue LED technique	
Superimposition	Mechanically aided by online overlay procedure		LASER aided mechanically	Software aided on top of all
Measurement area	Inner side of the forearm	Crow's-feet, under the eye, cheek, glabella, lips, nasolabial, dé colleté, forearm, leg		
Area of inspection	875 mm <sup>2</sup> (25 mm $\times$ 35 mm)		1200 mm <sup>2</sup> (30 mm $\times$ 40 mm)	
Area of analysis	20 mm $\times$ 20 mm		20 mm $\times$ 20 mm (or as needed)	
Resolution x-direction	~40 mm		~30 mm	
y-direction	~40 mm		~30 mm	
z-direction	4 mm		4 mm	
Time to digitize the fine structure	~320 msec		~260 msec	

Figure 2.3    Synopsis of the Technical Side Parameters of FOITS.

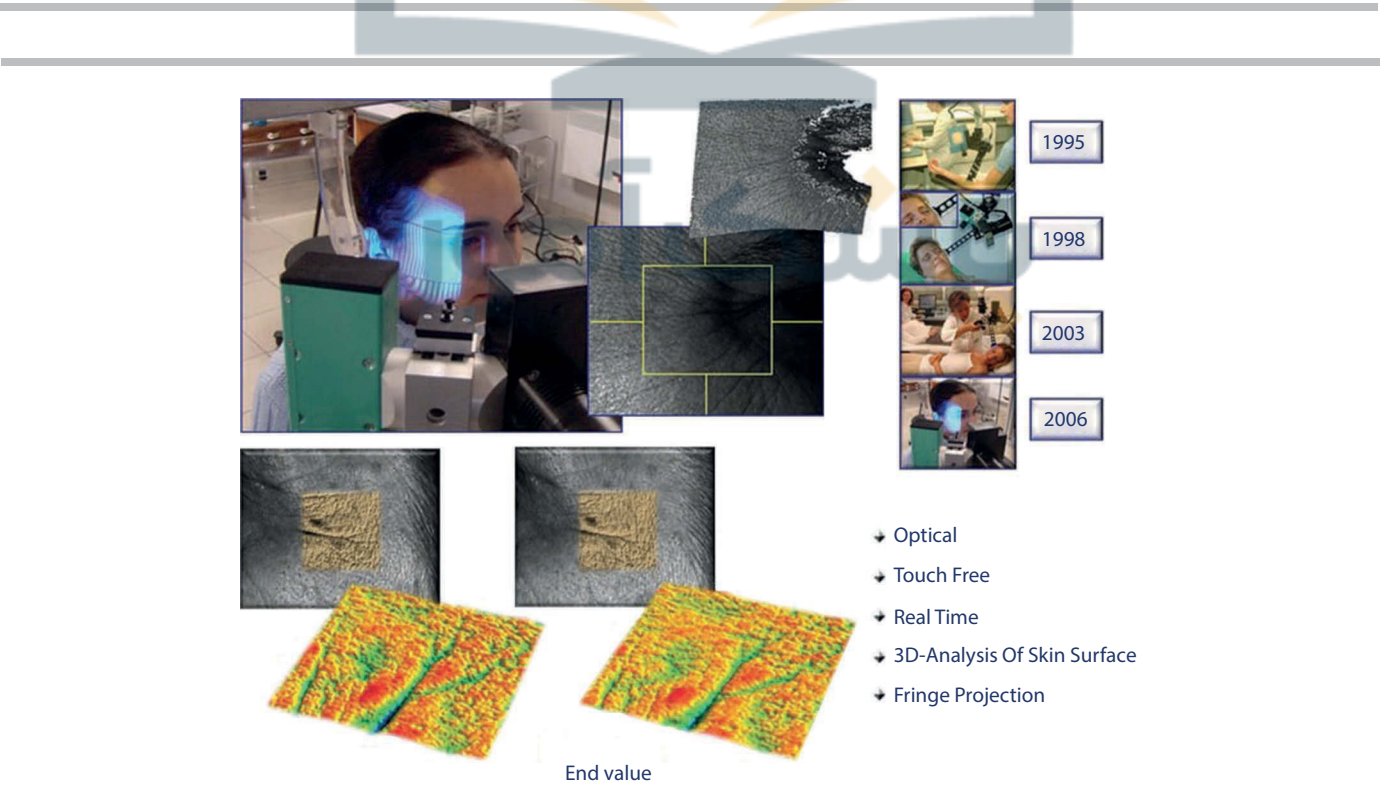


Figure 2.4    Presentation of various FOITS system from 1995 to today; example of FOITS data presentation on an individual subject. 3-DIM data presentation of the crow's-feet area before and after 4 weeks of product application.



Starting with analysis of the inner side of the forearm, the crow's-feet area eventually became the area of most interest. Increasing the power of FOITS technique as described in Figure 2.3, more areas could be investigated such as the cheek, glabella area, under the eye, nasolabial area, lips, or all body areas such as the décolleté and legs. The latest technique combines the fastest data measurement with the best superimposition technique to guarantee a perfect comparison of baseline and end-value data. Superimposition is realized in a combination of laser-aided mechanical alignment of the subject in a first step followed by a software-driven rotation and shifting procedure of measured data/pictures to find the optimum superimposition.

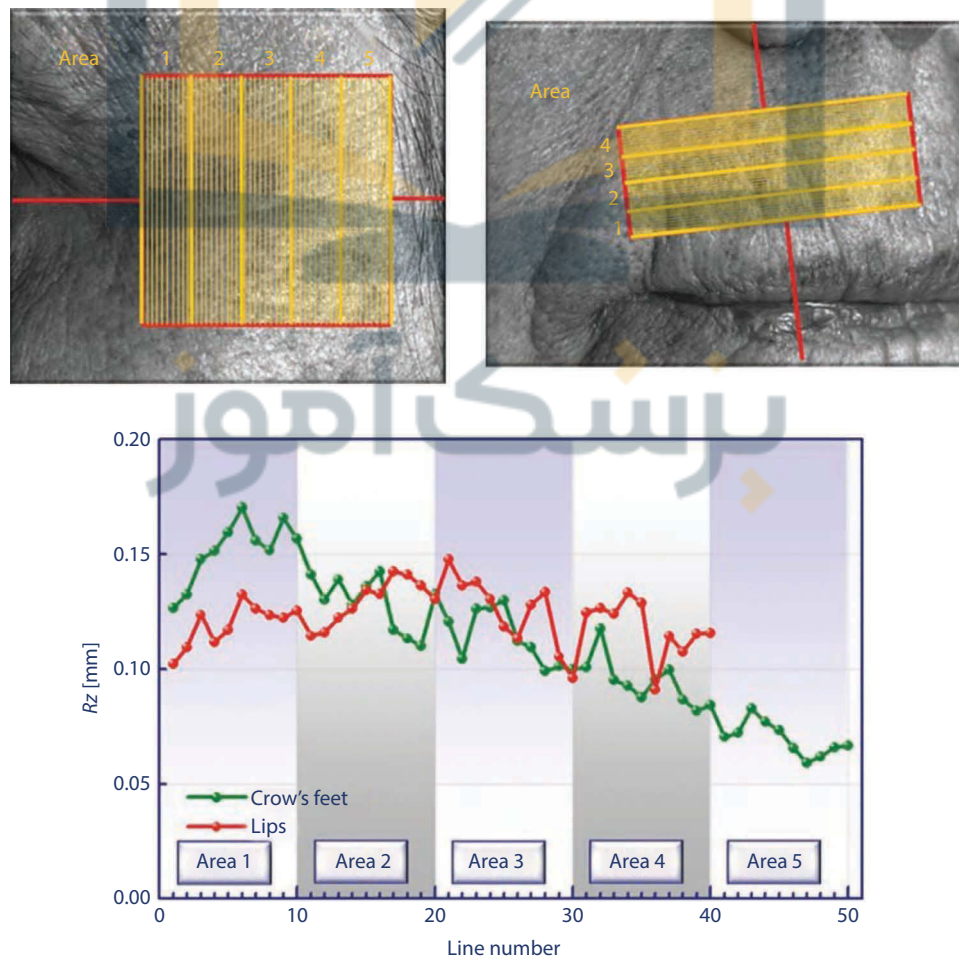
### Parameter of Analysis

Bringing into focus the periorbital wrinkle area (crow's-feet), the morphological structure of this test area has to be taken into account if wrinkles are investigated. Having this in mind, analysis is carried out perpendicular to the main wrinkle direction based on the Rz parameter (according to DIN 4668 [12]) or the frequency distribution of depth (FDD) analysis. Starting close to the eye, 50 separate lines with a distance of 400  $\mu\text{m}$  are analyzed. The resulting roughness is shown as a function of line number (Figure 2.5). Ten successive lines are averaged, resulting in five areas of evaluation. Separating

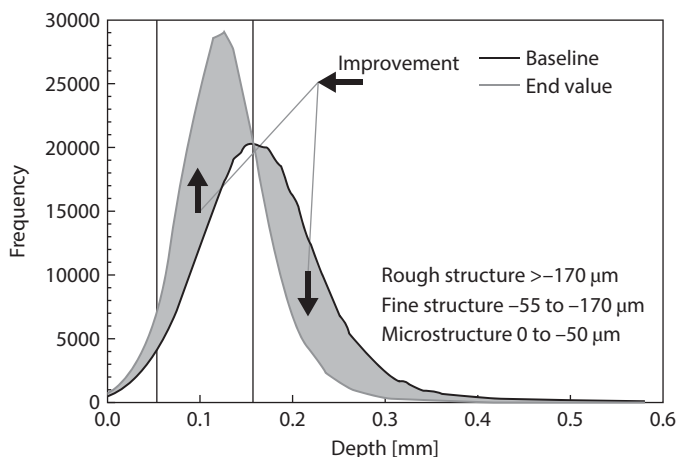
the area of analysis into these five subareas (areas 1 to 5, see Figure 2.5), the area close to the eye, called area 1, represents the deepest structures, while with area 5 smaller structures are quantified. An example of this analysis is given in Figure 2.4. In comparison, analysis of the lip area is shown. Because of the smaller test area, only four areas are defined with 40 separate lines with a distance of 250  $\mu\text{m}$ . As shown by Figure 2.1, correlation of line number and Rz results in a more flat link for the lip area in comparison to the crow's-feet area.

To document the surface structure by a global parameter, the frequency distribution of all depths is used. The FDD is calculated in the range from  $-600 \mu\text{m}$  to  $600 \mu\text{m}$  (after polynomial correction) by using interval steps of 5  $\mu\text{m}$ . The defined evaluation area is equivalent to a surface of  $2 \text{ cm} \times 2 \text{ cm}$  and according to the technical resolution of the camera represents 640,000 single points. Therefore, a calculated FDD parameter is based on a rearrangement within these 640,000 values of depth.

Working with a distribution function, the zero level has to be kept in mind. Thus, the zero level of each volunteer is defined as the first plane representing a level of about 0.1% of all single values (about 600 counts). This plane is set as zero and all further calculations are done with these resulting standardized values. From the surface structure, a frequency distribution of all depths is obtained, as shown exemplarily in Figure 2.6 (left curve).



**Figure 2.5** Definition of subarea of analysis. Rz as a function of subarea lines of an individual example in the crow's-feet area and lip area.



**Figure 2.6** Histogram of depth of a surface profile (crow's-feet area), classification of structural regions as well as visualization of smoothing effect/age effect—baseline: 65-year-old subject, end value: 15-year-old subject.

According to the selected zero level, a classification of depth is made as follows:

- 0 to 50 mm → Microstructure (about 5%)
- 55 to 170 mm → Fine structure (about 65%)
- <170 mm → Rough structure (about 30%)

The given proportion will give a rough estimation of structure ranges found in the crow's-feet area of women with distinct wrinkles and Caucasian skin. Taking into account a product's smoothing effect, the green FDD curve as shown in Figure 2.4 can be expected. Consequently, an improvement of skin structure is defined by a shift of maximum and a change of width of the distribution function. A reduction of rough structures can be expected, while for fine- and microstructures an increase is obtained in the case of structural improvements.

## Corneometer

Water differs markedly from most substances as far as its dielectric constant is concerned. A quantitative proof of changes to the water content of the skin can thus be achieved in a noninvasive manner by means of capacity measurements (17,18).

A corneometer (Courage + Khazaka Co., Köln, Germany) is used to measure the water content (Table 2.2). A measuring capacitor reacts to the samples in the volume to be measured by way of capacitance changes (depending on water content). Those capacitance changes registered by the measuring head capacitor are processed fully automatically by the equipment to form a digital measured value. There is no conductive (galvanic) connection between the object measured and the measuring equipment. Consequently, almost no electricity flows through the object measured. Properties such as ionic conductivity and polarization effects have no influence on the measurement result. The fact that the electronics adapt to the moisture circumstances almost without inertia means that the measuring process is very fast and that it is possible, to a considerable extent, to eliminate effects on the results caused by involuntary movements or moisture accumulation during the measuring process.

**Table 2.2** Summary of Experimental Conditions for the Various Skin Physiology Tests

Investigation brief description corneometer 20–30 volunteers
2–3 wk of application; twice a day
Baseline measurement on the forearm
Final value 12 h after the last application
Statistical analysis of data
Corneometer kinetic frequent measurements up to 5 h
Laser profilometry 30 volunteers
3 wk of application; twice a day
Silicone replica of the forearm (baseline)
Silicone replica 12 h after the last application (final value)
Robot-controlled laser profilometry
Analysis of Ra and Rz
FOITS frequent measurements up to 4 h
No replica
Analysis of Ra and Rz
Washing test on the bend of the elbow 20 volunteers 5 days of application
Twice a day, 2 × 1 min of washing
Subjective rating of side effects in a direct comparison
Reddening/stinging/skin tautness/itchiness
Skin roughness/dull feeling/bad skin feeling
Statistical analysis of reaction points
DHA decoloring 20 volunteers, aged >50 years
Measurement of skin color by chromameter (baseline)
Application of DHA to inner side of forearm
Application of test product twice a day for 18 days
Measurement of skin color every day
Analysis of decay curves

Abbreviation: DHA, dihydroxyacetone.

All tests mentioned in this discussion were carried out in an electronically controlled air-conditioned laboratory that ensures that room temperature and air humidity are kept constant. The volunteers were kept seated in this laboratory at 22°C (±1) and 60% or 50% (±5%) relative humidity for 45 minutes before the test and during the complete standard test procedure.

To quantify the influence of this procedure of standardization, frequent measurements were carried out immediately after the volunteers arrived at the institute and for up to 5 hours. To show the basic influence of the indoor climate, no product application was performed during the time of the investigation. In a second series of measurements, five different brands and five different formulations with an increasing amount of glycerine (3%–25%) as an active ingredient were investigated in a short time test design up to 4 hours after product application. To quantify the influence of the indoor climate on the product rating, the second test series was carried out twice. In a first run, the relative humidity was set at 60%; in a second run the relative humidity was reduced to 50%.

Transient individual side effects that may have an influence on the skin are standardized in this way. However, this procedure does not compensate for climatic conditions such as winter or summer.

## Regeneration

Dihydroxyacetone (DHA) is a substance that is tolerated very well and is approved in the cosmetics industry as a suntan substance. It tans by means of the Maillard reaction, forming combinations with amino acids in the skin that do not wash off. The color disappears within approximately 3 weeks as a result of desquamation of the colored horny cells. The tan of the skin decreases accordingly.



For this investigation the desquamation effect, and consequently the rate of regeneration, is measured in the laboratory color room by measuring the decoloring with a Minolta Chromameter CR 300 (L-a-b color room). The yellow value b differentiates best, and this is used to establish the color decay curves (19,20).

The region that is tested is again the volar forearm. Areas of 4 cm × 4 cm in the middle of the region of application are colored with DHA after a defined washing procedure to standardize the baseline conditions. In the coloring process, a special emulsion with 10% DHA is applied to the area to be tested. The amount applied is 6 mg/cm<sup>2</sup>. In addition, an adhesive bandage saturated with DHA emulsion is applied for 24 hours. Over the next 18 days, the volunteers continue to use the products twice a day. The forearms are permitted to be washed only twice a day with warm water. Surfactants and abrasive cleansing agents are not allowed to be used. Measurements are taken directly before DHA coloring, and then every day over the next 18 days with the exception of weekends. For each time and area of measurement, three values are recorded at different places in the measurement area and averaged. The b-values of all 30 volunteers per product are averaged, and the standard deviations, percentage changes, and percentage differences standardized to the coloring are calculated. The color decay curves can be described under normal conditions with the following exponential function:

$$b = a_1 e^{-a_2 t} + a_3$$

Further statistical treatment is described in detail in Refs. 3 and 9.

### Washing Test on the Bend of the Elbow

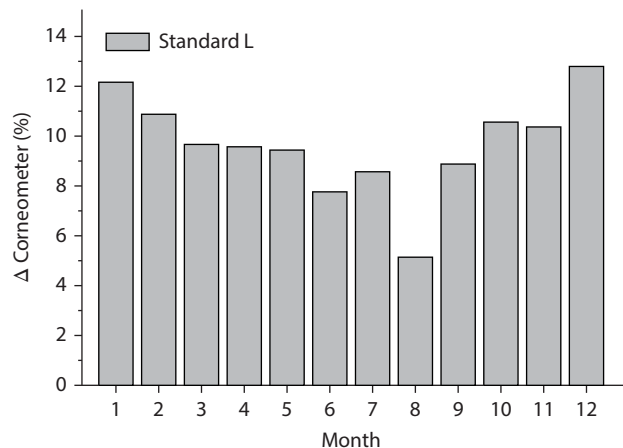
To assess the skin tolerance, the cleansing effect, and the acceptance of surfactant products, we carry out the washing test on the bend of the elbow. In a practical test, the bend of the elbow is washed under intensive conditions. Twenty volunteers take part in this test. In each application, the bend of one elbow is lathered vigorously with the first sample and washed for 2 minutes by hand. After being rinsed with lukewarm water, this bend of the elbow is again lathered and washed for 2 minutes. This is followed by a period of drying also lasting 2 minutes. After the second rinsing with lukewarm water, the area is carefully dabbed dry with a towel, ensuring that there is no rubbing. The bend of the other elbow is treated in exactly the same way with the negative standard SDS (21,22).

To determine any side effects induced by the test products, the volunteers are asked at the end of the test about any reactions they noticed directly after washing. The following parameters are ascertained: reddening, stinging, skin tautness, itchiness, skin roughness, dull feeling, and dehydrated skin feeling. The ratings are given on the basis of a coded volunteer questionnaire.

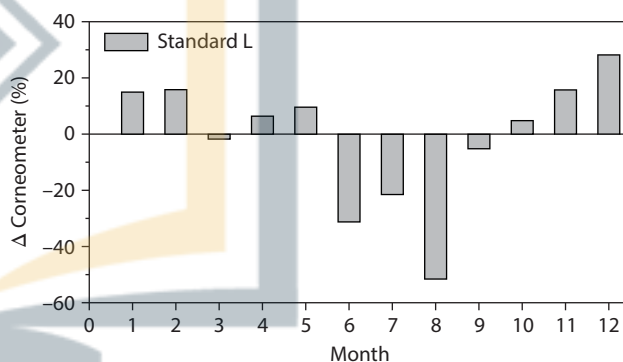
## RESULTS AND DISCUSSION

### Outdoor Climate

One of the major factors in cosmetic skin physiology is the moisture-retaining effect of a product. Figure 2.7 shows a summary of this for 1992–1995. The data have been summarized on a monthly basis in each case. The percentage increase in moisture induced by the positive standard L after correction for changes in the corresponding untreated area is shown. The recorded averages are based on at least 100 volunteers a month.



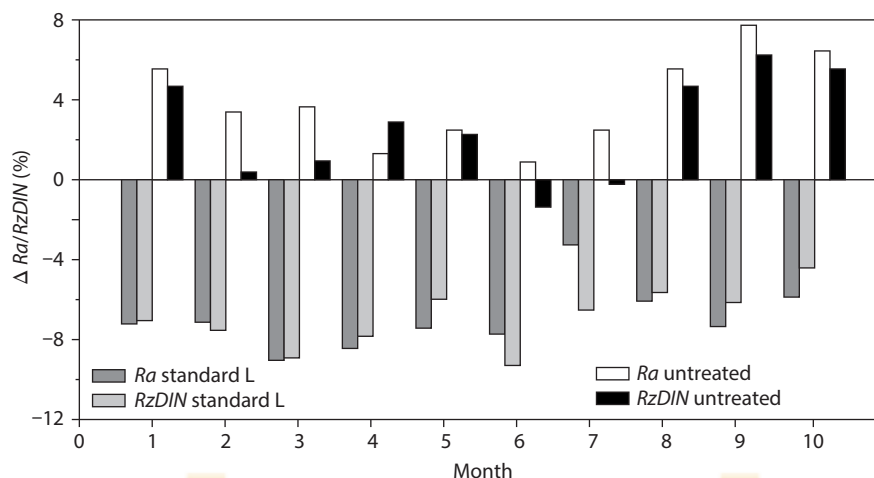
**Figure 2.7** Percentage increase in moisture, after correction for the untreated area, of positive standard L monthly summary (12 hours after last application, 4460 volunteers, 1992–1999).



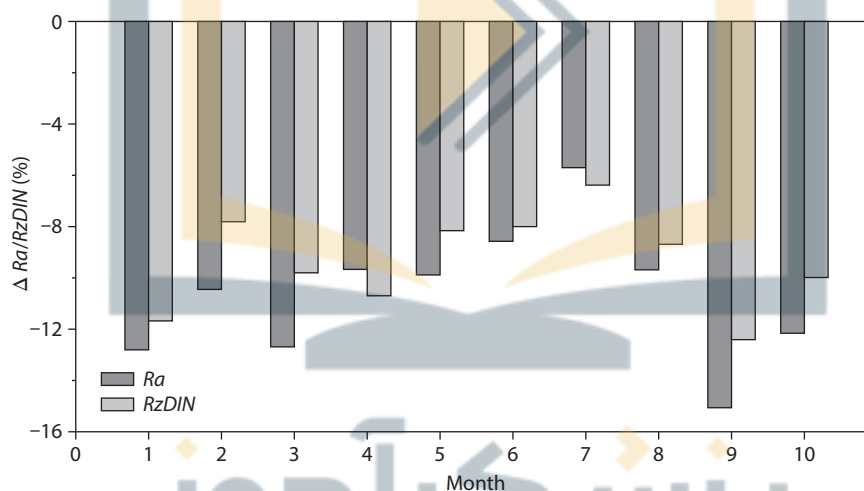
**Figure 2.8** Standardized differences of moisture for the positive standard L after correction for the untreated area (12 hours after the last application, 3100 volunteers, 1992–1995).

Calculations led to an average moisture increase of approximately 12.7% for all data recorded. To make it easier to compare seasonal dependency of the achievable moisture increase, Figure 2.8 shows the difference from the overall average after the data have been standardized on the basis of the overall average. A change of 0% corresponds to the above-mentioned overall average of approximately 12.7% moisture increase. A bar in the positive direction thus shows an increase in moisture that is higher than the average, whereas a bar in the negative direction indicates a reduced level of effectiveness. Figure 2.8 shows that from November to February, there was about 15% above the average moisture increase, whereas in the summer months of June, July, and August, the level of effectiveness was approximately 50% below the average achievable moisture increase.

Figure 2.9 shows the relative change of the laser profilometry parameters Ra and Rz both for the positive standard L and for the untreated area in a way that is comparable to Figure 2.7. The area referred to as “untreated” has not been treated with a cosmetic but has been subjected to a washing procedure to obtain better results, as described in the “Materials and



**Figure 2.9** Percentage of differences for the DIN parameters Ra and Rz for the positive standard L and the untreated area in a summary of laser profilometry data (1000 volunteers in general, 12 hours after the last application, 1994–1996).



**Figure 2.10** Differences of the DIN parameters Ra and RzDIN after correction for the untreated area in laser profilometry (12 hours after last application, 1994–1996).

Methods” section below. Figure 2.9 shows clearly how important this prior treatment is. Whereas the Ra and Rz parameters for the positive standard fluctuate between –6% and –8% from January to October 1994 to 1996 without showing a definite trend, these parameters fall noticeably for the untreated area from January to August, followed by a rise in September and October. After allowing for the untreated area, the profilometry tests result in the dependency that is shown in Figure 2.10. Again, the positive standard L was found to be less effective on average in the summer months of June, July, and August than in the other months.

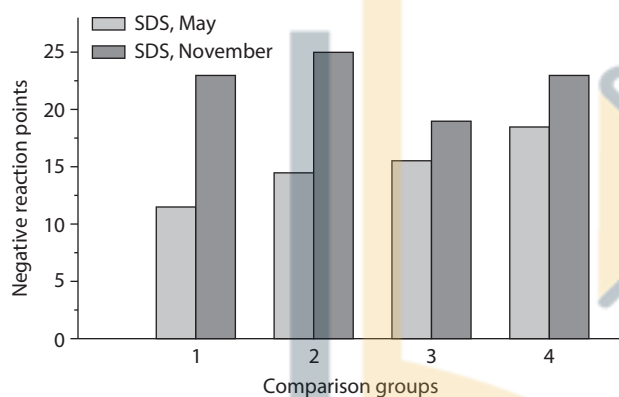
The data clearly show that the seasonal dependency was based on both the reduced positive effectiveness of standard L in the summer and the reduced negative sensitivity of the untreated area (prior treatment with a surfactant of all areas tested). External climatic conditions thus have a distinct influence on the cosmetic effects that can be achieved. The basic

level of the skin is increased in the summer months to such an extent that, first, skin moisture and smoothing can be increased further by cosmetics to only a limited degree and, second, that the deliberate use of substances that are detrimental to the skin also has a limited negative effect. This leads to an apparent reduction of cosmetic effectiveness.

In addition to these objective skin physiology parameters, subjective information gained from volunteers’ answers to questions indicates a comparable dependency on external climatic conditions. Figure 2.11 shows the total negative reaction points that volunteers gave for reddening, stinging, skin tension, itchiness, skin roughness, dull feeling, and bad skin feeling in the elbow washing test. The negative reaction points for the negative standard fluctuated between 11 and 18 in May, depending on the comparative product. Since the comparative product is of crucial importance in rating effects subjectively, the same test setup was repeated in November with the same

comparative products. Here, the average total negative reaction points for the comparative product SDS were distinctly higher in all four groups taking part in the test. Whereas the average for May was approximately 15 negative reaction points, this rose to approximately 23 reaction points in November under otherwise identical conditions as far as the volunteers' subjective feelings were concerned. These data, based on 80 volunteers, clearly show that it is possible and necessary to correlate information derived from volunteers' subjective ratings with climatic conditions and to consider this along with the objectively demonstrable parameters for skin physiology.

Another example of how external climatic conditions make it almost impossible to evaluate the results of skin physiology investigations is the turnover of the stratum corneum on the basis of DHA decoloring tests. When the stratum corneum has been colored with DHA, it can generally be expected that



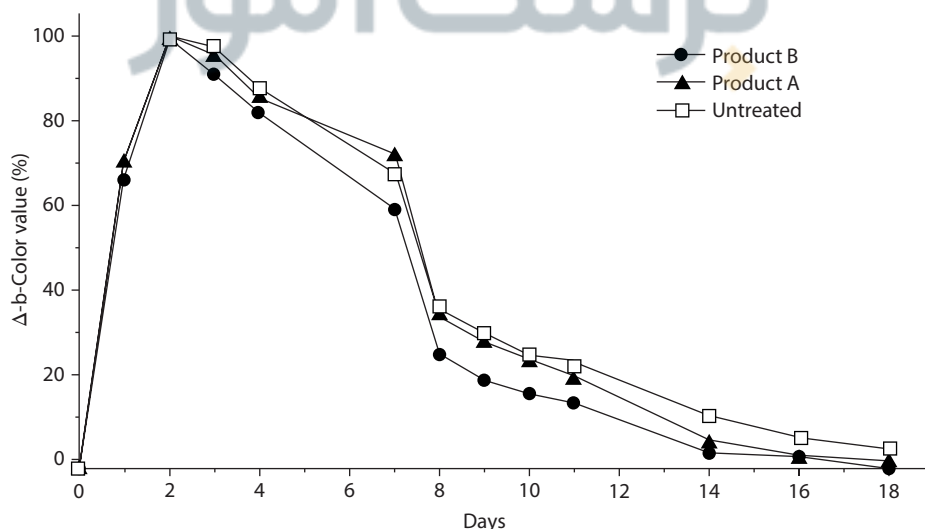
**Figure 2.11** Negative reaction points in a subjective rating system for four individual comparisons of the negative standard sodium dodecyl sulfate (SDS) to four different products in a washing test on the bend of the elbow (20 volunteers in each comparison).

there will be a constant exponential reduction of skin coloring of both the untreated area and the areas that have been treated with the test products (19). Figure 2.12 shows average curves that have been standardized to the maximum coloring, on the basis of 20 volunteers for two test products (A and B) containing  $\alpha$ -hydroxy acids and one untreated area. The observation period was 18 days. In contrast to theoretical expectations and preliminary experiments, this investigation revealed a reduction in skin coloring from about 70% to about 30% on day 8. Both before and after this sudden change, the curve is in keeping with theoretical expectations. When all potential technical sources of error had been eliminated, the solution to this problem was found in the temperature and relative humidity data for the days of measurement, as shown in Figure 2.13. As the curves show, relative humidity fell from about 90% to about 60%, whereas the temperature rose from about 0 to 68°C over the same period of just a few hours, and then fell to 18°C after a short time. Since temperature/humidity fluctuations were far less extreme in the rest of the test period, it seems reasonable to suppose that the strong fluctuations of temperature and humidity correlate with the recorded inconsistency in the DHA color decay curves. This inconsistency induced by extreme climatic fluctuations made it necessary to repeat the test, because it was no longer possible to carry out an exponential analysis of the decay curves.

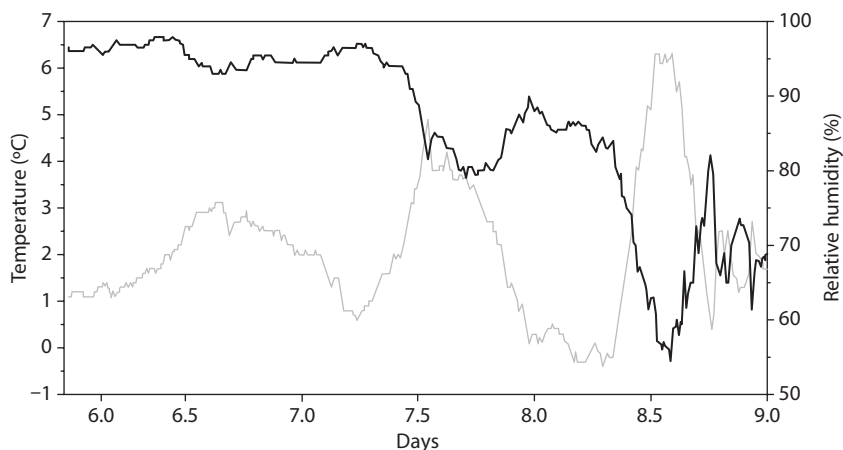
As the measured curve was constant before and after day 8 but higher humidity fluctuations accompanied by lower temperature fluctuations were recorded on day 7, it can be assumed that humidity is of greater importance in examining the regeneration of the stratum corneum and that the outside temperature plays only a subordinate part in the quality of this skin physiology investigation.

### Indoor Climate

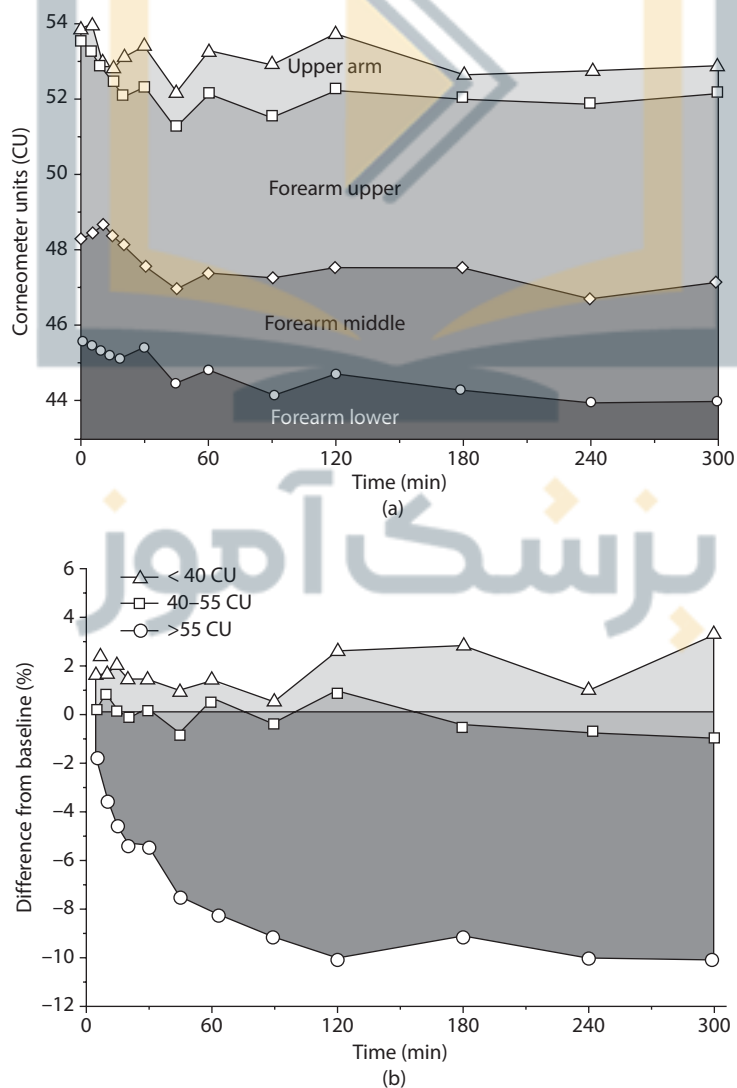
Figure 2.14a presents the results of the “no-product corneometer kinetic” (i.e., without application of a product). The kinetic measurements were carried out on four different test areas (forearm—lower, middle, and upper—and upper arm). In Figure 2.14b, the forearm data are summarized on the basis



**Figure 2.12** Exponential decay curves of the dihydroxyacetone (DHA) decoloring test standardized to the maximum coloring characterized by changing of the b-value of the L-a-b color room.



**Figure 2.13** Climatic data of temperature (grey) and relative humidity (black) from day 6 to day 8 during the dihydroxyacetone (DHA) investigation.



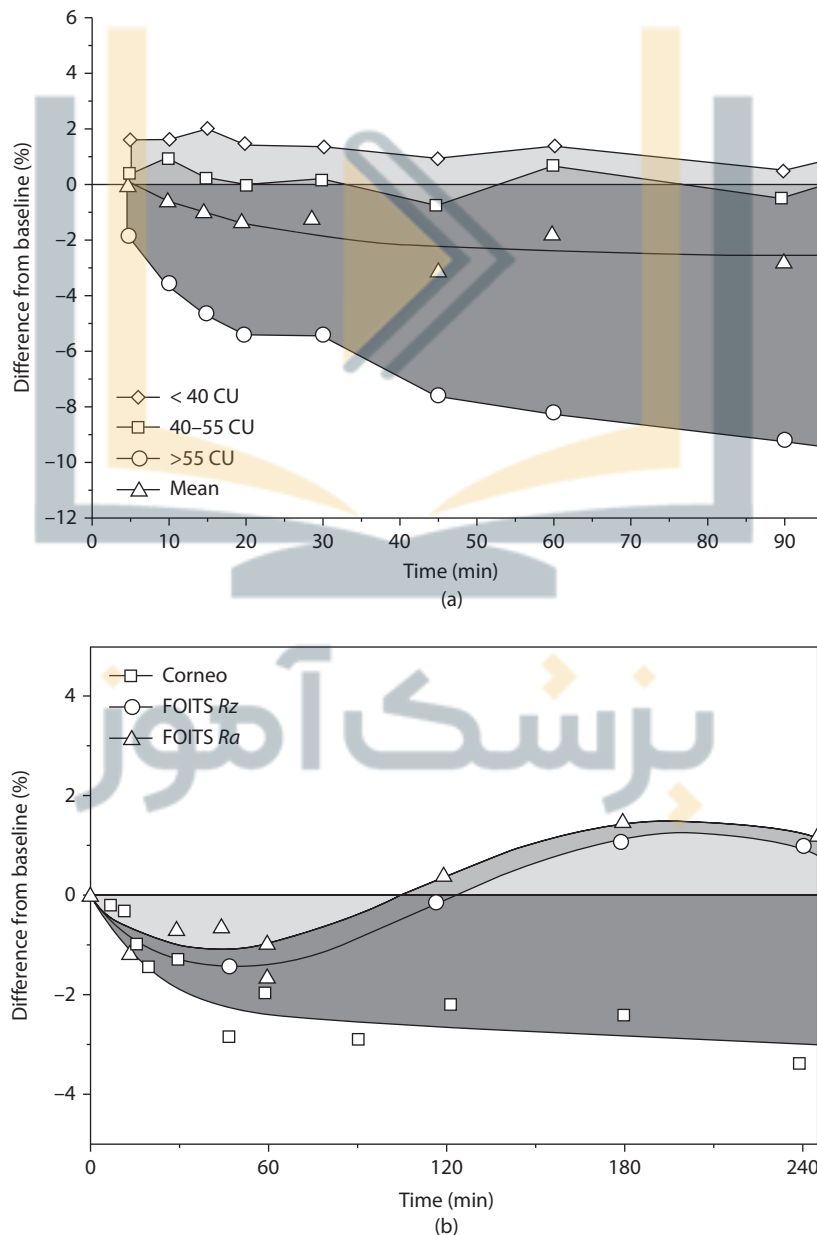
**Figure 2.14** (a) Kinetic corneometer—data summarized for different test areas, without any product application ( $n = 120$ ). (b) Kinetic corneometer—difference from baseline; data summarized for different volunteers, without any product application ( $n = 120$ ).

of the first measured value. The first group had starting values below 40 corneometer units (CU), the second group summarized the volunteers between 40 and 55 CU, and the third group was based on starting values above 55 CU.

Analyzing the data of different test areas resulted in a decrease of about 2 CU for the upper forearm and a little less for the other test areas independent of the absolute level, which was different for each test site (lower forearm < middle forearm < upper forearm = upper arm). These data were calculated without taking into account the individual skin type of the volunteers. Figure 2.14b reflects this, showing the individual starting conditions. As can be seen from the differences from baseline, the group with 40 to 55 CU did not show any changes above about 1% during 5 hours of investigation. The group

below 40 CU showed a constant increase of approximately 2%, whereas for the group with high starting values above 55 CU, a decrease of up to 10% was obtained. Independent of the test site, the preconditioning phase seems to be most effective for a high skin moisture level at the beginning of the study. A dry skin might be less influenced by the indoor climate. The data to determine the optimal time of preconditioning to generate stable skin conditions are represented in Figure 2.15.

As shown in Figure 2.15a, the difference from baseline (–D– curve: mean overall) became stabilized at 30 minutes and remained constant from 60 minutes on. Thus, 45 minutes of acclimatization seems to be the best choice—a time not too short for “moist” skin and not too long to reflect a reliable test design.



**Figure 2.15** (a) Kinetic corneometer—difference from baseline; data summarized for different volunteers up to 90 minutes, without any product application ( $n = 120$ ). (b) Kinetic FOITS and corneometer—difference from baseline mean overall up to 240 minutes, without any product application ( $n = 120/40$ ).



The data describing the skin surface are given in Figure 2.15b. No significant changes occurred during the 4-hour kinetic investigation. Differences between lower and upper forearm were comparable to the corneometer measurements. Summing up the Rz and Ra values for up to 4 hours, no trend in the changes was observed. Consequently, the influence of the indoor climate seems to be of minor impact if compared to skin moisture. In any case, changes of the skin structure are obviously on a much slower time scale if the producing event is as indirect as the indoor climate.

Changing the kinetic view to more static analysis, the data of five different brands are summarized in Figure 2.16. Figure 2.16a shows the difference between baseline and end value 4 hours after unique product application in absolute CU. The dark gray bars represent the data at an indoor climate of 60% relative humidity, whereas the light gray bars are obtained at 50% relative humidity.

With the exception of product no. 1, no difference occurred from changing the indoor humidity. For product no. 1, a tendency was calculated for the comparison of both measurements. Taking product no. 1 as a hint that an influence might

be possible, a second run of five formulations with an increasing amount of glycerine was carried out under the same conditions. In this case, significant changes occurred for the first two low-glycerine concentrations (concentration below saturation). At 50% relative humidity, the level of measured absolute units decreased significantly. Thus, the selectivity became better if the relative humidity was reduced and the product contained hygroscopic active ingredients. The hygroscopic ingredient seems to pick up the air humidity like a sponge as long as it is in the upper stratum corneum. Nevertheless, the origin of moisture should be irrelevant for the skin, but in the case of ranking and differentiating products as quickly as possible after the product application, it might be helpful to measure at 50% relative humidity.

## CONCLUSION

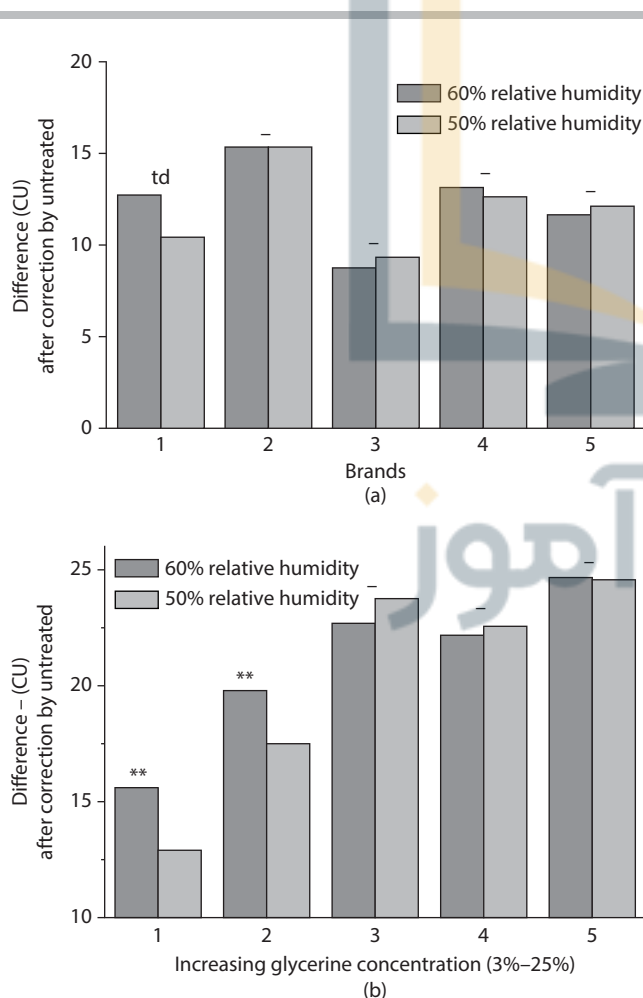
The data recorded, from both objective skin physiology parameters such as moisture and smoothness and subjective factors in the elbow-washing test, clearly show that such tests are influenced considerably by climatic conditions. Differences, such as between summer and winter, cannot be compensated for by acclimatization in air-conditioned laboratories. Alongside standardized measurement conditions, it is therefore essential to record the quality of the test panel not only by including an untreated area but also by means of a positive or negative control. Only in this way is it possible to establish a classification system for test products that is not dependent on a particular season and allows the quality of cosmetic products to be rated objectively.

As demonstrated by the obtained results, the indoor climate also plays an important part in cosmetic efficacy testing. In addition to the outdoor climate, which might have an effect on a long-term basis, the indoor climate (especially the time of preconditioning) is decisive for short-term and kinetic investigations. While the influence of the moisture level is strongly dependent on the starting value, the changes of the skin topometry seem to be not so marked. On the basis of the corneometer kinetic data, 45 minutes of preconditioning appears to be an optimal compromise between effect, standardization, and costs. The laboratory conditions (relative humidity) may also be of great influence. Depending on the active ingredients (hygroscopic or not), a ranking of products might be of greater selectivity if a lower level of relative humidity is used.

The data presented underline the importance of a standardized procedure to investigate cosmetic effects on a statistical and reproducible level.

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**Figure 2.16** (a) Corneometer for brands 1–5. Difference from baseline after correction by untreated. (b) Corneometer for increasing concentration of glycerine (bar 1, 3% increasing to bar 5, 25%). Difference from baseline after correction by the untreated test area. \*\*, significant difference; –, no significant difference.

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# Transepidermal Water Loss

Jan Kottner and Annika Vogt

## INTRODUCTION

The physician Santorio Sanctorius (1561–1636) who lived more than 500 years ago in Italy is considered to be the first person to work systematically on the phenomenon called *perspiratio insensibilis*, the invisible loss of water from the human body. Since then, it was widely agreed that water gets lost via the respiratory system and via sweating, but alternative passages of water through the skin were less clear (1). In 1911 Loewy and Wechselsmann proposed for the first time that besides sweating there is the possibility of passive water diffusion through the skin (2). Why and how this water penetrates and leaves the skin was another issue of debate for the following 40 years (3,4). Irrespective of these discussions, the American dermatologist Stephen Rothman introduced the term *transepidermal water loss* (TEWL) in 1955, characterizing continuous water loss through the epidermis excluding sweat secretion (5). Today TEWL plays an important role in characterizing the skin barrier function in physiological and pathological conditions. This chapter gives a brief overview about the structural and functional determinants of TEWL, how it can be measured, and what this parameter means.

## BIOLOGICAL BACKGROUND

Without effective protection of the “moist inside” of the human body, life in the dry environment on land would be impossible. The main anatomical barrier limiting the amount of water loss is in the stratum corneum (SC). At most skin areas the SC consists of 15 to 20 stacked layers of flat, terminally differentiated keratinocytes connected by desmosomes embedded in a matrix of lipid bilayers. Depending on the hydration state the diameter of the corneocytes is approximately 20 to 50  $\mu\text{m}$  and the thickness is approximately 1 to a maximum of 3  $\mu\text{m}$  if fully hydrated (6,7). The thickness of the intercellular lipid bilayer is up to 0.1  $\mu\text{m}$  (Figure 3.1) (8–10). This paper-thin but dense and compact structure separates the outermost boundary, the skin surface, from the living epidermal cells underneath. From the deeper highly hydrated tissue there is a constant flux of water molecules to the skin surface where it evaporates, resulting in equilibrium between the ongoing process of water loss at the surface and replenishment from beneath. Under physiological conditions the water concentration underneath and in the innermost layer of the SC is approximately 70%, and it is approximately 10% to 30% in the outermost layer of the SC depending on environmental conditions (7,11,12).

In theory, water molecules may take two routes through the SC: diffusion via the transcellular route through the corneocytes or intercellular penetration (Figure 3.1). Because the water diffusion coefficient of the intercellular lipids is considered to be ten times higher compared to the corneocytes, the intercellular water flux might also be higher (13). The structure

and order of these lipids is considered most important for water barrier function (8,14) and they are widely considered to be the major barriers for perpendicular water transport (6,9,15). The composition of the intercellular matrix, however, is complex and includes lipophilic, but also hydrophilic subcompartments. Although hardly detectable under normal conditions or on tissue sections, studies suggest that such aqueous regions can be expanded to tube-like structures, e.g., by means of sonophoresis, which enable entry of large molecules (16). Such phenomena are further supported by work that focuses on pathways of deformable carrier systems across intact skin (17).

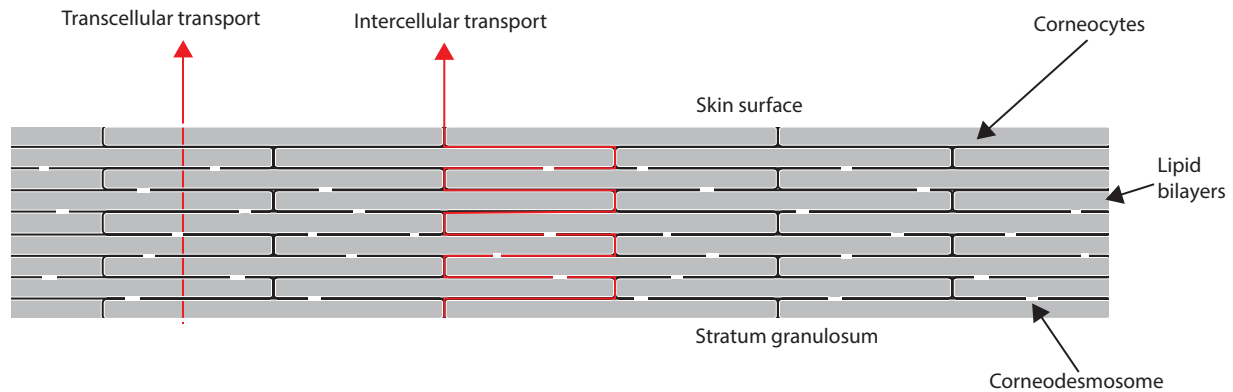
Finite element and microscopic transport models increasingly support the importance of the transcellular permeation pathway for hydrophilic chemicals, including water (6,13,15). The surface area of the corneocytes is also much larger compared to the intercellular lipids and therefore the total amount of transcellular water transport and the subsequent evaporation are major determinants of TEWL in healthy skin under physiological conditions (13).

## MEASUREMENT OF TRANSEPIDERMAL WATER LOSS

The flux of water through the SC cannot be observed directly. Today the established method of measuring TEWL is by placing a measurement probe on the skin and recording the amount of water that evaporates from the skin surface. The measured water flux density is expressed in  $\text{g}/\text{m}^2/\text{h}$ . In order to infer that this amount of evaporated water per area and time equals the water flux within the SC several assumptions need to be met: two of the most important are that sweat gland activity is reduced as much as possible, and that the water flux within the SC is in a steady state. In clinical and research practice this is accomplished by standardizing the measurement conditions and procedures, but total control of these variables is impossible (18).

Several TEWL measurement devices are commercially available. They can be classified as open-chamber and closed-chamber instruments (12,19). Open-chamber instruments consist of a small hollow cylinder which is placed onto the skin. In still environmental conditions the water vapor from the skin surface diffuses through the lower end of the chamber to the upper end into the ambient atmosphere. The steady-state humidity gradient between the skin surface and the ambient air is the physical basis for the water flux measurement. Using these types of devices continuous measurements are possible, but their accuracy depends on the ambient conditions.

Closed-chamber instruments have only one orifice. The open end is placed onto the skin surface and the water vapor diffuses into the closed chamber. The change of the constantly increasing water concentration inside the closed-chamber is



**Figure 3.1** Two-dimensional model of the human stratum corneum and possible inside-out diffusion pathways of water.

used for TEWL estimation. The accuracy of the TEWL estimates is much more independent from ambient conditions, but continuous measurements are not possible. The probe needs to be dried between measurements. The condenser-chamber method also uses a closed-chamber but the water vapor inside the chamber is controlled. Water vapor diffuses from the skin surface toward a condenser, creating a humidity gradient which, comparable to the open-chamber method, is used to TEWL estimation. Continuous measurements are possible.

Based on the different measurement principles, technical specifications, measurement ranges, and degree of precision, different instruments produce slightly different results (20). Even placing a probe onto the skin surface will influence the TEWL, because the water flux density on uncovered skin is different from the flux density within small measurement chambers (12,21). However, available method comparison studies indicate that TEWL estimates seem to be comparable and are highly correlated (22).

In order to achieve valid and reliable TEWL estimates, measurement guidelines have been proposed (18,23–25). Selected key recommendations are:

- An acclimatization period prior to TEWL measurement is required. During this time the skin areas that will be measured must be uncovered by any material (e.g., clothing). Recommended durations vary but they should not be shorter than 15 minutes.
- Acclimatization and measurements should be conducted under standardized ambient conditions. Room temperatures should be lower than 22°C and the relative humidity should be around 50% or lower.
- A calibration according to the manufacturer's instructions must be conducted regularly and/or before a larger set of measurements.
- If the “baseline” or “normal” TEWL is the parameter of interest, no cleansing procedures, leave-on products, or similar applications must be used before measurement.
- Neither too much nor too little pressure must be applied when placing the probe head onto the skin surface. It must be ensured that no gaps arise between the lower orifice and the skin.
- The probe head should be placed vertically onto the horizontal skin surface.

In research or clinical settings these and other details are usually specified in the standard operating procedures. These protocols are especially important when measurements are conducted by many different persons, at multiple centers, and over long durations.

### WHAT DO TRANSEPIDERMAL WATER LOSS VALUES REALLY TELL US ABOUT THE SKIN?

TEWL is considered one of the most important parameters indicating the state and integrity of the epidermal (water) barrier. It is elevated in a large range of dermatological conditions (e.g., atopic dermatitis), and TEWL decreases indicate barrier restoration and skin health (18,23). This parameter is also very sensitive and can indicate functional changes before clinical signs become obvious (26,27). On the other hand, there are numerous non-pathologic factors influencing the TEWL. Despite the measurement instruments and measurement conditions, TEWL is dependent on many factors, including age, skin area, skin temperature, circadian rhythm, season, or cigarette smoking (23,25). Because of this complexity and interdependence of the various factors it is strongly recommended that TEWL values be interpreted very cautiously, and not necessarily in absolute terms (14,28).

Hundreds of descriptive and experimental studies measuring TEWL have been conducted, and larger scale studies reporting skin barrier characteristics of hundreds of persons are now available (29,30). Two systematic reviews and meta-analyses have been conducted to summarize the available evidence about TEWL in adults of 18+ years (22) and infants from 0 to 24 months (31). Despite large variations, these reviews provide reference TEWL values for more than 50 skin areas in healthy individuals. These reference values are useful for study planning and for comparison with existing data, but nevertheless it must be emphasized that a “normal” TEWL seems to be unknown so far (22,23). Consequently, cutoff values or possible TEWL thresholds are also arbitrary. According to Menon and Kligman “... there is no single optimal TEWL value for the entire skin and that what is important is not how ‘tight’ a barrier is but rather when the barrier is good enough to allow for survival and subserve other biological functions...” (p. 180) (32).



## TRANSEPIDERMAL WATER LOSS AS A PARAMETER IN SKIN RESEARCH

In clinical research changes ( $\Delta$ ) of TEWL over time, e.g., during the course of disease, during artificial irritation tests, or during cosmetic treatments, are much better parameters for measuring skin barrier changes or effects of treatments compared to single measurements (33,34). Whether these changes are large enough to be considered clinically relevant is a matter of debate, but they should be larger than random variation (e.g., two standard deviations) in the data. While there is a clear relationship between substantially increased or decreased TEWL with skin barrier function and SC integrity, smaller TEWL changes might rather indicate biological variability only. For instance, a mean TEWL decrease of 1 g/m<sup>2</sup>/h is unlikely to indicate "... an improvement of the skin barrier..." (p. e368) (35). Similarly, lower TEWL in aged skin or senile xerosis should not be interpreted as a "better" skin barrier function (p. 93) (36,37). It must be emphasized that the applied TEWL measurements on the skin surface are indirect (12). Besides random variation or bias, the exact biological or functional reason for value changes must not be obvious. Water diffusion through the SC is for instance determined by the horizontal corneocyte overlap, the corneocyte volume and composition, or the number of layers (10). All these parameters might not necessarily have clinical relevance.

In the course of cosmetic or dermatological studies, comprehensive and transparent reporting of results is of utmost importance to ensure reproducibility, meaningful communication, and comparisons within and between studies. Details must be reported about demographic and health characteristics of the sample including dermatological conditions, season, geographical area, procedures, prior measurements (e.g., use of products, acclimatization), measurement device, the exact anatomical location, the method and number of measurements, and ambient measurement conditions (23). Irrespective of the subsequent statistical analysis, TEWL values must be reported at least using arithmetic means and standard deviations (22).

In clinical controlled intervention studies, randomization is a key design element to reduce bias. Because the TEWL is strongly dependent on the anatomical location, baseline comparability even between adjacent skin areas is limited. For instance, available empirical data indicate increasing TEWL from proximal to distal on the volar forearm skin (22,38) which was supported recently by new empirical data (39). Consequently, randomization even within small skin areas is complicated. Contralateral skin areas are very well comparable, and randomization should be preferred in such so-called "split body designs."

## CONCLUSIONS

TEWL is a valuable, straightforward parameter to measure the SC integrity and the inside-out water barrier. Highly standardized settings are required to yield accurate and reliable data. This parameter is extremely sensitive to environmental factors as well as intra-individual variations among body regions. TEWL is only one parameter to characterize the skin barrier function. Other techniques might be used in addition to obtain a comprehensive picture of the skin barrier structure and function.

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# Nail Penetration

Rania Elkeeb, Xiaoying Hui, and Howard I. Maibach

## INTRODUCTION

Human nail, equivalent to claws and hooves in other mammals, acts as a protective covering for the delicate tips of the fingers and toes against trauma, enhances the sensation of fine touch, and enables one to retrieve and manipulate objects. The nail is also used for scratching and grooming, as a cosmetic organ, and, by some, to communicate social status. The nail plate appears as a thin, hard, yet slightly elastic, translucent, convex structure (1).

Disorders of the nail resulting from conditions such as infections or physical-chemical damage can result in painful and debilitating states, and often change the nail plate's appearance. Onychomycosis, the most common nail plate disorder (2) thickens the nail, makes it white and opaque, and in the toenails it may cause pain while wearing shoes. Onychomycosis is a fungal infection of the nail plate—usually caused by the species *Epidermophyton*, *Microsporum* and *Trichophyton* – and affects 14% of the human population. Aging increases the incidence significantly, with the rate estimated at 48% in persons 70 years of age (3).

To cure the infection, the patient is obliged to take oral systemic medication for an extended period, generally months, or undergo surgical nail removal (4). These treatments have adverse effects such as pain (surgery) and systemic side effects (oral treatment). Thus, topical therapy is a desirable approach, but has met with limited success. Topical therapy is limited by the infection's deep-seated nature and by the ineffective penetration of the deep nail plate by topically applied drugs (5,6).

How can topical drugs be delivered effectively into the nail? And, perhaps as importantly, how can the drug content in the human nail be assessed in order to validate nail drug delivery? Our challenge was to develop a system to assay drug content in the inner nail bed in which infection often resides. We developed a micrometer-controlled drilling instrument that removes and collects from the inner nail bed a powder sample from which—by mass balance recovery—we assay the amount of penetrated radio-labeled drug. With this procedure, the effectiveness of topical nail drug delivery can be assessed (7,8). This paper reviews the results of studies undertaken with drilling system.

## REVIEW OF NAIL PHYSICAL AND CHEMICAL PROPERTIES THAT AFFECT TOPICAL PENETRATION

The human nail anatomy consists of nail plate, nail bed, and nail matrix. The nail plate consists of three layers: the dorsal and intermediate layers derived from the matrix, and the ventral layer from the nail bed (9,10). The upper (dorsal) layer, is only a few cell layers thick and consists of hard keratin. It constitutes a main barrier to drug diffusion into and through the nail plate. The intermediate layer constitutes three-quarters of the whole nail thickness, and consists of soft keratin. Below the intermediate layer is the ventral layer of soft keratin—a few cells thick—that

connects to the underlying nail bed, in which many pathological changes occur. Thus, in the treatment of nail diseases, achieving an effective drug concentration in the ventral nail plate is of great importance. The nail bed consists of non-cornified soft tissue under the nail plate. It is highly vascularized. Beneath the nail bed is the nail matrix, which is a heavily vascularized thick layer of highly proliferative epithelial tissue that forms the nail plate.

The human nail is approximately 100 times thicker than the stratum corneum, and both are rich in keratin. However, they exhibit some physical and chemical differences (11,12). The nail possesses high sulphur content (cystine) in its hard keratin domain, whereas the stratum corneum does not. The total lipid content of the nail ranges from 0.1% to 1%, as opposed to approximately 10% for the stratum corneum. This suggests that the role of the lipid pathway in the nail plate is probably of much less importance than that in the stratum corneum. The human nail acts like a hydrophilic gel membrane, while the stratum corneum acts like a lipophilic partition membrane.

Under average conditions, the nail contains 7%–12% water, in comparison to 25% in the stratum corneum. At 100% relative humidity, the maximal water content in the nail is approximately 25%, in sharp contrast to the stratum corneum that can increase its water content to 200%–300%. The rate of chemical penetration into/through the human nail depends upon its water solubility (11) and its molecular size (12).

Topical therapy for onychomycosis has been largely ineffective, and this failure may be due to minimal drug penetration into the nail plate (5). The nail's unique properties, particularly its thickness and relatively compact construction, make it a formidable barrier to the entry of topically applied agents (6). The concentration of an applied drug across the nail drops about 1000-fold from the outer to the inner surface (13). As a result, the drug concentration presumably does not reach a therapeutically effective level in the inner ventral layer. The existing clinical evidence suggests that a key to successful treatment of onychomycosis by a topical antifungal product lies in effectively overcoming the nail barrier. Currently available topical treatments have limited effectiveness, possibly because they cannot sufficiently penetrate the nail plate to transport a therapeutically sufficient quantity of antifungal drug to the target sites (14) and eradicate the infection.

To achieve an effective chemical concentration into/through the human nail plate, penetration enhancers that tend to promote diffusion through the skin's horny layer have been studied. However, these studies were conducted on a few limited nail penetration models that may not provide an intimate contact between the receptor compartment and the nail surface, and the nail plate can be easily hydrated beyond normal levels (6,11,12,14,15). Moreover, nail samples prepared with scalpel or sandpaper are time consuming, and may not accurately represent the three nail compartment structures (10,16).

## Challenges in Overcoming the Nail Permeation Barrier

Overcoming low ungual permeability to xenobiotics can be achieved by several approaches, and is an active field of research. Their effectiveness and applicability will vary from drug to drug depending on the physicochemical nature of the compound. A drug absorbed topically must partition into the nail bed and maintain concentration higher than the corresponding minimum inhibitory concentration (MIC) to be effective.

A hypothesized quantitative structure–activity relationship (QSPR) model was developed based on the available in vitro data set for eight drugs studied in the laboratory, listed in Table 4.2, later in the chapter. (17) This small sample size weakens the predictive power of the model, and a larger sample is needed to improve predictability. As this model has been derived from data in one laboratory where the study design and methodology of the nail study and nail sampling have been the same with similar sensitivity, precision, and robustness of the analytical method using radioactivity; thus one main factor contributing to a variation in ungual drug delivery in vitro has been eliminated.

## Limitations of Current Ungual Drug Permeability Studies (18)

1. Animal hooves: May not provide a representative model for nail penetration in which diffusion through the human nail can be evaluated.
2. Nail clippings: May not provide a complete representative model for nail permeation studies as they are not connected to the nail bed.
3. Hydration-controlled method: Modified diffusion cells are a commonly used in vitro method in ungual drug permeation. These assays super hydrate the nail and possibly alter the physical property and nail permeability. Hui et al. modified the conventional modified diffusion cells commonly used for in vitro studies by using a cotton ball soaked in saline to provide moisture (but not saturation), and hydrating the nail throughout the experiment.
4. Correlation of in vitro onychopharmacokinetics assays and the in vivo onychopharmacokinetics studies: The in vitro data obtained with the use of modified diffusion requires comparison to human in vivo data with the use of radioisotopes and possibly attenuated mass spectroscopy. Until these correlations are defined and until we enhance our understanding of the pathophysiology of the disease, biological interpretation remains tenuous (19). Additional references are found in Murdan 2002 and 2008 (1,20).

## ADVANCES IN NAIL PERMEATION TESTING AND SAMPLING

Nail topical therapy has been a challenge for scientists due to the minimally permeable nature of the nail plate; thus much interest has been directed toward finding a more efficient and robust screening method.

As mentioned earlier, Hui et al. modified the conventional Franz diffusion cell by using a Teflon one-chamber diffusion cell (Permeagear, Inc., Hellertown, PA) along with a cotton ball soaked in normal saline in order to maintain the nail at a physiological levels of temperature and humidity. A novel sampling

technique developed by Hui et al. enables the determination of drug concentration within the plate, where fungi reside. This method relies on a drilling system which samples the nail core without disturbing its surface. This is achieved by the use of a micrometer-precision nail sampling instrument that enables finely controlled drilling into the nail as well as the collection of the powder created by the drilling process. Drilling of the nail occurs through the ventral surface. The dorsal surface and ventrally-accessed nail core can be assayed separately. The core from the ventral side provides drug measurement at the site of disease while the dorsal surface sample contains residual drug. This method allows for drug measurement in the intermediate nail plate, which was previously impossible (21,22).

Murthy et al. developed the TranScreen-N™ method, based on a simple and rapid technique for screening that uses a simple microwell plate based high throughput (23).

A novel model of infected nail plate made of human hair keratin for testing the efficacy of topical antimicrobial formulation was recently developed by Luisiana and Muller-Goymann (24). This was subsequently infected by *Trichophyton rubrum*, the common causative agent of onychomycosis. The infected keratin films were treated with selected topical formulations—cream, gel, and nail laquer—and compared to bovine hoof. They demonstrated that the keratin film model was comparable to the bovine hoof, as both gave an equivalent response to the tested antifungal. The gel formulations were superior to the cream. Thus this model may be able to differentiate between efficacies of different topical antifungal formulations based on their activities against *T. rubrum*.

Infrared (IR) and impedance microscopy (IS) tools have been studied to show their usefulness in the development and optimization of topical drug delivery systems in treating nail diseases. The authors used IR and IS to characterize the effects of drug transport enhancement techniques such as hydration, iontophoresis, and N-acetyl-L-cysteine on human nail plate. The findings utilized nail clippings of healthy human volunteers; further testing will be needed to characterize the spectroscopic properties of diseased nail and the extent to which they are modified by the drug transport enhancement technique used in the study (25).

## ADVANCES IN METHODS OF IDENTIFICATION OF THE FUNGAL ELEMENTS IN THE NAIL

The conventional method for the identification of a fungus is the use of KOH preparation of nail samples with consecutive culture of the nail samples on agarose plates as well as histological examination of the nail plate (26). These methods are known to produce false negative results ranging from 20% to 40%. If false negative results are obtained in a clinically evident onychomycosis case, an invasive nail plate punch biopsy for giemsa staining may be performed.

## EMERGING NONINVASIVE DIAGNOSTIC TOOLS FOR ONYCHOMYCOSIS Optical Coherence Tomography

This method allows for noninvasive cross-sectional imaging of the nail plate. Abuzahra et al. conducted a pilot study to compare optical coherence tomography (OCT) with conventional methods. They examined the potential of OCT as a noninvasive tool for the diagnosis of onychomycosis. The authors were able to demonstrate a reliable pattern for identifying onychomycosis using OCT where it detected fungal elements in all histologically positive specimens, and no false positive results



were seen in their controls (27). In contrast to Abuzahra's findings, Rothmund et al. conducted a study to evaluate the use of confocal laser scanning microscopy (CLSM) and OCT as a noninvasive diagnostic tool and compare them to established techniques. Rothmund et al. had a very low specificity and a high number of false positive results with the use of OCT, possibly due to the lower resolution, which may not allow a clear-cut differentiation between hyphae/spores and other nail creases/artifacts, like trapped air, that may appear similarly (28). Contradictions are an indication for further, larger, and more detailed investigations.

## Confocal Laser Scanning Microscopy

CLSM is regarded as a helpful noninvasive tool in the diagnosis of skin lesions of the human skin, primarily for pigmented lesions, as well as other skin tumors and diseases. It has been investigated as a noninvasive tool in diagnosis of onychomycosis possibly offering higher precision and faster results compared with KOH preparations. The study by Rothmund et al. showed that CLSM had the best specificity superior to KOH preparations, culture, and OCT (28). One major drawback to this technique is the cost.

## Methodology

### Chemicals/Formulations

[<sup>14</sup>C]-Urea (specific activity 55 mCi/mmol, 99% purity), [<sup>7-14</sup>C]-salicylic acid (specific activity 55 mCi/mmol, 99% purity), and [<sup>3</sup>H(G)]-ketoconazole (specific activity 5 Ci/mmol, 99% purity) were purchased from American Radiolabeled Chemicals, Inc. (ARC, St. Louis, MO). [<sup>14</sup>C]-AN2690 was synthesized by Amersham Biosciences UK Limited (Buckinghamshire, UK). [<sup>14</sup>C]-Econazole [chlorophenyl benzyl-<sup>14</sup>C]-(D,L)-econazole (chemical name: 1-[2-[[4-chlorophenyl]-methoxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole), [<sup>14</sup>C]-ciclopirox (pyridinone-6-(<sup>14</sup>C)-ciclopirox) was obtained from Perkin-Elmer Life Sciences, Inc. (Boston, MA). [<sup>14</sup>C]-Terbinafine freebase and [<sup>14</sup>C]-terbinafine hydrogen malate were obtained from Novartis Pharma AG (Basel, Switzerland). The radiochemical purities and specific activities of all chemicals were >98% and >98%, respectively. Penlac<sup>®</sup> nail lacquer (ciclopirox 8% topical solution) was manufactured by Dermik (Berwyn, PA). 2-n-nonyl-1,3-Dioxolane (SEPA), a penetraton enhancer, and all necessary lacquer components were provided by MacroChem Corp. (Lexington, MA).

### Formulations

Nails have a high content of disulfide bonds (10.6% versus 1.2% for human skin), which make the nails both strong and impenetrable. To deliver a therapeutically sufficient quantity of an antifungal drug to fungally-infected sites, such as nail plate, bed, and matrix, a suitable carrier is needed to enhance drug penetration through the nail barrier. In the case of urea, ketoconazole, and salicylic acid, a lotion (Pennsaid lotion, Dimethaid Research Inc., Markham, Ontario, Canada.) containing the penetration enhancer dimethylsulfoxide (DMSO) had previously been shown to enhance skin penetration (7,8,29). To test these three drugs, we prepared three formulations with [<sup>14</sup>C]-urea, [<sup>3</sup>H]-ketoconazole and [<sup>14</sup>C]-salicylic acid at 0.002%, 0.1%, and 0.07%, respectively, and corresponding saline controls with each drug at the same concentrations (7).

For the antifungal drugs econazole, terbinafine free base, and terbinafine salt, we used a nail lacquer formulation, which is a popular choice for topical antifungal treatment. Nail lacquer contains a film-forming agent and a solvent, in addition

to the antifungal drug, and possibly a penetration enhancer. Once the lacquer is applied, it forms a thin, water-insoluble film containing the supersaturated antifungal drug. This provides a chemical gradient to drive drug flux as the drug is released. Thus, a lacquer formulation is suitable for topical treatment of nail diseases. We selected a commercial lacquer formulation, EcoNail<sup>™</sup> (MacroChem Corp). The components of this lacquer formulation include econazole with penetration enhancer, 2-n-1,3-nonyl-dioxolane (18%) and were assembled into a test formulation in the lab prior to use (8). The control is the same formulation minus 2-n-1,3-nonyl-dioxolane.

[<sup>14</sup>C]-Ketoconazole was mixed with a filming solution, Time Off Nail (Neutrogena Corporation, Los Angeles, CA) to be a final 2% nail lacquer formulation. 2% [<sup>14</sup>C]-Ketoconazole commercial cream was purchased from TEVA Pharmaceuticals USA (Sellersville, PA) and used for control.

AN2690 (5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole) was obtained from Anacor Pharmaceuticals, Inc. (Palo Alto, CA). Trace amount of [<sup>14</sup>C]-AN2690 was mixed with propylene glycol/ethanol (1:4, v/v) to a final 10% (w/v) test formulation (30). Penlac<sup>®</sup> nail lacquer (ciclopirox 8% topical solution) was manufactured by Dermik (Berwyn, PA) (31). Trace amount of [<sup>14</sup>C]-ciclopirox was mixed with the lacquer to be a control.

### Human Fingernail Plates

Nail plates were collected from adult human cadavers and stored in a closed container at 0°C. Before each experiment, nail samples were gently washed with normal saline to remove any contamination, then rehydrated by placing them for 3 hours on a cloth wetted with normal saline. Nail samples were randomly selected and allocated to test groups. Nail thickness was measured by a Sony microdigital meter (Sony Magnescale Inc., Japan) before testing in order to determine the drilling depth for each nail. Five nails were used for each formulation tested.

### Dosing and Surface Washing Procedures

A 10-μl dosing aliquot of each of the test formulations was applied to the surface of a nail plate with a microsyringe. Topical application was usually conducted in the morning. For twice daily dosing, a second one was done in the evening, 8 hours after morning application. Surface washing to remove the residue dose was done in the morning, 24 hours after the previous morning application, and 10 minutes prior to the next one if necessary. The nail was washed with cotton tip swabs in a cycle as follows to simulate daily bathing: a dry swab, then a swab wetted with 50% Ivory liquid soap (Procter & Gamble, Cincinnati, Ohio), then a swab wetted with distilled water, then another swab wetted with distilled water, then a final dry swab. The nails treated with lacquer also received an alcohol wash to remove residual lacquer that was insoluble in soap and water. The samples from each cycle from each nail were pooled and collected by breaking off the cotton swab into scintillation glass vials. An aliquot of 5.0 ml methanol was added to each vial to extract the test material. The radioactivity of each sample was measured in a liquid scintillation counter.

### Nail Incubation

To keep the nail at physiological levels of temperature and humidity, we incubated it in a Teflon one-chamber diffusion cell (PermeGear, Inc., Hellertown, PA). The nail surface (top center) was open to air and the inner surface made contact with a small cotton ball acting as a nail supporting bed (Figure 4.1). The cotton ball was wetted with normal saline. The incubation period started 24 hours prior to the first dose, and ended 24

hours after the final dose. A small cotton ball wetted with 0.1 mL normal saline was placed in the chamber beneath the nail plate to serve as a “nail bed” and provide moisture for the nail plate, and hydration was monitored and controlled during the experiment (7,8). This cotton ball method prevented overhydration of a liquid interface. Cotton is a hydrophilic fiber which can contain a high degree of moisture content and absorbs water-soluble substances. It also absorbs lipophilic substances, which can fill the lumen and lie between the numerous internal layers of the cotton (32). Thus the cotton ball is an ideal receiving medium for in vitro transungual delivery.

#### Nail Sampling

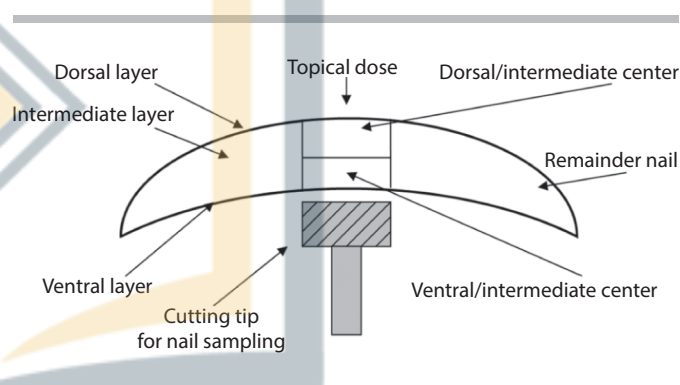
The objective was to determine drug concentration within the nail where the disease resides. Treatment is applied to the nail surface. The drilling system samples the inner core of the nail without disturbing the nail surface. The two parts (surface and inner core) can be assayed separately. The surface contains only residual drug after washing. The drilled-out core (from the ventral side) is thus a true drug measurement at the target site where the disease resides (Figure 4.2). Drug penetration into the nail was sampled by a unique micrometer-controlled nail sampling instrument that enabled finely controlled drilling into the nail and collection of the powder created by the drilling process (7,8). The nail sampling instrument (Figure 4.3) has two parts, a nail sample stage and a drill. The nail sampling stage consists of a copper nail holder, three adjustments, and a nail powder capture. The three adjustments control vertical movement. The first coarse adjustment (on the top) is for changing the copper cell and taking powder samples from the capture. The other two adjustments (lower) are used in sampling. The second coarse adjustment allows movement of 25 mm while the fine adjustment provides movement of 0.20 mm. The nail powder capture is located between the copper cell and the cutter. The inner shape of the capture is an inverted funnel with the end connected to a vacuum pump. By placing a filter paper inside the funnel, nail powder samples can be captured on the filter paper during sampling. The nail is fastened in a cutting holder below the cutter and surrounded by a funnel containing a filter paper. The funnel is attached to a vacuum pump. During drilling, the vacuum draws the powder debris onto the filter paper so it can be collected and measured and increase total collection for mass balance determination.



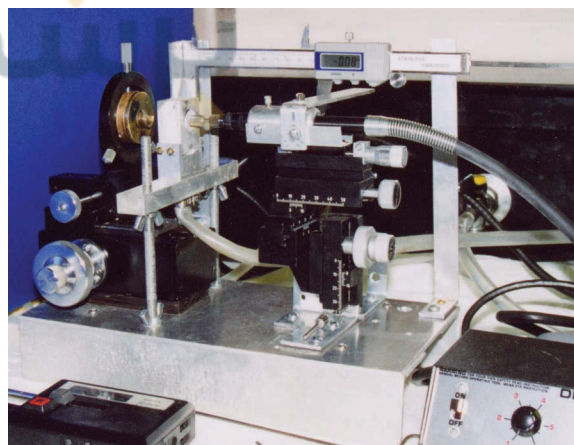
**Figure 4.1** Nail support and incubation system in a Teflon one-chamber diffusion cell. The cotton ball prevented the over hydration of a liquid interface. Cotton is a hydrophilic fiber which can contain a high degree of moisture content and absorbs water-soluble substances. It also absorbs lipophilic substances, which can fill the lumen and lie between the numerous internal layers of the cotton (32). Thus the cotton ball is an ideal receiving medium for in vitro transungual delivery.

After completion of the dosing and incubation phase, the nail plate was transferred from the diffusion cell to a clean cutting holder for sampling. The nail plate was secured in position so that the ventral surface faced the cutter and the dorsal-dosed surface faced the holder. The cutting holder was moved to bring the plate surface just barely in contact with the cutter tip. The drill was then turned on and a fine adjustment moved the stage toward the cutter tip, removing a powder sample from the nail. In this way, a hole approximately 0.3–0.4 mm in depth and 7.9 mm in diameter was drilled in each nail, enabling the harvest of powder sample from the center of each nail's ventral surface. We refer to these samples as having been taken from the “ventral/intermediate nail plate.”

After the nail had delivered its ventral/intermediate nail plate powder samples, it was removed from the sampling instrument. The nail outside the dosing area was cut away and discarded. The nail within the dosing area but outside the sampling area was trimmed away and saved; we refer to this as the “remainder nail plate.” It surrounds the dorsal layer above the sampling area where the powder samples were taken; we refer to this as the “sampling area dorsal nail plate.”



**Figure 4.2** Nail and nail drilling tip.



**Figure 4.3** The nail sampling system. The instrument has two parts, a stage and a drill. The stage consists of a copper nail holder, three adjustments, and a nail powder capture. During drilling, the vacuum draws the powder debris onto the filter paper so it can be collected and measured and increase total collection for mass balance determination.



The ventral/intermediate nail plate powdered samples, the sampling area dorsal nail plate, and the remainder nail plate were individually collected into a glass scintillation vial and weighed. The nail samples were then dissolved by adding 5.0 mL of Packard's Soluene®-350 (Packard Instrument Company, Meriden, CT). The total mass of nail collected was measured by the difference in weight of the plate before and after drilling (7,8).

Results

Nail Incubation Conditions

Table 4.1 shows that the average hydration of the wetted cotton balls  $109 \pm 6.2$  AU (arbitrary units [a digital expression of capacitance]) that resembles the average hydration of human nail bed,  $99.9 \pm 8.9$  AU measured from fresh human cadavers. During the experiment, the holding tank temperature was  $25 \pm 2^\circ\text{C}$  and relative humidity was  $44 \pm 8\%$ . Thus, there was no statistical difference between hydration conditions for nails treated with either the test formulation or the saline control. This incubation device is nonocclusive and hydration controlled, and approximate normal physical condition is reached.

Accuracy of Nail Sampling Process

The advantage of the micrometer-controlled drilling and nail powder removal system is the accuracy of the sampling process. The sampling instrument allowed well-controlled, accurate, and reproducible sampling of the inside of the nail. Table 4.2 shows that the average depth of nail sampling from the inner center surface was well controlled at  $0.26 \pm 0.05$  mm, which was close to the expected depth of 0.24 mm. The weight of the nail samples collected was consistent for all experiments.

Mass Balance of Radioactivity Recovery

Table 4.3 summarizes the econazole mass balance recovery following the 14-day nail treatment. Overall recovery of applied dose was  $90.8 \pm 16.4\%$  for the test formulation and  $96.4 \pm 7.3\%$  for the saline control, indicating that essentially the entire dose was accounted for.

Table 4.3 also indicates what happens to chemicals applied to the nail. Approximately 72% was washed from the surface. The dose absorbed from the surface of the nail penetrated to the sampling area dorsal nail plate (11.4%), the ventral/intermediate nail plate (1.4%), and the supporting bed (0.7%), which is the cotton ball upon which the nail rested. Note that econazole recovery in the test formulation is greater for both the ventral/intermediate nail plate and the supporting bed,

which is an effect of the drug delivery enhancer. At the sampling area dorsal nail plate, there is more econazole from the saline control because the dose remained on the nail surface.

Effects of Dosing and Washing Frequency

Figures 4.4 and 4.5 show the weight normalized of  $^{14}\text{C}$  econazole equivalent in different layers of the nail plate and cumulative  $^{14}\text{C}$  econazole equivalent collected in the cotton ball supporting bed following different frequency of topical dosing or surface washing treatments. As expected, after twice daily application (and surface washing once daily) for 14 days, the  $^{14}\text{C}$  econazole content in all nail and cotton ball samples was significantly higher than that in the dosing and washing once-daily group ( $p < 0.05$ ). Comparison of the once-daily dosing and washing treatment group and the once-daily dosing and once-weekly washing group did not show significant difference for all collected samples ( $p > 0.05$ ).

Enhancer Effects

Table 4.4 summarizes the penetration of ketoconazole, urea, and salicylic acid into the human inner nail plate. Each test formulation contained a drug delivery enhancer (7,8) and was compared to a control formulation without any penetration enhancer. In each case the test formulation enhanced drug delivery ( $p < 0.05$ ). Table 4.5 compares antifungal efficacy of econazole, terbinafine base, and terbinafine salt following topical applications. Each test antifungal agent contains 18% 2-*n*-nonyl-1,3-dioxolane (SEPA), a skin penetration enhancer, and the control (no 18% 2-*n*-nonyl-1,3-dioxolane). All test formulations show the antifungal efficacy coefficient (E) was significantly higher than that of corresponding control ( $p < 0.05$ ).

Topical Formulation Comparison

Table 4.6 compares [ $^{14}\text{C}$ ]-ciclopirox penetration into/through nail plate in vitro from three topical formulations: market gel, experimental gel, and lacquer. The order of deeper penetration, amount detected in the ventral/intermediate nail center, and cotton ball supporting bed samples was from the highest to the lowestmarket gel, lacquer, and experimental gel, respectively. Figure 4.6, however, shows that the deeper penetration of a lacquer formulation of ketoconazole was greater than that of cream when comparing their antifungal efficacy coefficient ( $p < 0.05$ ).

DISCUSSION

Topical therapy for onychomycosis is not yet maximally effective, and this failure may be due to inadequate penetration of drugs

Table 4.1    Hydration of Nail Plate and Nail Bed

Source	Measurement*		Hydration (AU)**	
	N	Time	Nail plate	Nail bed
Human cadavers	6	24-hr post mortem	$7.6 \pm 0.9$	$99.9 \pm 8.9$
Diffusion cells	8	Twice/day for 7 days	$8.5 \pm 2.4$	$109.9 \pm 6.2$

Note: During the experiment, the holding tank temperature was  $25 \pm 2^\circ\text{C}$  and relative humidity was  $44 \pm 8\%$ . The importance of this controlled temperature and humidity is to mimic normal physiological condition of the human nail to prevent over hydration which was easily occurred in nail *in vitro* studies.

\*Hydration of the nail plate and the supporting cotton bed was measured with a Corneometer CM 820 (Courage & Khazaka, Cologne, Germany). \*\*AU, arbitrary units—a digital expression of capacitance. Thus the Corneometer CM 820 gives only an estimate of the nail (or other membrane such as stratum corneum) hydration. Agache et al. (33) used a sorption-desorption test to assess the water content of the stratum corneum. They correlated the results measured with the Corneometer CM 820 (as AU unit) and TEWL ( $\text{g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ) measured with an evaporimeter and found that water retained in the SC( $\mu\text{g}\cdot\text{cm}^{-2}$ ) =  $\ln(\text{AC}/3.8)/0.0436$ .

**Table 4.2** Nail Core Sampled from the Ventral (Inner) Surface Center of the Human Nail Plate

Test	Whole nail thickness (mm)	Nail core sampled from the ventral (inner) surface center of the nail plate <sup>a</sup>			
		Depth of core (mm)	% Whole nail thickness	Total core sample removed (mg)	Powder sample collected (mg)
Urea (control)	0.65 (0.09)	0.25 (0.03)	39.52 (8.05)	16.4 (4.3)	5.2 (0.8)
Urea (test)	0.71 (0.07)	0.27 (0.03)	37.97 (2.69)	17.6 (4.3)	6.4 (1.3)
Ketoconazole (control)	0.68 (0.05)	0.28 (0.03)	41.88 (1.16)	14.3 (6.7)	6.7 (2.6)
Ketoconazole (test)	0.73 (0.03)	0.28 (0.02)	38.62 (2.69)	14.1 (5.1)	4.3 (1.6)
Salicylic acid (control)	0.77 (0.07)	0.25 (0.08)	32.62 (9.38)	12.1 (2.4)	6.0 (0.5)
Salicylic acid (test)	0.60 (0.12)	0.21 (0.06)	35.03 (6.45)	23.4 (8.3)	4.7 (0.8)
Average	0.69 (0.09)	0.26 (0.05)	37.61 (6.20)	16.3 (6.2)	5.5 (1.6)

Source: Hui X et al., *J Pharm Sci*; 91:189–95, 2002.

<sup>a</sup>Nail sample, approximately 0.24 mm in depth and 7.9 mm in diameter, was drilled from the center of the ventral surface of the nail. The amount of nail sample removed was measured by difference in weight and depth of the drilled area before and after sampling. Each number represents mean ( $\pm$  S.D.) of 5 samples. The data demonstrated the repeatability and accuracy of the nail sampling system.

**Table 4.3** Mass Balance Recovery of Econazole following 14-Day Human Nail Treatment with a Test Formulation Containing a Penetration Enhancer and a Control without a Penetration Enhancer

Sampling Area	Carbon-14 recovery as percent of dose	
	Test Formulation	Control Formulation
Dorsal/intermediate nail plate	11.4 (3.6)	20.1 (2.9)
Ventral/intermediate nail plate (powdered samples)	1.3 (1.1)	0.22 (2.9)
Remainder nail plate	5.6 (3.9)	3.2 (2.3)
Supporting bed (cotton ball)	0.7 (0.3)	0.0 (0.0)
Surface washes	71.7 (12.5)	72.8 (5.1)
Total	90.8 (16.4)	96.4 (7.3)

Source: Hui X et al., *J Pharm Sci*; 92:142–8, 2003.

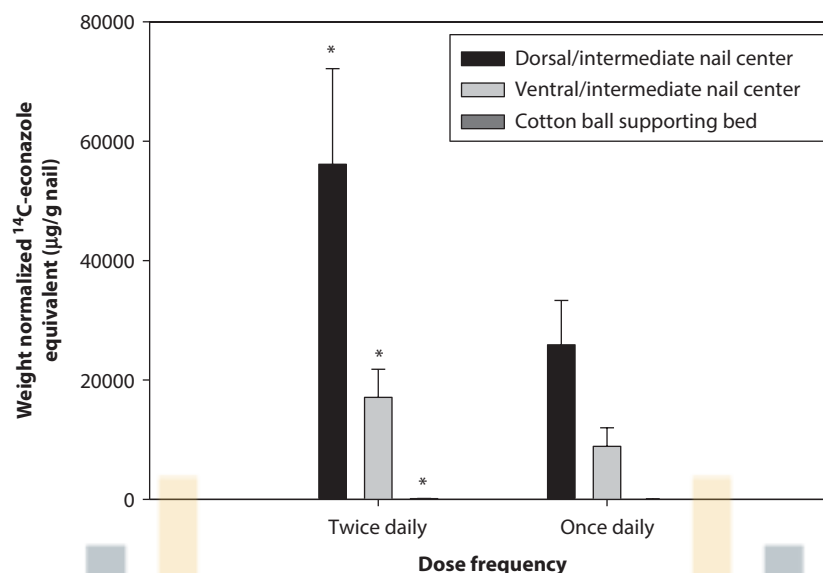
Note: The data represent the mean (SD) of each group ( $n = 5$ ). The test formulation group contains 18% 2-*n*-nonyl-1,3-dioxolane and the control formulation contains no 2-*n*-nonyl-1,3-dioxolane. The data demonstrated not only the importance of the penetration enhancer but the high recovery rate of two groups when using the nail sampling system.

into the nail plate. The nail's unique properties, particularly its thickness and relatively compact construction, make it a formidable barrier to the entry of topically applied agents. The concentration of an applied drug across the nail drops about 1000-fold from the outer surface to the inner surface. As a result, the drug concentration presumably does not reach a therapeutically effective level in the ventral/intermediate layers. To optimize the nail penetration of topical treatments, it is important to consider the nail's unique barrier properties and develop an antifungal drug formulation that has matching physicochemical properties.

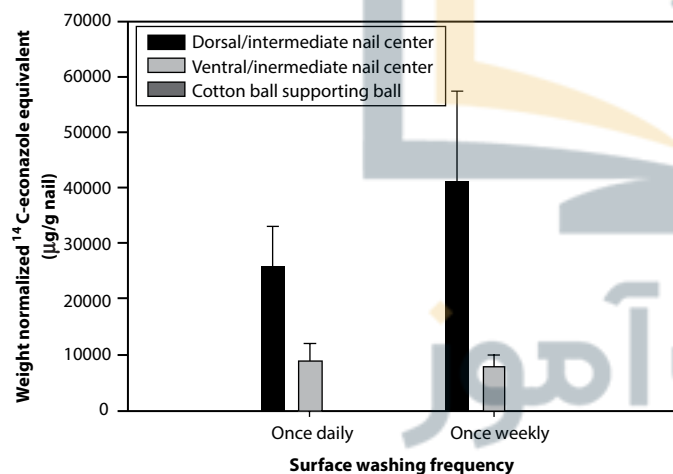
Nail lacquer formulations, a popular choice for topical antifungal treatment, typically contain a film-forming agent, solvent, antifungal drug, and possibly a penetration enhancer. Once the lacquer is applied, it forms a thin film containing a supersaturated antifungal drug. This film provides a chemical gradient to drive drug flux as the drug is released. For

example, <sup>14</sup>C-ketoconazole lacquer yields a significantly higher antifungal efficacy coefficient than that in cream ( $p < 0.05$ ; Figure 4.6). However, the deeper penetration of [<sup>14</sup>C]-ciclopirox from the commercially available formulation, Penlac nail lacquer (ciclopirox 8% topical solution), was statistically lower than that of market gel formulation (ciclopirox 0.77 % topical solution) (Table 4.6) (31). When compared with other antifungal topical formulations, such as [<sup>14</sup>C]-AN2690, 10% (w/v) in propylene glycol/ethanol solution, it decreased ( $p < 0.05$ ) the deeper nail layer penetration rate (Figure 4.7) (30).

Transungual enhancement has been examined: DMSO and 2-*n*-nonyl-1,3-dioxolane used as transdermal delivery enhancers were tested for enhancement of antifungal agents penetrating into and through the nail plate in vitro. The two enhancers increased the deeper nail penetration of [<sup>14</sup>C]-ketoconazole (Table 4.3), [<sup>14</sup>C]-econazole, [<sup>14</sup>C]-terbinafine



**Figure 4.4** Nail penetration profile of  $^{14}\text{C}$ -econazole following a 14-day treatment/incubation period in vitro. Each bar represents the mean (SD) of 6 samples. The frequency of topical dosing was different but the surface wash was the same, once daily. \*The group that received twice-daily topical doses was statistically significantly higher than the one treated with a once-daily dose ( $p < 0.05$ ).



**Figure 4.5** Nail penetration profile of  $^{14}\text{C}$ -econazole following a 14-day treatment/incubation period in vitro. Each bar represents the mean (SD) of 6 samples/group. Each group received once-daily topical application for 14 days. The frequency of surface washing for each group was different. The group that received daily washing was not statistically significant from the corresponding bar from the once-weekly washing group ( $p > 0.05$ ).

salt,  $^{14}\text{C}$ -terbinafine base (Table 4.5) significantly compared to the controls ( $p < 0.05$ ). DMSO has been previously reported to facilitate the penetration of some topical antimycotics (15,35). The enhancement function of 2-*n*-nonyl-1,3-dioxolane has not previously been determined. The mechanism of 2-*n*-nonyl-1,3-dioxolane in skin penetration was suggested to reversibly fluidize the stratum corneum lipids and alter barrier function

(36). However,  $^{14}\text{C}$ -dioxolane, the radiolabeled 2-*n*-nonyl-1,3-dioxolane, had minimal penetration to and through the human nail plate in vitro (8). 2-*n*-nonyl-1,3-dioxolane can function as an adhesion promoter and a plasticizer for the film-forming polymer of the nail lacquer (37,38). The enhancement function of 2-*n*-nonyl-1,3-dioxolane for the tested antifungal agents was possibly to soften the lacquer film to increase releasing per-unit time (8). As shown in Table 4.5, the amounts of  $^{14}\text{C}$ -econazole,  $^{14}\text{C}$ -terbinafine free base, and  $^{14}\text{C}$ -terbinafine salt detected from the deeper layer, ventral/intermediate nail layer in the test groups, which the lacquer formulation contains 18% 2-*n*-nonyl-1,3-dioxolane were significantly greater than that in the controls ( $p < 0.05$ ). The results suggest that the enhanced level of these antifungal agents in the ventral/intermediate layers and supporting bed dramatically increased, which exceeds the minimum inhibitory concentration (MIC) of econazole for most common onychomycosis organisms (Table 4.5).

MIC is a laboratory index in the determination of antifungal potency. Mertin and Lippold (11) introduced an efficacy coefficient *E* to better estimate and compare the relative efficacy of antifungal agents. The efficacy coefficient *E* is the ratio of the flux of an antimycotic drug through the nail plate to the MIC. For econazole, the range of MIC for the dermatophyte species is 0.1 to 1.0  $\mu\text{g}/\text{mL}$  and for yeast species it is 1.0 to 100  $\mu\text{g}/\text{mL}$  (39). After 14 days of exposure, the econazole content measured in the test group was  $11.15 \pm 2.56 \mu\text{g}/\text{mg}$  for the ventral/intermediate layers. This content, multiplied by the density of the nail sample ( $1.332 \text{ mg}/\text{cm}^3$ , measured under current experimental conditions), yields  $14,830 \pm 340 \mu\text{g}/\text{cm}^3$  of econazole, almost 15,000 times the MIC for most dermatophyte species and 150 times that for most yeast species (Table 4.5).

This study demonstrated that with our in vitro nail study methodology, the nail plate can be scientifically studied, and with proper formulation one can deliver a variety of chemicals,

**Table 4.4** Radiolabeled Drug Penetration into Human Nail from a Test Formulation Containing DMSO, a Penetration Enhancer, versus a Control without a Penetration Enhancer

Test chemicals	Penetration Enhancer <sup>*</sup>	Radioactivity content in ventral/intermediate center layer of the nail plate			
		Unit <sup>**</sup>	Test formulation	Control formulation	Significant ( $p < 0.05$ )
Ketoconazole	Dimethylsulfoxide	µg Eq/g	53.9 (10.6)	34.0 (15.9)	Yes
Urea	Dimethylsulfoxide	µg Eq/g	0.3 (0.1)	0.2 (0.1)	Yes
Salicylic acid	Dimethylsulfoxide	µg Eq/g	10.2 (0.6)	7.0 (1.1)	Yes

Source: Hui X et al., *J Pharm Sci* 91:189–95, 2002.

Note: The data represent the mean (SD) of 5 samples per formulation group. The nail sample drilled as powder from ventral/intermediate layer of human nail plate.

<sup>\*</sup>Dimethylsulfoxide (DMSO) is a transdermal delivery enhancer. The enhancement mechanism of transungual delivery is not clear. However, it did enhance the nail penetration of the test chemicals.

<sup>\*\*</sup>µg Eq/g = microgram equivalents drug per gram of nail sample. Because radioactivity is used, the drug mass is referred to as “equivalents” because radioactivity was measured, not the drug itself.

**Table 4.5** Comparison of Econazole Concentration and Relative Antifungal Efficacy with a Test Formulation Containing 2-N-Nonyl-1,3-Dioxolane, a Penetration Enhancer, and a Control

Parameter <sup>*</sup>	Test formulation	Control formulation	Significant ( $p < 0.05$ )
Econazole in the deeper layer (µg/cm <sup>3</sup> )	14,830 (341)	2,371 (426)	Yes
E <sub>D</sub> (MIC <sub>D</sub> = 1 µg/mL)	14,830	2,371	Yes
E <sub>Y</sub> (MIC <sub>Y</sub> = 100 µg/mL)	148	23.7	Yes
Terbinafine salt in the deeper layer (µg/cm <sup>3</sup> )	5946 (1029)	463 (271)	Yes
E <sub>D</sub> (MIC <sub>D</sub> = 0.04 µg/mL) <sup>**</sup>	5946	463	Yes
E <sub>Y</sub> (MIC <sub>Y</sub> = 1.77 µg/mL) <sup>**</sup>	134	10	Yes
Terbinafine base in the deeper layer (µg/cm <sup>3</sup> )	727 (372)	407 (106)	Yes
E <sub>D</sub> (MIC <sub>D</sub> = 0.04 µg/mL) <sup>c</sup>	1527	156	Yes
E <sub>Y</sub> (MIC <sub>Y</sub> = 1.77 µg/mL) <sup>c</sup>	34	3	Yes

Note: The data represent the mean (SD) of each group ( $n = 5$ ). The test formulation group contains 18% 2-n-nonyl-1,3-dioxolane and the control formulation contains no 2-n-nonyl-1,3-dioxolane.

Abbreviations: E, antifungal efficacy coefficient; MIC, minimum inhibitory concentration (of the tested antifungal agent); D, dermatophytes; Y, yeast. (Reference 8)

<sup>\*</sup>The deeper layer is the center of the ventral/intermediate layer of the nail plate. The data represent the amount of drug in the sample after a 14-day dosing period. The amount of antifungal agents in the tested nail layer (µg/cm<sup>3</sup>) was computed from the average of the cumulative amount of the test agent permeated into the area (area × thickness) of the deeper layer of the nail (dorsal/intermediate layer).

<sup>\*\*</sup>See Reference 34.

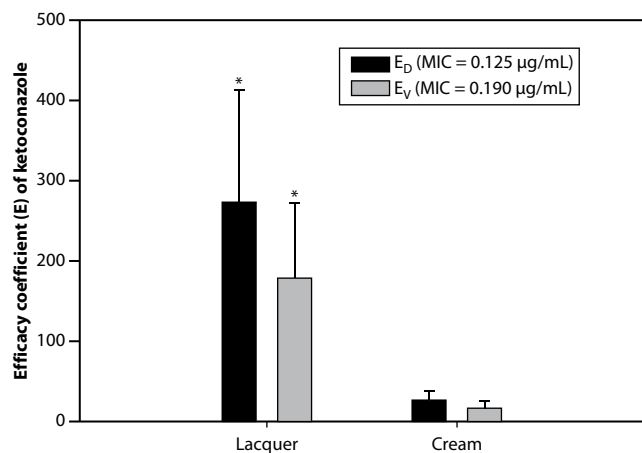
**Table 4.6** Summary of Weight Normalized <sup>14</sup>C-Ciclopirox Equivalent in the Nail and Supporting Bed Samples after 14-Day Treatment

Items (Unit)	Normalized <sup>14</sup> C-Ciclopirox Equivalent			Significant (if $P$ value < 0.05)
	Marketed Gel	Experimental Gel	Lacquer	
Dorsal/intermediate center within surface of nail (µg eq/mg)	71.7 (16.8)	103.4 (38.1)	2162.1 (526.4)	Lacquer vs experimental gel Lacquer vs marketed gel
Ventral/intermediate center within infection-prone area (µg eq/mg)	0.6 (0.3)	0.2 (0.1)	0.3 (0.1)	Marketed gel vs lacquer Marketed gel vs experimental gel Experimental gel vs lacquer
Penetration through the nail into the supporting bed cotton ball (µg eq/sample)	46.3 (4.1)	6.0 (1.3)	15.5 (3.1)	Marketed gel vs lacquer Marketed gel vs Experimental gel Experimental gel vs lacquer

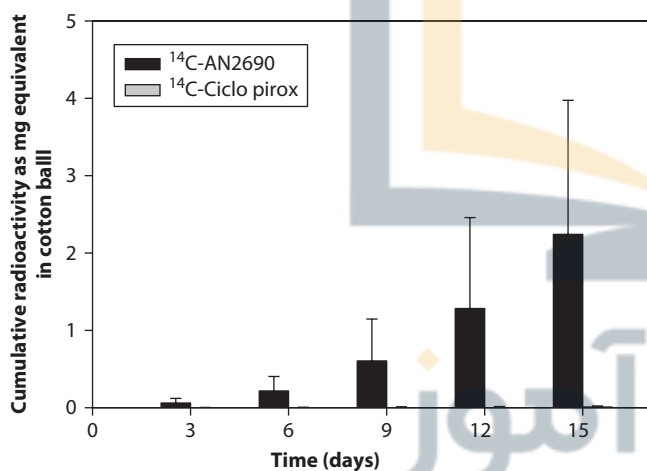
Source: Hui X et al., *J Pharmaceut. Sci*; 93:2545–8, 2004.

Note: The data represents the mean (SD) of each group ( $n = 5$ ).





**Figure 4.6** Comparison of antifungal efficacy coefficient of two ketoconazole formulations, lacquer and cream. Each bar represents the mean (SD) of 5 samples/group. Each group received a once-daily topical dose and washing for a 7-day treatment. The lacquer group shows that the antifungal efficacy coefficient was statistically significantly higher than that of the cream group ( $p < 0.05$ ).



**Figure 4.7** Cumulative amounts of AN 2690 and ciclopirox (mg equivalent) in cotton ball supporting bed samples following a 14-day treatment. The test were dosed daily and the dose residue was washed 24 hours later and 10 minutes prior to the next application. Each bar represents the mean (SD) of 6 samples/group. Each bar of the AN2690 group was statistically higher than the corresponding one from the ciclopirox group ( $p < 0.05$ ). (From Hui X et al., *J Pharmaceut Sci*; 96:2622–31, 2007.)

including drugs or nail modifiers (cosmetics). The nail is now ready for serious attention and treatment just as hair and skin have been in the past. The nail barrier can be breached.

These findings presumably relate to delivery of cosmetic agents for the management of nail abnormalities, such as nails that are peeling or fragile. R&D on these agents will be simplified when the rules describing the relationship of physical chemistry to flux are developed for the nail, as they have been in part for the skin.

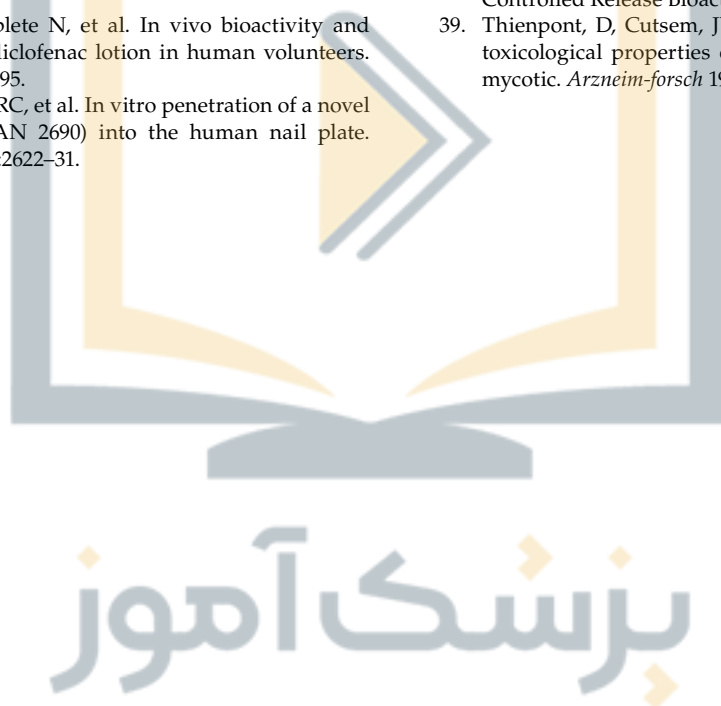
Taken together, the methodology appears robust; as with other models, inclusion of other chemicals with varying physicochemical properties, and in vivo replication will add strength to the results.

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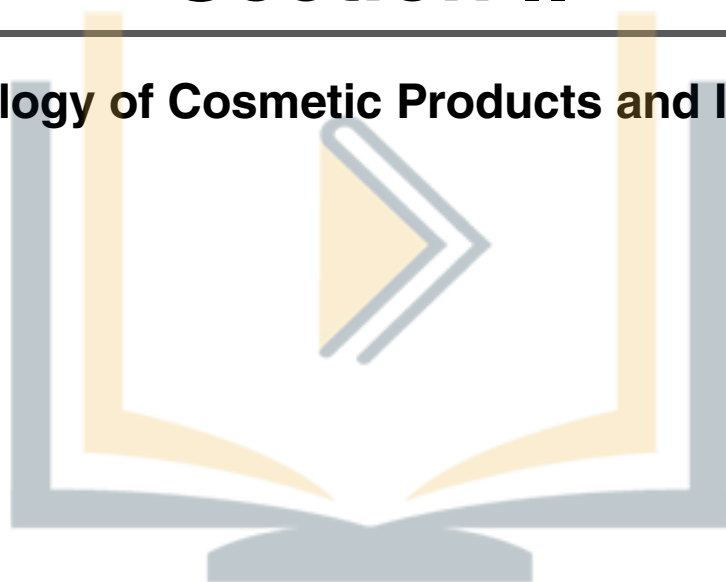


پزسک آموز

# Section II

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**Pharmacology of Cosmetic Products and Ingredients**



پزشک آموز



پزسک آموز

# Sensitive Skin: New Findings Yield New Insights

Miranda A. Farage and Howard I. Maibach

## INTRODUCTION

The goal of product testing is to ensure that consumer products are free of irritant potential and to prevent unexpected consumer reactions to the product once it reaches the marketplace. It is not uncommon, nonetheless, for postmarketing surveillance efforts to receive reports of sensory perceptions not predicted by even the most robust methodology (1). These sensory perceptions, though often transient and not accompanied by a visual dermatological response in rigorous experimental testing, strongly influence consumer product preference (1). In fact, 78% of consumers who profess sensitive skin report avoiding some products because of unpleasant sensory effects associated with their use (2).

Unpleasant and subjective sensory effects, often unaccompanied by objective physical signs, define a controversial and still evolving dermatological condition known as sensitive skin. Consumer reports of sensitive skin are typically self-diagnosed and may be increasing (3). The concept of sensitive skin arose in the 1970s with the observation that despite the fact that previous safety evaluations had found no evidence of toxicity, some patients reported stinging sensations upon using a particular sunscreen that contained a derivative of P-aminobenzoic acid (4). Growing consumer awareness of the potential for irritation from common products fueled a huge increase in products marketed for the supposedly rare individual with susceptibility to components of everyday products, particularly since no requirement for proof of safety and efficacy for such products has existed (5). The initial conceptualization of sensitive skin as a minority complaint has not been borne out by epidemiological surveys, which consistently find a high prevalence of sensitive skin across the world (Table 5.1).

Sensitive skin is a term whose definition continues to be refined. It is now generally agreed to describe unpleasant subjective sensory reactions (such as, prickling, burning, tingling, or pain) in response to common external factors (such as ultraviolet light, heat, cold, wind, cosmetics, cleaning products, etc.) and intrinsic stressors (such as stress or hormones) (6). Sensory effects are only occasionally accompanied by erythema or other demonstrable irritation or immunological responses (6).

Although sensitive skin is now generally accepted as a real physiological disorder, there is still no consensus regarding its etiology, classification, or criteria for diagnosis. Several issues have hindered a better understanding of this condition. It is typically self-diagnosed (7). Patients may interpret an underlying dermatological condition as well as any reaction to product use as sensitive skin (8). There are also some psychological disorders characterized by similar symptoms (e.g., cosmetic intolerance syndrome, dermatological non-disease) (9). Many people who profess sensitive skin do not predictably experience visible signs of the sensations reported, whereas some who describe themselves as non-sensitive react strongly

to tests of objective irritation (10). The diversity of methodology approaches employed in sensitive-skin research has also contributed to interpretive difficulties. Further, much of the research to date has been published in cosmetic journals that are inaccessible through major databases and may be ignored by leading dermatological publications (5).

Irritant testing reveals profound interpersonal variability in individual response to specific irritants (11,12), even among chemicals with similar modes of action (13). Some have reported sizeable variation within the same individual at different anatomic sites (12), and even at the same anatomical site on symmetric limbs (14).

The etiology of sensitive skin is unknown, but the disorder is believed to be the product of multiple etiologies with multiple components, including deficiencies in barrier function, neurosensory dysfunction, compound-specific irritancy, and cultural influences (15).

Deficiencies in barrier function have been shown to be a critical component of skin discomfort (16). Increased permeability is believed to be the result of a functional compromise of barrier function in the sensitive-skin patient (17). Lipid content of the stratum corneum has been shown to be a more accurate predictor of skin permeability than stratum corneum thickness or cell number (12). The permeability barrier in the stratum corneum requires the presence of well-organized intracellular lipids (7,16) and depends highly on lipid composition (12). Increased neutral lipids and decreased sphingolipids are associated with superior barrier properties (12). A weak barrier inadequately protects nerve endings and facilitates access to antigen presenting cells, a mechanism which would support an association with atopic conditions (16). Irritation results from the abnormal penetration in skin of potentially irritating substances and a resulting decrease in the skin tolerance threshold (7). Alterations in barrier function in sensitive-skin patients have been observed (18,19). Alterations of baseline capacitance values imply barrier impairment and support the view that hyperreactivity to water-soluble irritants results from increased absorption (20). A derangement of intercellular lipids, specifically, was also associated with a decline in barrier function in sensitive skin (21). The pain sensations, which are the hallmark of the disorder, also imply possible integration dysfunctions in the central nervous system. Cho et al. (22) found that individuals were classified into sensitive skin and non-sensitive skin based on a lactic acid sting test performed on the face. There were no differences in transepidermal water loss (TEWL) and erythema index values between the two groups. However, the mean value of the quantity of stratum corneum ceramides on the face was significantly lower in the sensitive skin group compared to the non-sensitive group.

The pain and other unpleasant sensations that are the hallmark of sensitive skin imply a likely relationship to



**Table 5.1** Prevalence of Sensitive-Skin Perception in Various Geographies

Population(s) studied	Population characteristics	Definition of sensitive skin	Percentage of people who claimed sensitive skin	Reference(s)
France (2000)	319 women, conducted by interviews	Cutaneous discomfort in the absence of clinical and histological evidence of skin lesions	90% (23% very sensitive skin)	4
France (2005)	1006 men and women, identified by questionnaire	Sensitive skin	59% women, 44% men	36
France (2006)	8522 men and women, identified by questionnaire	Sensitive skin	61% women, 32% men	67
France (2008)	1004 men and women, opinion poll survey	Sensitive scalp	44.2% (47.4% women, 40.8% men)	70
France (2008)	18 women, identified by questionnaire	Sensitive skin	50% (facial skin, specifically)	65
France (2008)	400 identified by questionnaire	Sensitive skin	85% (facial skin, specifically)	6
Germany (2001)	420 men and women	Sensitive skin	75% (48% severe)	60
England (2001)	3300 men and women, conducted by mailed questionnaire	Intolerance to cosmetics and toiletries, including both sensory and visible signs	51.4% men (5.8% very sensitive skin) 38.2% women (10% very sensitive skin)	32
Italy (2005)	1870 women	Sensitive skin	56.50%	31
Greece (2008)	25 women, identified by questionnaire	Sensitive skin	64%	61
Greece (2008)	25 women with atopic dermatitis	Sensitive skin	100%	61
USA (San Francisco) (2002)	800 women, conducted by telephone interview	Sensitive facial skin	52%	2
USA (Cincinnati) (2009)	1039 men and women surveyed by questionnaire	Sensitive skin	68.4% reported some degree of sensitivity ("slight"–"very") (69.0% women, 64.4% men)	3
USA (Cincinnati) (2006)	29 women with light incontinence	Sensitive skin	83% reported some degree of sensitivity ("slight"–"very")	71
USA (2011)	994 subjects by phone survey (495 men and 499 women)	Rate their skin as: "very sensitive," "sensitive," "slightly sensitive," or "not sensitive at all"	44.6% reported "very sensitive" or "sensitive" (38.2% men, 50.9% women)	70
USA (2013) primarily rural southern states	89 women by questionnaire	Sensitive skin	77.5% reported some degree of sensitivity ("slight"–"very")	72
Japan, USA, Europe (1992)	15 000 men and women, conducted by questionnaire	People whose skin reacts to particular insults more than the majority of people	50% women (25% very sensitive skin) 30% men	73
China (2012)	408 women surveyed by questionnaire	Sensitive skin	23%	74
China (urban dwellers) (2012)*	9154 surveyed by questionnaire (3931 men and 5223 women)	Sensitive skin	13% (8.62% men, 15.93% women)	75
Mexico (2013)	246 subjects (78 man and 168 women)	Sensitive skin	36%	76
Dermatologist opinion survey				
USA (2013)	300 dermatologists surveyed by email questionnaire	Sensitive facial skin	58.3% have noticed an increase in male patients reporting sensitive facial skin over the past 5 years	77
Europe (2013)	1531 dermatologists surveyed by questionnaire	Sensitive facial skin	82% have noticed an increase in male patients reporting sensitive facial skin over the past 5 years	77

\*Year of publication, not year of study.

dysfunctions in the central nervous system, possibly involving several neuromediators and sensory receptors (23). Of the two studies reviewed that did evaluate the relationship between neurosensory responses and objective clinical irritation and included only subjects with demonstrated sensory sensitivity, both showed a correlation between sensory and objective signs (18,19). In a study regarding sensitivity to facial tissue which did not exclude non-sensitive individuals, sensory effects were demonstrated to be the most reliable measure of product differences (18,19).

Although no predictive value was demonstrated for any individual sensitivity when subjects were tested with a seven-irritant panel, a weak association between tests was demonstrated by statistical analysis of binomial probability (13). However, studies which evaluated the association of barrier function and sensitivity have yielded arguably the most conclusive results. A high baseline TEWL was associated with increased susceptibility to numerous cutaneous irritants by numerous studies and a variety of assessment methods (14).

Most methods focused on objective assessment of physical effects to skin rather than the sensory effects reported (24), and few reports have quantified sensory effects or correlate sensory effects to degree of irritation. Most testing has included few subjects, and few have restricted subjects to those with demonstrated sensitivity (25). Few have attempted to evaluate the influence of endogenous hormones or lifestyle factors.

Ultimately, traditional irritant-testing methodologies have not proven to be good predictors of consumer response (1). Response to one irritant does not predict sensitivity to another, and has not correlated well with evaluation of objective signs (26).

## FACTORS IN SKIN SENSITIVITY

Numerous potential host factors (Table 5.2) undoubtedly play a role in experimental variability observed in sensitive skin. To date, no constitutional factors have been identified (23).

### Gender

Sensitive skin is self-reported far more often in women than in men (Table 5.1). There is biological plausibility for greater

sensitivity, as thickness of the epidermis was observed to be greater in males than in females ( $p < 0.0001$ ) (27), and hormonal differences, which may produce increased inflammatory sensitivity in females, have also been demonstrated (14,28). Irritant testing, however, generally finds no differences (12). One study, though, found among 1039 subjects a 68.4% prevalence of self-reported sensitive skin, with no difference between men and women (3). It may be that with increased marketing of products for sensitive skin in men it has become more culturally acceptable for males to define themselves as having sensitive skin.

### Age

The physiological changes that occur as skin ages would predict an increased susceptibility to irritants (29). Existing studies, however, are ambiguous with regard to the influence of age on skin sensitivity. Clinical assessment of the erythematous response to irritants in older people suggest that susceptibility generally decreases with age (29). However, objective signs of irritation often show little correlation with the intensity of subjective complaints (29). A study of sensory perceptions of sensitive skin conducted on 1029 individuals in Ohio stratified subjects into four age groups (subjects under 30, in their 30s, in their 40s, and over 50), and evaluated subjective data according to age (29). Those over 50 were more likely to claim sensitive skin than younger adults and more likely to perceive genital skin (to the exclusion of other body sites) to be more sensitive (29). Older adults also stated that their skin had become more sensitive over time (46%) (29). The turnover rate of the stratum corneum has been reported as longer in aged skin (30), which may contribute to increased sensitivity. However, in a large Italian study that performed lactic acid sting tests on more than 100 elderly subjects, the intensity of the stinging response was inversely proportional to age (31).

### Ethnicity

There are pronounced differences in skin structure depending on skin type (Table 5.3), and racial differences, with regard to skin susceptibility to irritants, are among the fundamental questions in dermatotoxicology (25). Two large epidemiological studies reported no observed racial differences in reporting product sensitivity (2,32). Most testing, however, has focused on Caucasian females (25).

Differences have been observed in sensory perceptions, although substantive conclusions are hard to provide. Asians have been reported to complain of unpleasant sensory responses more often than Caucasians, supported by the observation that a higher incidence of dropouts in a Japanese clinical study withdrew because of adverse skin effects as compared with those in Caucasian studies (33). There have also been reports of an increased sensory response, as well as speed of response in Asian subjects versus Caucasian in sensory testing (33). Another study, however, found fair-skinned subjects prone to sunburn had higher sensory responses to chemical probes than those with darker skin tones (34). No racial differences in innervation on an architectural or biochemical level have been observed (13).

Studies of racial differences with regard to irritants have yielded conflicting evidence. Although black skin was demonstrated to have greater potential for irritant susceptibility than white skin (12), another study found blacks to be less

**Table 5.2** Possible Contributors to Sensitive Skin

Factor	Reference(s)
Female sex	32
Hormonal status	44
Cultural expectations in technologically advanced countries	60
Fair skin which is susceptible to sunburn	34
Susceptibility to blushing and/or flushing	32
Skin pigmentation	34
Thin stratum corneum	25, 33, 78, 79
Decreased hydration of stratum corneum	7, 80, 81
Disruption of stratum corneum	34
Increased epidermal innervations	7, 63
Increased sweat glands	33
Increase neutral lipids and decreased sphingolipids	82
Decreased lipids	20, 83–86
High-baseline TEWL	14
Atopy	59, 61

Abbreviation: TEWL, transepidermal water loss

**Table 5.3** Comparison of Racial Differences in Functional Skin Properties

Skin property	Types	Racial differences	Reference(s)
Permeability	In vitro penetration of fluocinolone acetonide	Lower in blacks than in Caucasians	87
	In vitro penetration of water	No differences	87, 88
	Topical application of anesthetic mixture	Less efficacy in blacks than in Caucasians	89
	In vivo penetration of C-labeled dipyrithione	Lower in blacks (34%) than in Caucasians	90
	Methyl nicotinate-induced vasodilation	Time-to-peak response equal	91
TEWL		Slower in blacks	35
	Baseline TEWL (in vitro)	Higher in blacks	35
		Higher in blacks (in vitro)	92
	TEWL in response to SLS irritation (in vivo)	Higher in blacks and Hispanics	81
	Baseline TEWL (in vivo)	Blacks > Caucasians > Asians	93
	Return to baseline TEWL after tape stripping	Blacks faster than whites	94
	Reactivity to SLS (measured by TEWL)	Higher in blacks than Caucasians	92
Skin irritant reactivity	Reactivity to dichlorethylsulfide (1%)	Lower in blacks (measured by erythema, 15% vs. 58%) than Caucasians	95
	Reactivity to o-chlorobenzylidene malonitrile	Lower, longer time to response in blacks than Caucasians	96
	Reactivity to dinitrochlorobenzene	Lower in blacks, but trend towards equalization after removal of stratum corneum than Caucasians	97
	Reactivity to octanoic acid, 20% SLS, 100% decanol, and 10% acetic acid	Asians more reactive than Caucasians (react more quickly)	98
Stinging response	Stinging response	Lower in blacks than whites	99
		Equal in blacks and whites	100
		Higher in Asians than whites	33, 101
Skin transparency	UV protection factor of stratum corneum	Higher in blacks (about 50% higher) than in Caucasians	102
	UVB transmission in stratum corneum	Lower in blacks (about 50% lower)	102
	Spectral emittance	Lower in blacks (above 300 nm: 2–3-fold)	103
	UV protection factor of epidermis	Higher in blacks (4-fold)	102
	UVA transmission through epidermis	Lower in blacks (almost 4-fold)	102
	UVB transmission through epidermis	Lower in blacks (4-fold)	102
	Contribution of malpighian layer	Black skin: twice as effective in absorbing UVB as white skin	102
Photoprotection of epidermis	Skin extensibility on dorsal (sun exposed) and volar (sun protected) forearms	Black skin maintains extensibility on sun-exposed sites, but Hispanic skin extensibility is reduced on sun-exposed sites	104
Consequence of photoaging	Elastic recovery	Black skin maintains recovery on sun-exposed sites, white and Hispanic skins reduced	104
	Drying	Higher in Caucasian and Asians than in Hispanics and blacks	105
	Hypertrophic scarring	Higher in Asians than Caucasians	106
Response to insult	Pigmented dermatoses	Higher in Asians than Caucasians	106, 107
	Wrinkling	Average onset is 10 years later in Asians than Caucasians	107
	Wrinkling	Average onset 20 years later in blacks than Caucasians	108
	Thermal tolerance	Blacks have a lower threshold than whites	109
Somatosensory function	Elastic recovery (tested on the cheek)	1.5 times greater in black as compared with white subjects	110, 111

Abbreviations: SLS, sodium lauryl sulfate; TEWL, transepidermal water loss; UV, ultraviolet; UVA, ultraviolet band A; UVB, ultraviolet band B.

reactive than Caucasians (11). Asians seemed to be more reactive than Caucasians in some studies and less in others, even within studies done under the same investigator and protocol (25). Tristimulus colorimeter assessment of skin reflectance observed that skin pigmentation was inversely associated with susceptibility to irritation (14), supported by the finding that irritant susceptibility to sodium lauryl sulfate (SLS) is decreased after ultraviolet B (UVB) exposure (tanning) (14).

Methyl nicotinate assessment of vasoactive response suggests that there may be genuine racial differences in permeability (35). Increased percutaneous absorption of benzoic acid, caffeine, and acetylsalicylic acid was demonstrated in Asians when compared with Caucasians, and decreased percutaneous absorption was observed in blacks (33).

More studies have observed that, while overall prevalence of skin sensitivity is similar across skin types and ethnic groups, there are some observable differences with regard to what triggers discomfort and how discomfort is experienced. Caucasians report visual effects more than African-Americans, while African-Americans are more likely to report sensory effects (26). In addition, African-Americans of both genders were more likely to report sensitivity in the genital area than other groups ( $p = 0.0008$ ) (3).

A study of 800 women in San Francisco enrolled 200 subjects in each of four ethnic groups to interview by phone and found no significant difference in overall prevalence between ethnic groups studied. Euro-Americans, however, were found to have a relatively higher susceptibility to wind than other ethnic groups, Asians had significantly higher sensitivity to spicy food, and Hispanics had relatively less reactivity to alcohol (2).

## Cultural Factors

Cultural factors may play a role. Fastidious cleansing routines (with douches, perfumes, medication, antifungal medications, and contraceptives), which often precede irritation (28), undoubtedly have some cultural component. Hygiene practices are the most common cause of vulvar irritation (28).

Confounding lifestyle factors should be considered with regard to some observed differences, as cultural practices may produce widely different exposures to potential irritants (23). For example, older women were found to be more likely to report irritation due to incontinence products than younger women, who were more likely to report irritation due to tampons (29). Air conditioning is more likely to be reported as a trigger for sensitive skin in July than in March (36,37). These findings are quite likely to be based on increased levels of exposure than on actual physiological differences.

## Environmental Factors

A majority of sensitive-skin sufferers report unpleasant sensory responses to cold temperatures, wind, sun, pollution, and heat (2,7). An increased susceptibility to SLS was observed in the winter compared with the summer (14). Low temperatures and humidity characteristic of winter cause lower water content in the stratum corneum (14). Large-scale epidemiological testing in France conducted phone interviews of over 1000 people each in March and then in July, and observed the frequency of sensitive skin in women to be significantly higher in summer than in winter (71.2% in July vs. 59.39% in March) (36).

Numerous other host factors that could influence skin sensitivity include unusual occupational or leisure exposures to chemicals and home climate-control measures (26). Long-term or excessive use of personal-care products can also create

sensitivities (7). Daily topical use of corticosteroids has been demonstrated to produce fragile skin (7), and excessive use of topical medications has been demonstrated to be the source of up to 29% of vulvar dermatitis (38). Drug-induced sensitivity is also possible, although no reports on that issue were uncovered. Interestingly, the thickness of the epidermis in one study was demonstrated to be inversely proportional to the number of years that the subject had smoked, with a  $p = 0.0001$  (27).

The specific methodologies and conditions involved in the testing of skin sensitivity introduce a significant amount of variability into the published results; however, one study reveals that parameters of the testing can themselves induce sensitivity apart from that of the specific irritant employed. Sahlin et al. (39) evaluated the sting potential of the vehicle used in testing the adverse stinging reaction related to lactic acid application. The results showed that the ordinary oil-in-water emulsion induced stinging in and of itself; use of a water-in-oil emulsion created less discomfort. It was also observed that decreasing the mineral oil content in the oil-in-water emulsion resulted in decrease in the degree of sting experienced (39).

## Anatomic Site

Differences in skin sensitivity between anatomical regions have been observed. Analysis of structural differences found that stratum corneum density varies tremendously by anatomical site: palms and soles are the thickest, whereas the genital area is the thinnest (30). The face is the most common site of skin sensitivity. In a study of 1039 men and women, 77.3% reported facial sensitivity, compared with 60.7% for the body, and 56.2% specifically with regard to genital skin (3). Saint-Martory also found the face to be the most commonly reported site of sensitivity, with hands, scalp, feet, neck, torso, and back also reported, in order of frequency (6). The nasolabial fold has been reported to be the most sensitive region (13) of the facial area, followed by the malar eminence (13), chin, forehead, and upper lip (6). Misery et al. (40) found 44.22% of sensitive-skin subjects questioned experienced sensitivity of the scalp. Factors contributing to facial sensitivity are likely the number of products used on the face (particularly in women), a thinner barrier in facial skin, and a plentitude of nerve endings as well (9). Individual susceptibility appears to be dependent on anatomical site (41). Most studies have been conducted in facial skin because of its sensitivity. Stinging sensations, particularly, are readily elicited on facial skin (42). Further, the face is readily accessible for both visual (43) and biophysical assessments (20).

The vulva is an area of particular interest, since it is formed partially from embryonic endoderm; it differs from skin at exposed body sites (10). Differences in irritation seem to be dependent on relative permeability of the irritant in vulvar skin; vulvar skin is significantly more reactive than forearm skin to benzalkonium chloride and maleic acid (44), but less reactive than the forearm to SLS (10). When both venous blood and menses were evaluated for irritant potential, the vulva was less responsive to both than was the upper arm (45).

Nonkeratinized vulvar skin exhibits clearly increased permeability related to the absence of keratin and loosely packed, less structured lipid barrier (10). In addition, the inner epithelia are thinner, representing a shorter distance to penetrate (10). Buccal tissue is often employed in a surrogate model for vulvar testing, as it has very similar structure and biochemistry (10). Buccal skin has been demonstrated to be 10 times more permeable than keratinized skin (46).

Although the vulvar area may be particularly susceptible to cutaneous irritation (47), little objective published data



exists with regard to sensitive skin (42). Irritant reactions to feminine-care products have been reported (38) with a few feminine products that contain chemicals known to be irritants in certain doses (48). However, the potential for heightened vulvar susceptibility to topical agents is not widely reported in literature (10). The contribution to irritation by topical agents, though, is substantial (11) and often underestimated (28). In fact, 29% of patients with chronic vulvar irritation were demonstrated to have contact hypersensitivity, and 94% of those were determined to have developed secondary sensitization to topical medications (38). Thus, reported sensitivity in the vulvar area may often be related to underlying contact hypersensitivity because of excessive use of topical hygienic and medicinal preparations.

Some studies have evaluated skin sensitivity in the vulvar area with regard to sensory responses to consumer products meant for the vulvar area. It was hypothesized that patients with erythema related to a previous genital infection may represent a population of sensitive subjects; however, no increase in sensory effects to exposure to feminine hygiene pads was observed (42).

In a similar population, however, in which observed erythema was evaluated against perceived sensory effects, women who perceived themselves as particularly susceptible to facial erythema were significantly more likely to have medically diagnosed vulvar erythema, a potential indicator of a underlying biological origin (42).

Interestingly, a separate study evaluated perceptions of sensitive skin in women with urinary incontinence, expecting to observe an increased sensitivity of genital skin (49). Increased sensitivity specific to the genital area was not observed, but incontinent women were significantly more likely to assess themselves as having overall skin sensitivity than continent subjects ( $p = 0.014$ : 86.2% in incontinent subjects vs. 68.3% in controls) (49).

## SENSORY EFFECTS AND OBJECTIVE SIGNS

It was observed early on that some subjects report a greater incidence of adverse reactions to certain products because of higher sensitivity (2,32). Some individuals possess exaggerated sensitivity to specific individual irritants (34). Despite the fact, however, that studies have demonstrated that sensitive-skin patients are capable of distinguishing products on the basis of blinded sensory endpoints (13,24), a clinically satisfactory description of observed sensitivities remains out of reach.

Tantalizing clues to the underlying mechanisms of sensitive skin, however, continue to be reported. If deficits in barrier function do play a role in skin sensitivity, regular use of moisturizer should improve sensitivity; patients who completed four daily treatments with moisturizer improved (8). Evaluation of the potential role of the stratum corneum in sensitive skin using corneografometry demonstrated that subjects with demonstrated sensitivity to detergents had an increased reactivity to tested products as compared with the control group. It may be a specific subgroup of sensitive skin with some sort of defect in the stratum corneum that caused weakened resistance to surfactants (18).

Local anesthetics block response in lactic acid sting tests; stingers respond more vigorously to vasodilators (9). An Italian study compared self-reports of sensitivity with response in the lactic acid test as follows: stingers were found at very similar prevalences to self-reported sensitivity (56.9% of women perceived skin as sensitive, 54.3% revealed to be stingers). In

addition, those who believe skin to be sensitive were revealed to be more likely to be stingers (59%) than among nonstingers (48.9%) (31).

Simion et al., by exaggerated arm-washing with synthetic detergent bars, observed signs that correlated statistically with sensory perceptions (dryness, tightness, and itching). In addition, consumers were able to reproducibly distinguish between test products purely on the basis of sensory effects (50).

Another study evaluated specific biophysical parameters in 32 subjects medically diagnosed with sensitive skin in parallel with a non-sensitive skin control group. Patch testing, skin hydration, sebum production, alkali resistance test, lactic acid sting test, methyl nicotinate 0.5%, methacholine chloride 1:1000, pH, dermographism, and measurement of total and specific Ig were performed (19). Patch testing found that patients with sensitive skin were ten times more likely to respond to allergens in the European standard series ( $p < 0.01$ ) and three times more likely to respond to cosmetic allergens ( $p < 0.01$ ) than those without sensitive skin. Sensitive subjects also had significantly less sebum production ( $p < 0.01$ ) and dryer skin ( $p < 0.05$ ). Sensitive patients had a four-fold risk of a decrease of alkali resistance ( $p < 0.05$ ).

Vascular reactions to methyl nicotinate and methacholine chloride in sensitive-skin patients were observed to be characterized by a significant hyperreaction of skin blood vessels, with a more intense erythema after methyl nicotinate application (19). The risk of and intense vascular reaction to methyl nicotinate was 75 times higher in sensitive patients than in non-sensitive subjects, and nearly one-third of sensitive-skin subjects experienced an abnormal vascular reaction (skin blanching) after application of methacholine chloride. A strong association of sensitivity with fair skin was also observed. This may relate to well-established differences in skin structure and permeability across different skin types.

## SENSITIVE SKIN: ZEROING IN ON BIOLOGICAL ORIGIN

Part of the reason for the observed breakdown between sensory effects and objective signs is the fact that an objective sign like erythema is the end result of a complex, multistep physiological process. Numerous underlying processes (e.g., changes in blood flow, moisture content, pH) would be expected to occur before the appearance of visible external changes (1). A goal of our research has been to increase the ability to predict and quantify these subjective consumer responses. Our approach has been three-fold: to exaggerate testing conditions to elicit corroborating physical findings, to increase the sensitivity of assessment of physical findings, and to find a way to quantify sensory endpoints (1).

## Exaggeration of Test Conditions

One study evaluated four versions of facial tissues, with and without coating, with repeated wiping to accentuate irritation (48). Affected skin had been compromised by tape stripping prior to wiping protocol initiation. Erythema as well as dryness were evaluated daily by trained graders. In addition, panelists were interviewed about specific aspects of product preferences. Statistical analysis revealed that the panelists' subjective product preferences were more consistent in distinguishing between the test products than were either erythema or dryness.

A second method of accentuating test conditions, developed in our laboratories specifically for testing paper such as



catamenial products, has proven very effective at accentuating irritant response to inherently mild products. The behind-the-knee (BTK) protocol uses the popliteal fossa as a test site and adds a relevant mechanical friction component to old testing (51). BTK testing consists of a test product placed behind the knee and held securely by an elastic knee band. Levels of irritation produced in BTK testing are consistently higher than those achieved with standard patch testing, and have proven to be consistently reproducible (51). BTK testing, in conjunction with the other two approaches below, has proven useful in the development of potentially valuable protocols for sensitive-skin testing.

### Quantifying Sensory Responses

A study similar to the facial tissue study above tested feminine hygiene products according to four combinations of test conditions (wet/dry, intact/compromised skin). Products tested were inherently nonirritating and were tested in parallel in arm patches and BTK. In addition, the study evaluated observed erythema grading against a patient log of sensory effects. Although no differences were observed between any combinations tested, a significant correlation of reported sensory discomfort with mean irritant scores was observed. Skin sites where patients experienced burning, itching, or sticking had consistently higher mean irritant scores (52). Ultimately, eight separate comparison studies were able to statistically associate perceived sensory effects with an increase in irritant scores (51).

Companion papers that utilized only BTK methodology (53) but also evaluated patient diaries in conjunction with the irritant testing observed correlation between sensory effects and mean irritant scores as well (52).

### Increasing Sensitivity of Assessment of Physical Response

Our laboratories evaluated several new methodologies in the pursuit of an increased sensitivity of the evaluation of the physical response. Visual grading of erythema has been relied on for a number of years; trained graders achieve a high degree of reproducibility with no specialized equipment (54). A new approach in our laboratories, however, utilized cross-polarized light, which allows visualization of the skin at a depth of 1 mm below the surface (55). Testing was performed with SLS in a standard, 24-h patch test on the upper arm, and with two different feminine hygiene products (identified as A and B) in the BTK. It had been established previously that these two products differed in consumer preference, but no discernable difference had been found in objective measures of skin effects (24). With the minor irritation produced the SLS patch on the upper arm, both unaided visual scoring and subsurface visualization detected erythema associated with irritation. With multiple, shorter exposures (2-h and 6-h) to lower concentrations of SLS, the subsurface visualization detected erythema earlier than unaided visual scoring. Using the mild feminine hygiene products in BTK-enhanced visual scoring through subsurface visualization allowed the observation of significant differences in the irritation produced by the two different products, thus establishing a potential link between sensory effects and subclinical irritation.

A second approach evaluated the potential for changes in skin temperature related to inflammation to act as a subclinical measure of skin irritation. Previous research has demonstrated a correlation between surface temperature measurements and

inflammatory response (56). A high precision, handheld infrared thermographic scanner makes it feasible to conveniently measure local changes in skin temperature in situ (57). Two catamenial products were compared in a BTK protocol. Skin surface temperature was measured using an infrared thermographic scanner. Subjects were also asked to keep a diary of skin discomfort experienced at test sites, specifically including sticking, chafing, burning, itching, pain, edema, or any other issue. Skin temperature changes observed were closely associated with visual scores. In addition, the study incorporated diary-derived data on sensory effects experienced by panelists as an additional endpoint. The diaries of subjective sensory experiences over the course of the exposure made a clear distinction between the two test products that was consistent with both visual scoring and skin temperatures. A significant t-difference was also observed between mean visual scores of those who reported specific adverse sensations as compared with those who did not report negative sensations. Skin temperature means were significantly higher for those who reported the adverse sensations rubbing and chafing (interestingly, burning sensations were not associated with increase in skin surface temperature). Conditions in this protocol were optimized for using erythema as the primary endpoint; refining the protocol to optimize detection of differences in skin surface temperature would be a logical next step. Skin surface temperatures correlated well with visual signs of irritation; six of eight sensory effects were associated with higher visual scores.

An additional new technique in development uses a commercially available product, Sebutape® (CuDerm Corporation, Dallas, Texas), an absorbent tape, which is applied to skin for 60 seconds and then removed. Application of the tape to both healthy skin and compromised skin was followed by extraction of different cytokines from the Sebutape, which were then quantified. Levels of IL-1a, IL-1RA, and IL-8 were evaluated. Compromised skin was associated significantly with increased IL-1a levels, increased IL-8 levels, and increased IL-1RA:IL-1a ratio. This technique has not been substantially applied to the problem of sensitive skin yet, but shows potential (58).

### Links between Sensitive Skin and Immunology

Evidence for a link between atopy and sensitive skin has accumulated. An assessment of 1039 individuals (83.6% female) found that individuals who claimed overall to have sensitive skin were 5 times likely to have skin allergies confirmed by a doctor ( $p < 0.0001$ ), and more than 3.5 times more likely to have relatives with sensitive skin (59). In a study in older adults, those who claimed sensitive skin had a higher frequency of medically diagnosed skin allergies than younger people who claimed sensitive skin (29). Löffler et al. (60) observed a link between sensitive skin and nickel allergy.

A study compared 25 Greek women with medically diagnosed atopic dermatitis with 25 healthy women (61). A significant association was found between the clinical diagnosis of atopic dermatitis and the self-diagnosis of sensitive skin ( $p < 0.001$ ). All patients in the atopic dermatitis group described themselves as having sensitive skin to at least some degree, with 80% claiming either moderately or very sensitive. By contrast, 64% of individuals in the control group described their skin as sensitive to some degree, with only 16% claiming either very or moderately sensitive. Patients with atopic dermatitis were also significantly more likely to indicate a family history of sensitive skin than were non-sensitive individuals

(68%–24%,  $p = 0.004$ ) 76% of atopic patients who claimed a family history identified a parent as having sensitive skin.

Atopic individuals were significantly more likely to report genital sensitivity after contact with hygiene pads, although not more likely to experience sensitivity to genital cleansing products, fragrances, or antiperspirants (61). In addition, the study demonstrated a link between clinically diagnosed atopic dermatitis and sensitive skin, with the frequency, severity, and history of skin sensitivity in patients with atopic dermatitis far more pronounced than in controls. This link has substantial biological plausibility, as contact allergy and skin sensitivity are phenomena that share similar cytokine inductions (59).

Of potential utility for large-scale screening in industry, postmarket surveillance, and epidemiological testing, a rapid algorithm containing only three questions has been developed to quickly identify atopic individuals (62). Testing indicated the algorithm was capable of successfully categorizing individuals as “atopic” and “non-atopic” with a 90% success rate (45 out of 50 individuals in the test).

### Insight into Neurogenic Causality

Sensitive skin is predominantly sensory in nature and thus ultimately a neurological disorder. Sensory differences may be related to innervation (63). Dermal nerve fibers extend throughout viable epidermis as free nerve endings, but the epidermal component of this network is still poorly characterized (63). Epidermal nerve density variation could explain the different sensitivity thresholds in various anatomical sites (64). Hyperreactivity of the neural response of the skin is postulated to play a role. Possible mechanisms for neural system hyperreactivity include nerve fibers; endothelin receptors; burn, itch, and heat receptors; cold receptors; and neurotrophins (23).

Neurogenic inflammation probably results from release of neurotransmitters such as substance P, calcitonin gene-related peptide, and vasoactive intestinal peptide, which induce vasodilation and mast cell degranulation. Nonspecific inflammation may also be associated with the release of interleukins (IL-1, IL-8, prostaglandin E2, prostaglandin F2, and tumor necrosis factor- $\alpha$ ) (40). Other studies have evaluated what contribution neural dysfunction may play in the development of sensitive skin.

Functional magnetic resonance imaging, which measured cerebral activation associated with skin discomfort, was used to evaluate neural reaction to application of lactic acid to the face in 18 women, with and without sensitive skin (65). Lactic acid-induced skin discomfort resulted in increased activity in the primary sensorimotor cortex contralateral to the application site as well as in a bilateral frontoparietal network that included the parietal cortex, prefrontal areas around the superior frontal sulcus, and the supplementary motor activity. In addition, in sensitive skin patients only, group activity spread into the ipsilateral primary sensorimotor cortex and the bilateral periinsular secondary somatosensory area, a phenomenon which did not occur in the control group. Subjects with self-assessed sensitive skin were also observed to have significantly greater increases in neural activity than those without sensitive skin, demonstrating an increase in neural activity specifically associated with sensitive skin.

Another study measured calibrated electrical stimulation of the skin, which stimulates sensory nerve fibers such as

the myelinated A fiber, A delta fiber, and unmyelinated c-fiber independently (66). In subjects with clinically documented sensitive skin (lactic acid sting test, cosmetic compatibility tests) versus non-sensitive controls (all subjects male), nerve fibers were stimulated by three different current strengths, and capsaicin (0.075%) was applied to the zygomatic arch. Sensory perception was verbalized by the subject and recorded. Baseline perception of current revealed no significant differences between sensitive and non-sensitive subjects at either 2 kHz or 250 Hz, but at 5 Hz—a current known to selectively stimulate the c-fibers of sensory nerves. Sensitive skin subjects displayed a significantly lower perception threshold. In addition, stimulation of the skin by capsaicin, in non-sensitive subjects, had no effect on perception of the 5-Hz current, whereas sensitive subjects displayed a long-lived increase in the sensory perception threshold (still in place at last time point of 60 minutes). These findings imply that sensory perception in sensitive subjects is easily disturbed by weak stimulation, inducing a wide variability of response compared with non-sensitive subjects, an effect that appears to be c-fiber modulated. The study was conducted in only eight subjects (four with sensitive skin) and should be followed up in a larger population.

### CONCLUSION: A VALID SYNDROME WITH MULTIPLE ORIGINS?

Sensitive skin, though now largely recognized as genuine syndrome of physiological origin, is still a subjective complaint with no consistent associations (60), no predictable or classical visible signs of irritation, no immunologically verifiable response, and no accepted and reproducible diagnostic test (23). Although it is clear that specific individuals clearly have heightened sensitivity to different kinds of sensory and physical irritants, observed reactions are not predictive of generalized sensitivity, and the relationship between observed sensitivities is unclear (24,67). Evidence suggests that sensitive skin may not be a single condition, but the product of multiple etiologies with multiple components. Therefore, the condition may encompass different categories of subjects and sensitivities based on different mechanisms (20). Multiple etiologies would not be farfetched, as the nervous system does not act in isolation but is interdependent with both the immune system and the skin, sharing numerous cellular contact as well as the same language of cytokines and neurotransmitters. All three interact to affect cutaneous responses (19).

There is an urgent necessity to establish methodologies with the capacity to accurately identify sensitive skin (5), independent of self-assessed reports (23). Methods are needed that are capable of detecting very subtle skin benefits or potential for adverse effects. Testing has been done primarily on normal subjects, bringing into question the need to focus on examining populations that may be inherently more sensitive to irritant effects (10). Some studies did compare the irritation potential of products between self-declared sensitive skin to non-sensitive-skin subjects (68,69). A summary of current methodologies used to identify sensitive skin is shown in Table 5.4.

Subclinical irritation may be the key to understanding sensitive skin, as sensations elicited by product exposure are generally discerned long before observable differences (50). One significant advance in the understanding of sensitive skin is the development of new, noninvasive techniques; for example, cross-polarized light-enhanced visualization, which has demonstrated good correlation with sensory perceptions and the ability to measure subclinical damage (55).

**Table 5.4** Some Methodologies Used for Sensitive Skin

Methodology	Sensory affect evaluated	Physical effect evaluated	Relevant irritants	Advantages	Disadvantages
Lactic acid (8)	Stinging	None	Cosmetics, other personal preparations meant to be left on	Highly sensitive and specific*	Does not predict sensitivity to other irritants
Capsaicin (23)	Stinging	None	Cosmetics, other personal preparations meant to be left on	Sensitive, detection threshold well correlated (inversely) to perception of sensitive skin	Does not predict sensitivity to other irritants
Sodium lauryl sulfate (14)	Burning	Erythema	Industrial exposures, cleaning products	Cheap, quick, reliable assessment of individual susceptibility to specific irritant	Sensitivity to one irritant not predictive of general sensitivity, relationship to sensitive skin in question
Cross-polarized light (55)	None	Subclinical erythema	Any potential irritant	Permits detection of physical changes not apparent by standard visual scoring, noninvasive	Requires specialized equipment
Thermoscan (57)	None	Temperature increases resulting from inflammatory processes related to skin injury	Any potential irritant	Noninvasive, objective, quantitative	Requires specialized equipment
SebutapeX (58)	None	Measurement of cytokines produced by injured skin	Any potential irritant	Noninvasive, objective, quantitative, potentially very sensitive	Requires training, specialized equipment; utility for sensitive skin still unassessed

\*Lactic acid test positive in 90% of women who claim sensitive skin.

An immediate need is to build on what is known with improved techniques, carefully crafted protocols that evaluate appropriate exposures and study populations, and rigorous methodological and statistical procedures, bringing the study of sensitive skin out of the realm of fairy tales and into the realm of a genuine physiological disorder worthy of focused research. The challenge of the future is to unravel the biological link between subjective clinical signs and their physical sequelae as a means to develop appropriate diagnostic criteria as well as to understand the etiology of this still largely mysterious disorder.

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# Organic Acids with Novel Functions: Hydroxy, Bionic, N-acetylamino Acids and N-acylpeptide Derivatives

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## INTRODUCTION

Organic acids cover a wide range of organic compounds having an acidic group such as carboxyl, sulfonyl, or phosphoryl, which include retinoic acid, salicylic acid, benzoic acid, and its peroxide form, benzoyl peroxide. The present discussion will focus on certain organic carboxylic acids and related derivatives with unique cosmetic and dermatological effects on the skin. These acids are  $\alpha$ -hydroxyacids (AHAs),  $\beta$ -hydroxyacids (BHAs), polyhydroxy acids (PHAs), aldobionic acids (ABAs), N-acetylamino acids (NAAs), and N-acylpeptides (NAPs).

Abnormal keratinization is a principal event associated with a majority of dermatologic conditions including ichthyosis, xerosis, eczema, psoriasis, and acne. As a result, we estimate that over 50% of all skin problems are due to or are associated with disturbed formation and shedding of the stratum corneum, and in part attribute the cosmetic and therapeutic uses of AHAs on skin to their unique ability to modulate the process of keratinization and normalize stratum corneum exfoliation.

Hydroxyacids and related acids of nonphenolic origin are a group of natural and physiological substances which have profound effects on keratinization and the synthesis of dermal components. Many hydroxyacids and related acids occur in food, fruits such as sugar cane, tomato, oranges, lemons, grapes, apples, mangos, and body tissues. For many years, cosmetic chemists have used lactic acid along with other organic acids to adjust pH, and citric acid as a chelating and antioxidant stabilizer in topical formulations. In addition, lactic acid has been used as a stabilizer in urea formulations for topical treatment of dry skin.

In 1974 the term AHA was first introduced to dermatology when it was discovered that AHAs substantially improved the severe hyperkeratotic conditions of ichthyosis (1). AHAs are also beneficial for topical treatment of dry skin, dandruff, calluses, acne, keratoses, warts, wrinkles, photoaging skin, and for other cosmetic conditions and dermatological purposes (2–5). AHAs such as glycolic acid and lactic acid are routinely used in peel solutions by estheticians and dermatologists. In dermatological office procedures they are used for topical management and treatment of various skin conditions including skin smoothing, acne, and skin changes associated with intrinsic and extrinsic aging (6–9). AHAs, on topical application, have been shown to markedly increase biosynthesis of hyaluronic acid and collagen in the papillary dermis, although the mechanism of action is unknown (5,10).

Polyhydroxy AHAs constitute one of the two major components of hyaluronic acid, chondroitin sulfate, dermatan

sulfate, heparin, and heparan sulfate. In combination with numerous cosmetic or pharmaceutical agents, hydroxyacids have been found to enhance desirable topical effects, and also to reduce or prevent side effects caused by topical agents. Because most hydroxyacids are nontoxic, natural, and physiological they are used as primary or secondary ingredients in many cosmetic and pharmaceutical products.

NAAs and NAPs are derived from amino acids or peptides by substitution at the amino group. N-acetyl-L-cysteine (NAC) has been shown to be a potent antioxidant. We have found, for example, N-acetyl-L-proline, N-acetyl-L-glutamine, N-acetyl-L-valyl-L-alaninamide, N-acetyl-L-tyrosyl-L-tyrosinamide, and N-acetyl-L-tyrosyl-L-tyrosyl-L-tyrosinamide to be topically effective for relieving itch and improving lesions associated with eczema and xerosis. We have also found that NAAs and NAPs are effective for topical treatments of aging-related skin changes.

In this chapter we discuss the scientific basis for topical effects of certain organic acids in dermatological therapy and in various cosmetic applications.

## HYDROXYACIDS: NOMENCLATURE AND OCCURRENCE

Organic hydroxyacids of non-phenolic origin may be classified into the following four groups: AHAs, BHAs, PHAs, and ABAs. For group names, the spellings  $\alpha$ -hydroxyacid and  $\beta$ -hydroxyacid are preferred instead of  $\alpha$ -hydroxy acid and  $\beta$ -hydroxy acid because  $\alpha$  and  $\beta$  indicate the position of hydroxyl group in the hydroxyacid molecule. In contrast, the spelling polyhydroxy acid is preferred instead of poly-hydroxyacid or poly-hydroxy acid because in the word, poly indicates many or multiple hydroxyl groups, not the position of hydroxyl group in the acid (11).

### $\alpha$ -HYDROXYACIDS

AHAs are organic carboxylic acids having one hydroxyl group attached directly to the  $\alpha$  position of an aliphatic or alicyclic carbon atom, but not to a benzene or other aromatic ring. On a broader scope, AHAs may include those molecules having additional carboxyl groups (11). Glycolic acid, present in sugar cane juice, is the smallest molecule of all the hydroxyacids, and is a major ingredient in most AHA products on the market. All other AHAs may be considered derivatives or substituted glycolic acid. The AHAs may be divided into three subgroups: alkyl AHAs, aralkyl AHAs, and polycarboxyl AHAs.

**Table 6.1** Nomenclature and Occurrence of Glycolic Acid and Alkyl  $\alpha$ -Hydroxyacids

Systematic name Chemical structure	Common name	Occurrence
2-Hydroxyethanoic acid $\text{CH}_2\text{OHCOOH}$	Glycolic acid Hydroxyacetic acid	Sugar cane
2-Hydroxypropanoic acid $\text{CH}_3\text{CHOHCOOH}$	Lactic acid	Tomato
2-Methyl 2-hydroxypropanoic acid $(\text{CH}_3)_2\text{COHCOOH}$	Methylactic acid	Mango
2-Hydroxybutanoic acid $\text{CH}_3\text{CH}_2\text{CHOHCOOH}$	$\alpha$ -Hydroxybutyric acid	
2-Hydroxyoctanoic acid $\text{CH}_3(\text{CH}_2)_5\text{CHOHCOOH}$	$\alpha$ -Hydroxycaprylic acid	
2-Hydroxyeicosanoic acid $\text{CH}_3(\text{CH}_2)_{17}\text{CHOHCOOH}$	$\alpha$ -Hydroxyarachidonic acid	
2-Hydroxytetraeicosanoic acid $\text{CH}_3(\text{CH}_2)_{21}\text{CHOHCOOH}$	Cerebronic acid	Skin as ceramide

### Alkyl AHAs

A radical attached to the  $\alpha$  carbon of glycolic acid can be a simple hydrocarbon called alkyl group. The smallest alkyl group is a methyl group and in this case, the AHA is lactic acid (present in tomatoes). Representative alkyl AHAs are listed in Table 6.1.

### Aralkyl AHAs

Aralkyl is an abbreviation of aryl plus alkyl. Aralkyl AHA is formed when a phenyl group is attached to an alkyl AHA, and is represented by mandelic acid, benzilic acid, 3-phenyllactic acid, and atrolactic acid. Mandelic acid has been used in combination as methenamine mandelate for oral administration to treat urinary tract infections.

### Polycarboxy AHAs

AHA may consist of more than one carboxyl group, as shown in Table 6.2. Malic acid, occurring in apples, is also called apple acid, and tartaric acid, present in grapes, has been called fruit acid in the past. Citric acid, occurring in oranges and lemons, has one hydroxyl group and three carboxyl groups. The  $\alpha$ - or  $\beta$ -hydroxyacid refers to the position of a hydroxyl group as related to a carboxyl group in the hydroxyacid. When a hydroxyacid has more than one carboxyl group it can be an AHA and BHA at the same time. For example, malic acid, tartaric acid, and citric acid can be both AHA and BHA.

### $\beta$ -HYDROXYACIDS

BHAs are organic carboxylic acids having one hydroxyl group attached to a carbon atom at the  $\beta$  position, and are represented by  $\beta$ -hydroxybutanoic acid and tropic acid.  $\beta$ -Hydroxybutanoic acid, also known as  $\beta$ -hydroxybutyric acid, is excreted in amounts as much as 30 grams per day in the urine of diabetic subjects. Salicylic acid, 2-hydroxybenzoic acid, has both hydroxyl and carboxyl groups directly attached to a benzene ring. It is not chemically a true BHA, but it is erroneously referred to as a BHA (12) in casual jargon.

**Table 6.2** Nomenclature and Occurrence of Polycarboxy  $\alpha$ -Hydroxyacids

Systematic name Chemical structure	Common name	Occurrence
2-Hydroxypropane-1,3-dioic acid $\text{HOOC CHOH COOH}$	Tartronic acid	
2-Hydroxybutane-1,4-dioic acid $\text{HOOC CH}_2\text{ CHOH COOH}$	Malic acid	Apple
2-Methyl-2-hydroxybutane-1,4-dioic acid $\text{HOOC CH}_2\text{ C}(\text{CH}_3)\text{OH COOH}$	Citramalic acid	
2,3-Dihydroxybutane-1,4-dioic acid $\text{HOOC CHOH CHOH COOH}$	Tartaric acid	Grape
3-Carboxy-3-hydroxypentane-1,5-dioic acid $\text{C}(\text{OH})(\text{COOH})(\text{CH}_2\text{COOH})_2$	Citric acid	Orange
3-Carboxy-2-hydroxypentane-1,5-dioic acid $\text{HOOCCHOH CH}(\text{COOH})\text{CH}_2\text{COOH}$	Isocitric acid	Lemon
3-Carboxy-3-hydroxyhexane-1,6-dioic acid $\text{HOOCCH}_2\text{C}(\text{OH})(\text{COOH})\text{CH}_2\text{CH}_2\text{COOH}$	Homocitric acid	
3-Carboxy-2-hydroxyhexane-1,6-dioic acid $\text{HOOCCHOH CH}(\text{COOH})\text{CH}_2\text{CH}_2\text{COOH}$	Homoisocitric acid	
3-Carboxy-2-n-hexadecyl-3-hydroxypentane 1,5-dioic acid $\text{HOOCCH}_2\text{C}(\text{OH})(\text{COOH})\text{CH}(\text{C}_{16}\text{H}_{33})\text{COOH}$	Agaricic acid  n-Hexadecyl citric acid	

### POLYHYDROXY ACIDS

PHAs are organic carboxylic acids having multiple hydroxyl groups (13). Many PHAs are also AHAs; they are derived from carbohydrates, and are important intermediates in carbohydrate metabolism. PHAs may be divided into three groups: aldonic acid, aldaric acid, and alduronic acid.

### Aldonic Acid

An aldonic acid is a carbohydrate, called aldose, having the carbon atom at position 1 changed to a carboxyl group, and is represented by ribonic acid and gluconic acid, as shown in Table 6.3. Vitamin C, L-ascorbic acid, is a 1,4-lactone form of the AHA 2,4,5,6-tetrahydroxy-3-ketohexanoic acid, a keto PHA with chemical structure:  $\text{HOCH}_2\text{CHOHCHOHCOCHOHCOOH}$ . The lactone form of vitamin C has two acidic hydroxyl groups at carbon positions 2 and 3, and does not have an effect on keratinization comparable to that of  $\alpha$ -hydroxy PHAs.

### Aldaric Acid

An aldaric acid is a carbohydrate having two carbon atoms at the end positions changed to carboxyl groups, and is represented by glucaric acid (saccharic acid) and galactaric acid (mucic acid).

### Alduronic Acid

An alduronic acid is a carbohydrate having the terminal carbon changed to a carboxyl group, and is represented by glucuronic

**Table 6.3** Nomenclature and Occurrence of Aldonic Acids

Systematic name and chemical structure	Common name stereoisomer name	Occurrence
2,3-Dihydroxypropanoic acid $\text{HOCH}_2\text{CHOHCOOH}$	Glyceric acid	
3,3-Dimethyl-2,4-dihydroxybutanoic acid $\text{HOCH}_2\text{C}(\text{CH}_3)_2\text{CHOHCOOH}$	Pantoic acid	In vitamin B <sub>5</sub>
2,3,4-Trihydroxybutanoic acid $\text{HOCH}_2(\text{CHOH})_2\text{COOH}$	Erythronic acid Threonic acid	
2,3,4,5-Tetrahydroxypentanoic acid $\text{HOCH}_2(\text{CHOH})_3\text{COOH}$	Ribonic acid, arabinonic acid, xylonic acid, lyxonic acid	
2,3,4,5,6-Pentahydroxyhexanoic acid $\text{HOCH}_2(\text{CHOH})_4\text{COOH}$	Allonic acid, altronic acid, gluconic acid, mannoic acid, gulonic acid, idonic acid, galactonic acid, talonic acid	In skin
2,3,4,5,6,7-Hexahydroxyheptanoic acid $\text{HOCH}_2(\text{CHOH})_5\text{COOH}$	Alloheptonic acid, altoheptonic acid, glucoheptonic acid, mannoheptonic acid, guloheptonic acid, idoheptonic acid, galactoheptonic acid, taloheptonic acid	

acid which is produced in the body as a detoxifying agent, and forms hyaluronic acid with N-acetyl glucosamine.

### ALDOBIONIC ACIDS

ABA, also known as bionic acid, consists of one monosaccharide chemically linked through an ether bond to an aldonic acid, as shown in Table 6.4. An ABA may also be described as an oxidized form of a disaccharide or dimeric carbohydrate, such as lactobionic acid derived from lactose and maltobionic acid from maltose. Lactobionic acid solution is currently used in preservative media for organ transplants.

### AHA-RELATED COMPOUNDS

An  $\alpha$ -ketoacid has a keto instead of hydroxyl group at the alpha carbon of an organic carboxylic acid, and is related to its counterpart alpha-hydroxyacid. Pyruvic acid (2-ketopropanoic acid,  $\text{CH}_3\text{COCOOH}$ ) and lactic acid have such a biochemical relationship in that the latter can be converted to the former when the hydroxyl group is oxidized to keto group by lactate dehydrogenase.

### HYDROXYACIDS: PHYSICOCHEMICAL PROPERTIES

#### Stereoisomers

Stereoisomers are formed when a carbon in a hydroxyacid has a stereocenter in the molecule, i.e. the carbon has four non-identical radicals. Whereas glycolic acid, methylactic acid, and benzoic acid do not have stereoisomers, lactic acid and mandelic acid have stereoisomers, D and L forms. Tartronic acid and citric acid do not have stereoisomers, but malic acid has D and L, and tartaric acid has D, L, and meso forms. For PHAs and ABAs, stereoisomers usually result in different chemical names, such as ribonic acid and arabinonic acid, gluconic acid and galactonic acid, lactobionic acid and maltobionic acid, and often retain similar though not identical functions.

#### Lactone Form

In contrast to AHAs and BHAs, many PHAs can form spontaneous intramolecular lactones by elimination of water molecules between the carboxyl and hydroxyl groups, especially when these two functional groups are separated by two or three carbons. D-Gluconolactone, known as D-gluconic acid

**Table 6.4** Nomenclature and Source of Aldobionic Acids

Common name	Chemical structure	Source
Lactobionic acid	$\text{HOH}_2\text{CCHOHCHOR}(\text{CHOH})_2\text{COOH}$ R=galactose; 4-O- $\beta$ -D-Gal-D-gluconic acid	Lactose
Isolactobionic acid	6-O- $\beta$ -D-Gal-D-gluconic acid	Isolactose
Maltobionic acid	$\text{HOH}_2\text{CCHOHCHOR}(\text{CHOH})_2\text{COOH}$ R=glucose; 4-O- $\alpha$ -D-Glc-D-gluconic acid	Maltose
Isomaltobionic acid	6-O- $\alpha$ -D-Glc-D-gluconic acid	Isomaltose
Cellobionic acid	$\text{HOH}_2\text{CCHOHCHOR}(\text{CHOH})_2\text{COOH}$ R=glucose; 4-O- $\beta$ -D-Glc-D-gluconic acid	Cellobiose
Gentiobionic acid	$\text{ROH}_2\text{C}(\text{CHOH})_4\text{COOH}$ R=glucose; 6-O- $\beta$ -D-Glc-D-gluconic acid	Gentiobiose
Kojibionic acid	$\text{HOH}_2\text{C}(\text{CHOH})_3\text{CHORCOOH}$ R=glucose; 2-O- $\alpha$ -D-Glc-D-gluconic acid	Kojibiose
Laminarabionic acid	$\text{HOH}_2\text{C}(\text{CHOH})_2\text{CHORCHOHCOOH}$ R=glucose; 3-O- $\beta$ -D-Glc-D-gluconic acid	Laminarabiose
Melibionic acid	$\text{ROH}_2\text{C}(\text{CHOH})_4\text{COOH}$ R=galactose; 6-O- $\alpha$ -D-Gal-D-gluconic acid	Melibiose
Nigerobionic acid	3-O- $\alpha$ -D-Glc-D-gluconic acid	Nigerose
Sophorobionic acid	2-O- $\beta$ -D-Glc-D-gluconic acid	Sophorose

$\delta$ -lactone, is formed by eliminating one mole of water between the carboxyl group and the hydroxyl group at carbon 5 position of D-gluconic acid, forming a six-member ring lactone.

## Solubility and Gel Matrix

AHAs and BHAs with small molecular weight, and most PHAs and ABAs, are soluble in water. Certain AHAs and BHAs such as methylactic acid, mandelic acid, malic acid, phenyllactic acid, atrolactic acid, and tropic acid are also soluble in alcohol. Some aralkyl AHAs are lipophilic, and are more soluble in alcohol than water, such as benzilic acid.

One unique property of ABAs is their potential to form a gel matrix with water. Maltobionic acid can form a clear gel matrix containing 29% water molecules complexed with maltobionic molecules. Under the same conditions, lactobionic acid and cellobionic acid can form clear gels containing 14% and 7% water respectively. The gel matrix may add protective, soothing, and healing effects for inflamed skin or in wound healing.

## Acid Strength and $pK_a$

The acid strength of an organic hydroxyacid is determined by its proton dissociation from the carboxyl group in aqueous solution. After equilibrium is reached, the dissociation constant  $K_a$  is defined as hydroxyacid anion multiplied by proton ion, and divided by undissociated hydroxyacid based on molar concentration. The acid strength is expressed as  $pK_a$ , and the latter is a negative logarithm of the dissociation constant. The hydroxyacid is a stronger acid if its  $pK_a$  number is lower (14). The acid strength of a hydroxyacid may not be related to its topical action on keratinization, although its  $pK_a$  is crucial to the determination of bioavailability and bioavailable concentration.

## Antioxidant Property

Oxidation is defined as removal of electrons or reaction with oxygen. An antioxidant is defined as any substance capable of preventing or inhibiting oxidation. In biological systems, an antioxidant may be described as a substance capable of disposing, scavenging, or suppressing formation or actions of peroxide, superoxide, or free radicals. There are three simple screen methods which are useful to determine antioxidant properties: prevention or retardation of air oxidation of (1) anthralin, (2) hydroquinone, or (3) banana peel. Based on these three tests, all the PHAs and ABAs we have tested are antioxidants which include ribonolactone, gluconolactone, galactonolactone, gulonolactone, glucoheptonolactone, lactobionic acid, and maltobionic acid (11,15,16). Among AHAs and BHAs, citric acid, isocitric acid, tartaric acid, and malic acid are antioxidants.

## HYDROXYACIDS: BIOCHEMISTRY Relationship to Amino Acids

Many hydroxyacids are related to or derived from amino acids. Based on chemical structures, the only difference between an AHA and an amino acid is the hydroxyl group instead of the amino group, as shown in Table 6.5.

## Carbohydrate Metabolism and Citrate Cycle

Many hydroxyacids are intermediate products or end metabolites in carbohydrate metabolism; these include glyceric acid in glycolysis. In anaerobic glycolysis, D-glucose is converted to L-lactic acid as the end product. Gluconic acid and gluconolactone are important intermediates in the pentose phosphate pathway for the synthesis of nucleotides in DNA and RNA. Gulonic acid and gulonolactone are carbohydrate

**Table 6.5** Biochemical Relationship between Hydroxyacids and Amino Acids

Hydroxyacid and amino acid	Chemical structure
Glycolic acid	$\text{CH}_2\text{OHCOOH}$
Glycine	$\text{CH}_2\text{NH}_2\text{COOH}$
Lactic acid	$\text{CH}_3\text{CHOHCOOH}$
Alanine	$\text{CH}_3\text{CHNH}_2\text{COOH}$
Isopropylglycolic acid	$\text{C}_3\text{H}_7\text{CHOHCOOH}$
Valine	$\text{C}_3\text{H}_7\text{CHNH}_2\text{COOH}$
3-Isopropyllactic acid	$\text{C}_3\text{H}_7\text{CH}_2\text{CHOHCOOH}$
Leucine	$\text{C}_3\text{H}_7\text{CH}_2\text{CHNH}_2\text{COOH}$
3-Methyl-3-ethyl-lactic acid	$(\text{C}_2\text{H}_5)\text{CH}(\text{CH}_3)\text{CHOHCOOH}$
Isoleucine	$(\text{C}_2\text{H}_5)\text{CH}(\text{CH}_3)\text{CHNH}_2\text{COOH}$
Glyceric acid	$\text{CH}_2\text{OHCHOHCOOH}$
Serine	$\text{CH}_2\text{OHCHNH}_2\text{COOH}$
3-Methylglyceric acid	$\text{CH}_3\text{CHOHCHOHCOOH}$
Threonine	$\text{CH}_3\text{CHOHCHNH}_2\text{COOH}$
Malic acid	$\text{HOOCCH}_2\text{CHOHCOOH}$
Aspartic acid	$\text{HOOCCH}_2\text{CHNH}_2\text{COOH}$
3-Phenyllactic acid	$\text{C}_6\text{H}_5\text{CH}_2\text{CHOHCOOH}$
Phenylalanine	$\text{C}_6\text{H}_5\text{CH}_2\text{CHNH}_2\text{COOH}$

intermediates for the synthesis of vitamin C in plants and some animals. Citric acid, isocitric acid, and malic acid are important intermediates in the citrate cycle for energy production.

## Glycosaminoglycans

Glycosaminoglycans (GAGs) are large carbohydrates widely distributed in the body, for example in skin, fibroblasts, mast cells, cartilage, bones, synovial fluid, cornea, and loose connective tissues. Their physiological roles include formation of extracellular matrix, specific interactions with collagen and elastin, binding of water and ions, facilitating cell migration, formation of anticoagulants, and facilitating cell adhesion, cell interaction, and cell receptors. There are six different types of GAGs, namely hyaluronic acid, chondroitin sulfate, keratan sulfate I and II, dermatan sulfate, heparin, and heparan sulfate. Each GAG is formed from two major carbohydrate components which include PHAs. For example, glucuronic acid is one of the two major components of hyaluronic acid, chondroitin sulfate, and heparan sulfate. Iduronic acid is an important component of dermatan sulfate and heparin.

## HYDROXYACIDS: BIOAVAILABILITY AND BIOAVAILABLE CONCENTRATION Stratum Corneum Barrier

In normal human skin, the stratum corneum consists of 14 to 30 layers of corneocytes, including the inner level stratum compactum and the outer level stratum dysjunctum. The keratin-enriched corneocytes in the stratum corneum are embedded in a lipid matrix and are very resistant to penetration by ionic compounds or large molecules with molecular weight greater than 800 to 1000. While undissociated glycolic acid or lactic acid molecules can readily penetrate into the stratum corneum, the ionized glycolate or lactate anions from the metallic salt cannot. Although the active form of a hydroxyacid may be the anions once inside the skin, a topical formulation must contain



a bioavailable form which can penetrate into and through the stratum corneum.

### Partial Neutralization and Buffered Formulation

A topical formulation containing a hydroxyacid without neutralization has a pH below 2. Since the pH of skin surface is approximately 4.2 to 5.6, many commercial products containing glycolic acid or lactic acid are partially neutralized with sodium hydroxide or ammonium hydroxide to pH 3.5 to 4.5, and claim to be buffered formulations. A buffered system is designed to control pH changes of a formulation and does not effectively reduce or eliminate skin irritation without compromising topical efficacy. Therefore, lessened irritation is mainly due to decreased penetration of glycolic acid or lactic acid.

### Efficacy Potential

Cosmetic or therapeutic efficacy of a topical formulation containing a hydroxyacid is proportional to bioavailable concentration of the hydroxyacid in an optimal vehicle (13,14). The bioavailable concentration is obtained by bioavailability multiplied by initial total concentration of the hydroxyacid. The bioavailability is defined as a ratio or fraction of the undissociated hydroxyacid, because only the free acid, not the anion, can substantially penetrate the stratum corneum. Bioavailability decreases sharply when the pH is raised.

### HYDROXYACIDS: OPTIMAL RELEASE FORMULATION Skin Stinging and Irritation

A topical formulation containing a hydroxyacid without partial neutralization usually has pH of below 2. Such formulation, especially with a small molecular AHA, may provoke sensations of tingling, itching, stinging, or irritation when applied to sensitive, atopic, diseased, or inflamed skin. The undesired skin reactions may be due to the lower pH of the formulation, or uncontrolled release and fast penetration of hydroxyacid into the skin. We have found that faster penetration of an AHA is the major factor in causing skin stinging (17).

### Molecular Complex

In an amphoteric system, the control-release mechanism is based on intermolecular attracting forces between a hydroxyacid and an amphoteric substance to form a molecular complex. Amino acids are the best amphoteric substances, and the preferred ones are arginine, lysine, histidine, tryptophan, and ornithine. There are three major attracting forces between a hydroxyacid and an amphoteric substance: ionic/ionic, dipolar/ionic, and dipolar/dipolar (17). The amphoteric formulations are therapeutically effective with minimal or no irritations to the skin.

In a non-amphoteric system, the control-release mechanism is also based on intermolecular attracting forces between a hydroxyacid and a non-amphoteric substance to form a molecular complex. The non-amphoteric substances are multifunctional organic bases such as amino acid esters, amino acid amides, aminocarbohydrates, aminoalcohols, or aminocyclitols. Examples include glycine ethyl ester, glycineamide, arginineamide, lysineamide, ornithineamide, glucosamine, glucamine, meglumine, and streptamine. In contrast to that of an amphoteric system, the main attracting force of a non-amphoteric system is from ionic/ionic force between a hydroxyacid anion and a cation of a non-amphoteric substance such as glycine ethyl ester ammonium ion. The non-amphoteric formulation is also

therapeutically effective with minimal or no irritation to the skin.

### HYDROXYACIDS: TOPICAL ACTIONS Effects on Keratinization

On topical application, hydroxyacids exert a profound effect on desquamation. At low to moderate concentrations, the hydroxyacid, such as glycolic acid 10% cream, on topical application to ichthyotic skin (Figure 6.1) causes initial separation of stratum corneum at lower levels near stratum compactum (3,11). The separation of stratum corneum as a sheet indicates that topical action of the hydroxyacid is not a dispersive keratolysis, such as by salicylic acid. A similar event can also happen to normal skin. For example, DL-mandelic acid 10% cream on topical administration twice daily to normal skin causes sudden separation of stratum corneum as a thin sheet after a few days of application. The skin exposed after the separation of stratum corneum shows light pinkish coloration with a shiny smooth surface. With continued applications such desquamation returns to normal, i.e. cannot be perceived. Because of their marked effects on desquamation, hydroxyacids can be topically effective for various cosmetic objectives.

### Effects on Dermal Components and Skin Thickness

Hydroxyacids at concentrations of 10% to 25% on topical application have been shown to increase biosynthesis of glycosaminoglycans and collagen fibers, and also to improve the quality of photoaged elastic fibers (5,10,18,19). Hydroxyacid 10% to 35% creams topically applied twice daily to one forearm and control cream to the opposite forearm for 1 to 9 months have been found to increase skin thickness very substantially (4,8). The increased skin thickness is mainly due to increased biosynthesis of glycosaminoglycans and collagen fibers as shown by histological analysis (5,19). The degree of increase in skin thickness is quite variable, as shown in Table 6.6, and seems to depend on individual subject and the type of hydroxyacid used. In some cases hydroxyacids have been found to increase skin thickness more than 40%. Although epidermal thickness is also increased, the major part of the increase in thickness is the dermis. The increased skin thickness is not due to edema formation, because it persists for many weeks to months after discontinuation of topical application. Because of these dermal effects and increased skin thickness, hydroxyacids are found to be therapeutically effective for topical treatment of skin changes associated with aging, including wrinkles and photoaging.

In contrast, under the same test conditions salicylic acid at 5% concentration has been found to cause a reduction in skin thickness, as shown in Table 6.7. Forearm skin treated with salicylic acid clinically appeared thinner.

### Peel Solutions and Skin Peeling

Certain AHAs can be used in office procedures as peel solutions for topical treatment of various cosmetic and dermatological indications, including acne, keratoses, warts, wrinkles, and photoaging (20,21). The AHAs adaptable for such include glycolic acid, lactic acid, citric acid, and mandelic acid. In wide use is glycolic acid in 20, 35, 50, and 70% aqueous solutions containing small amount of ethanol and propylene glycol for uniform penetration with pH 1.6, 1.3, 1.2, and 0.6 respectively (7,8). DL-Lactic acid can be used in the same manner, and 90%





**Figure 6.1** Thirteen-year-old girl with lamellar ichthyosis before (a,b) and after (c,d) topical application of 10% glycolic acid in hydrophilic ointment twice daily for 3 weeks.

**Table 6.6** Increased Skin Thickness by Topical Application of Hydroxyacids and Related Compounds\*

Substance	Subject number	Age range (Years)	Duration (Months)	Percentage increase over control
Benzilic acid	2	68–72	2	22–45
Citric acid	13	50–83	5–9	7–55
Glycolic acid	4	58–77	4–8	11–43
Gluconolactone	6	62–81	2–7	7–19
Lactic acid	4	59–70	5–7	17–42
Lactobionic acid	7	49–76	1–3	5–58
Mandelic acid	2	55–62	1	22–27
Methylactic acid	3	65–76	1–3	14–20
Pyruvic acid	4	62–82	2	14–27

\*10%–35% Concentration twice daily on forearm skin.

**Table 6.7** Decreased Skin Thickness by Topical Application Of 5% Salicylic Acid Solution Twice Daily on Forearm Skin

Subject (Age and gender)	Duration (Weeks)	Percentage decrease over control
57F	8	-6
60F	3	-7
59F	6	-8
63F	10	-11
61F	2	-12
60F	5	-14
67F	6	-21
49F	3	-23
73F	7	-32

syrupe liquid having pH 0.5 is commercially available. Citric acid peel can be used as 20, 30, 40, and 50% aqueous solution with pH 1.5, 1.4, 1.3, and 1.2 respectively. DL-mandelic acid 50% in ethanol solution can be used for light desquamatory peeling.

Pyruvic acid, an  $\alpha$ -ketoacid, is the most powerful peeling agent but is unsuitable for clinical use because of its chemical instability. Glycolic acid 70% solution or DL-lactic acid 90% liquid can provoke epidermolysis on the facial skin, generally requiring several minutes of exposure depending on skin type. The clinical sign of epidermolysis is blanching of the skin, which indicates the threshold between superficial peeling and deeper peeling. For new patients it is best to begin with 20% or 35% glycolic acid solution and establish a reaction profile for each subject. In most cases the appearance of erythema is good indication that the skin will be peeled superficially. The peeling process should be terminated by neutralization with sodium bicarbonate solution. The patient may feel mild stinging but degrees of discomfort are mild to moderate and acceptable for the intended end result. Superficial peeling may be repeated at intervals of 2 to 3 weeks or longer to provide beneficial effects in acne, acne-prone skin of younger age, older skin prone to milia and comedones, post-acne scarring, wrinkle-prone skin, keratosis-prone skin, and photoaging skin.

For superficial peeling with milder reactions, glycolic acid 50% in ethanol may be used. Application of this solution to facial skin is associated with rapid onset of a burning sensation and erythema. After 1 minute, the skin is rinsed with sodium bicarbonate solution to relieve the burning sensation. In most cases, the erythema fades within 1 to several hours. Epidermolysis can occur if glycolic acid 50% in ethanol is left on facial skin for several minutes. The erythema may persist into the next day, with denudation of skin and degrees of serous oozing. In some cases, sheet-like separation of stratum corneum occurs 1 day after the procedure, leaving the skin light pink in color and smooth without other overt signs of an unwanted reaction. For most cosmetic procedures, superficial peeling with 50% or 70% glycolic acid is adequate and can be repeated every 2 to 3 weeks. For deeper peeling, glycolic acid 70% in water or 50% in ethanol can be left on the skin for longer periods, and can be so used in the treatment of seborrheic keratoses and actinic keratoses.

### Synergistic Compositions

Associated with the ability of hydroxyacids modulating keratinization and inducing biosynthesis of glycosaminoglycans

and collagen fibers is the capability of these natural and physiological substances to enhance or amplify pharmacologic actions of many topical agents. Such topical agents include corticosteroids, retinoic acid, hydroquinone, diphenhydramine, 5-fluorouracil, and antifungal agents (11,22). The mechanism of this synergistic action is not known. It may be because hydroxyacids disrupt skin barriers and promote better binding between a topical agent and its receptor molecule, resulting in enhanced topical effect. The enhanced therapeutic effects appear not due to an increased penetration of the topical agent into the skin. Hydroxyacids can also reduce or eliminate tachyphylaxis, as well as rebound worsening associated with topical corticosteroids. It has been found that certain side effects associated with topical corticosteroids, such as atrophy, can be reduced or avoided with concomitant use of an AHA (23).

### HYDROXYACIDS: MECHANISMS OF ACTION Specificity of Chemical Structure

Unique biological and biochemical actions of a hydroxyacid depend on specific chemical structure of the molecule, although its receptor molecule(s) in the skin has not been identified. Regarding the three attachment or binding sites, the hydroxyl group must be neutral and not acidic in chemical property, like that in alcohol but not like aromatic phenol, which is slightly acidic. The carboxyl group must be attached to a non-aromatic carbon, preferably an alkyl chain carbon. The amide or ester form is substantially less active than the free acid form. The side chain can be H, alkyl, or aryl, but the one preferred is a short chain. In the case of glycolic acid, the three attachment points or binding sites to a receptor molecule(s) in the skin are hydroxyl, carboxyl, and one of the two hydrogen atoms attached to the alpha carbon. Among related compounds, pyruvic acid is the most active and effective  $\alpha$ -ketoacid. In contrast with the hydroxyacid, the ester form of pyruvic acid, such as methyl pyruvate or ethyl pyruvate, can be topically active. It has been speculated that the ester form is hydrolyzed to free pyruvic acid by an esterase enzyme in the skin.

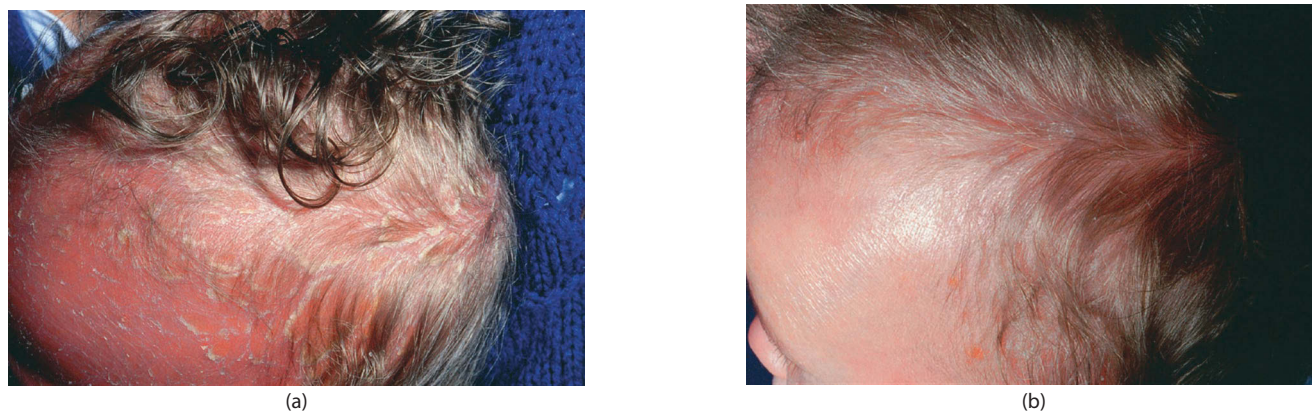
### Biological Action

The precise mechanisms of actions induced by the hydroxyacid are not known. Based on the available laboratory and clinical data, hydroxyacids at different concentrations provide the following actions: diminished corneocyte cohesion at lower level of stratum corneum, near the stratum compactum; a diminished number of desmosomes; reduced epidermal thickness in lamellar ichthyosis (Figure 6.2); increased epidermal and dermal skin thickness in aging skin; increased synthesis of glycosaminoglycans and collagen fibers; and increased activities of dermal dendrocytes (3,5,24).

### Biochemical Action

Biological actions are usually due to or caused by biochemical reactions. In normal stratum corneum, extractable lipids contain by weight approximately 45% to 50% ceramides, 25% cholesterol, 10% to 15% free fatty acids, and less than 5% each of other lipids including cholesterol-3-sulfate, which appears to be involved with cell cohesion in the lower layers of stratum corneum (25–27). The conversion of cholesterol-3-sulfate to cholesterol is required for normal desquamation of stratum corneum in the upper layers (28). It has been shown in X-linked ichthyosis that the skin is deficient in steroid sulfatase enzyme (29). While cholesterol is non-ionic, cholesterol-3-sulfate is an ionic compound which may cause stronger intercorneocyte





**Figure 6.2** Two-year-old girl with lamellar ichthyosis, including scaly skin and erythema, before (a) and after (b) twice daily topical application of 8% gluconolactone in control-release combination formulation for 4 weeks.

binding and cohesion, resulting in retarded desquamation. We might speculate that the hydroxyacid activates steroid sulfatase to enhance hydrolysis of cholesterol-3-sulfate to free cholesterol in the stratum compactum of ichthyotic skin. AHAs such as glycolic acid, lactic acid, and citric acid have been shown to activate factor XIIIa transglutaminase enzyme, tumor necrosis factor- $\alpha$ , and to stimulate mast cells, fibroblast cells, and dermal dendrocytes.

### HYDROXYACIDS: COSMETIC AND DERMATOLOGICAL INDICATIONS

#### Dry Skin and Skin Smoothing

Hydroxyacids can modulate keratinization at the levels of the stratum compactum, and such action is desirable for topical treatment of dry skin conditions. Most cosmetic products for dry skin contain humectants or moisturizers which tend to improve water content or prevent water loss from the stratum corneum. Although lactic acid has been claimed to be a moisturizer, most AHAs are not primary humectants or moisturizers. Rather, they modulate keratinization to normalize or improve the quality of stratum corneum so that water loss is minimized. Most AHAs and BHAs, 4% to 10% cream or lotion, are therapeutically effective for topical treatment or prevention of common dry skin or xerosis (30,31). PHAs and ABAs 5% to 10% concentration have extra benefits for dry skin because of multiple hydroxyl groups in the molecule, which seem to bind water molecules through hydrogen bonding. In addition, these hydroxyacids are gentle to sensitive or inflamed skin without causing skin stinging.

Hydroxyacids such as glycolic acid, lactic acid, and mandelic acid at 10% concentration are therapeutically effective for topical treatment of ichthyosis and other severe dry skin conditions. PHAs and ABAs such as gluconolactone, lactobionic acid, and maltobionic acid 10% to 15%, alone or in combination with other topical agents, are beneficial and soothing for topical treatment of eczema and psoriasis (58). Combination of several hydroxyacids seems to be the best for topical treatment of severe dry skin.

### Acne and Rosacea

All acne lesions involve retention of follicular stratum corneum. Hydroxyacids are therefore therapeutically effective for topical management of acne (32). Topical action of a hydroxyacid at lower concentrations can diminish corneocyte

cohesion and dislodge early comedones from follicular orifices (6). A gel or solution formulation in water, ethanol, propylene glycol 40:40:20 ratio containing 5% to 10% hydroxyacid and applied twice daily is usually quite beneficial for topical treatment or prevention of early acne. The hydroxyacids include glycolic acid, lactic acid, methylactic acid, mandelic acid, and benzilic acid. In general, improvement of acne lesions is discernible within a few weeks of starting topical treatment.

For moderate to severe acne, hydroxyacids at higher skin peeling concentrations can be used to cause epidermolysis to unroof pustules and beneficially modulate follicular epithelium to the level of sebaceous glands (7,33). Glycolic acid 50% or 70% aqueous solution containing small amounts of alcohol and propylene glycol can be effectively used as peeling solution. The solution is applied to acne-involved areas with a cotton ball or suitable brush. The patient will feel a mild to moderate sense of burning as erythema develops over a period of a few minutes. When skin blanching or perifollicular edema is detected, the skin is neutralized with 5% sodium bicarbonate solution to stop further epidermolysis. With this procedure, most pustules will become unroofed. The procedure may be repeated every 2 to 3 weeks. During intervening periods, a low concentration such as 5% to 8% hydroxyacid in gel or solution may be used by the patient once or twice daily to keep follicles opened.

Rosacea is characterized by vascular dilatation with erythema near the center of the face. The cause is unknown, although sunlight may play an important role in the development of rosacea lesions. Rosacea lesions can evolve into telangiectasia with acneiform papules and pustules, and the skin is quite sensitive to many topical agents. Metronidazole 0.75% gel is beneficial for topical treatment of rosacea but has no effect on telangiectasias. Because PHAs and ABAs are antioxidants and gentle to sensitive skin, substances such as gluconolactone, lactobionic acid, and maltobionic acid at 5% to 10% concentrations are beneficial for prophylactic as well as topical treatment of rosacea (34).

### Warts (Verrucae Vulgares)

Hydroxyacids are therapeutically effective for topical treatment of warts caused by human papillomavirus, which induces extreme degrees of hyperkeratosis (6). A rational approach to topical treatment includes removing the hyperkeratotic

“armor,” destroying the tissue harboring the virus, and introducing antiviral agent(s).

Much of the hyperkeratotic plate can be removed by scalpel paring. Thereupon an AHA in 70% solution or gel can be applied under occlusion twice daily by patients for a week or more to cause epidermolysis. In most cases, destruction of wart tissue occurs by epidermolysis and is sufficient to eradicate the virus as well as the lesion. However, combined use of an anti-metabolite such as 5-fluorouracil (5-FU) is more curative, and it can be applied concomitantly (7). For home use by patients, 0.5% 5-FU solution is prepared by dissolving the drug in 70% glycolic acid. The solution is applied twice daily with a cotton applicator to the center of the wart, which is then covered with tape. Applications are discontinued if discomfort occurs and resumed if the wart lesion is not yet resolved. We have found that this treatment usually results in complete resolution of lesions within 3 to 4 weeks.

### Eczema and Pruritus

Eczema may be defined as persistent inflammatory skin lesions with constant or repeated itch. Eczematous disorders can occur at any age and in various forms, such as nummular eczema and lichen simplex chronicus, and is a common skin disease in Asian countries. Eczema may be caused by endogenous and exogenous factors. Pruritus is the main disturbance. Corticosteroids have been used for topical treatment of eczema, and the pruritus diminishes secondarily as the inflammatory process is subdued. In cases of eczema wherein inflammation is not a primary event, topical corticosteroids are not very effective in eradicating the pruritus. Hydroxyacids such PHAs and ABAs incorporated into topical antipruritic formulations can greatly enhance efficacy. For example, addition of gluconolactone, lactobionic acid, and/or maltobionic acid to diphenhydramine topical formulations greatly enhances antipruritic efficacy. The best results are obtained when gluconolactone, lactobionic acid, and/or maltobionic acid are combined with hydrocortisone-17-valerate and diphenhydramine in cream or lotion vehicles.

### Onychomycosis

Onychomycosis is a paramount cosmetic affliction, heretofore difficult to treat. When an AHA is incorporated into a composition containing an antifungal drug, the formulation becomes topically very effective for nail infections. Improvement of fingernail and toenail infections progresses at the rate of nail growth; approximately 1 mm per week for a fingernail and 0.5 mm per week for a great toenail. The improvement rates also suggest that fungal infection of the nail is arrested by topical treatment with synergistic compositions containing both AHA and antifungal drug. Fungal infections of fingernails have been regularly eradicated with up to 6 months of topical treatment with solutions containing 2% clotrimazole and 20% glycolic acid.

### HYDROXYACIDS: INTRINSIC AND EXTRINSIC SKIN AGING

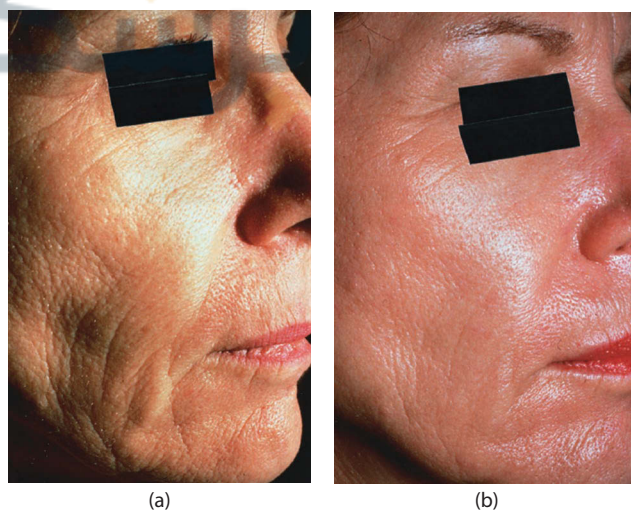
Cutaneous aging is caused by internal and external factors (35–38). Intrinsic aging is a physiological degeneration caused by declining ability and functions inherent with increasing age. Upper arms and buttocks are typical skin areas showing intrinsic aging, where the skin thins and develops fine wrinkles. Daily topical application of glycolic acid, gluconolactone, or lactobionic acid 5% to 10% cream or lotion has been found to be beneficial for prophylactic as well as topical treatment of sun-protected areas of aging skin.

Extrinsic aging is a combination of intrinsic aging and accelerated degeneration caused by ultraviolet (UV) radiation, ionizing radiation, air pollution, wind, cold, heat, dampness, chemicals, smoke, and cigarette smoking. Face and hands are typical skin areas showing extrinsic skin aging. Photoaged skin is rough, dry, mottled, yellowish, leathery, and thickened. It lacks elasticity, has keratoses and pigmented age spots and coarse and deep wrinkles. Aging of the face and the hands in elderly people is due to a combination of intrinsic aging and photoaging. Physiological aging cannot be stopped, but signs of extrinsic cutaneous aging can be modified by topical application of AHAs, PHAs, ABAs or combinations to improve appearance and slow the process (39–42) (Figure 6.3).

### Sunburn Cells

Sunburn cells are dead or dying keratinocytes caused by UV radiation, and are physiologically programmed for cell death (apoptosis). The cells are eosinophilic and appear in the epidermis within 30 minutes, and are maximal at 24 hours after UVB radiation (290–320 nm) (43). The action spectrum appears to be just below 300 nm, and UVA (320–400 nm) radiation does not seem to produce any detectable or significant numbers of sunburn cells in epidermis. The mechanism of action is unknown. It appears that DNA damages have occurred in certain keratinocytes at the lower epidermis after the UVB radiation, and these sunburn cells move rapidly upward through the epidermis into the stratum corneum.

Preliminary study showed that glycolic acid 10%, pH 3.5, on topical application for 12 weeks decreased minimal erythema dose by 18% and increased the number of sunburn cells by twofold, as compared to control group (44). Under the same test conditions, a cosmetic surfactant, sodium lauryl sulfate at 0.5% concentration, increased the number of sunburn cells sixfold. Topical formulations containing an AHA and a sunscreen agent with a sun protection factor (SPF) approximately 3–4 appeared to prevent any increase of sunburn cells (45). Topical formulations containing a PHA, 5% to 10% gluconolactone, without any sunscreen agent have been shown to provide



**Figure 6.3** Fifty-five-year-old woman with photoaged skin, including coarse wrinkles and textural signs of elastosis on her face, before (left) and after (right) 70% glycolic acid peels monthly and home use of twice daily 10% glycolic acid cream for 9 months.



therapeutic effects and protect the skin from any increase in sunburn cells (46).

Nevertheless, regular use of a sunscreen is advisable.

### Actinic Keratoses

Actinic keratosis is a precancerous lesion of keratinocytes, also called solar keratosis, which is caused by photodamage. The lesions are on the sun-exposed areas of skin. Actinic keratosis may progress to squamous cell carcinoma. One conventional treatment is continued topical application of 5-FU for several weeks. A combination of a hydroxyacid and 5-FU can shorten the treatment time and the period of discomfort from several weeks to just 1 week. First, the lesions or sites of actinic keratoses are identified by topical application of 5% 5-FU cream twice daily to affected areas for 5 to 7 days before the office procedure. Once the lesions are identified, 70% glycolic acid in ethanol:propylene glycol 80:20 is applied to the lesions. After 2 to 5 minutes when the lesions begin to blanch, 5% 5-FU solution is applied.

Alternatively, the lesions may be treated with 0.5% to 1% 5-FU dissolved in 30% glycolic acid aqueous solution (7). In most cases, this procedure results in complete eradication of the lesions.

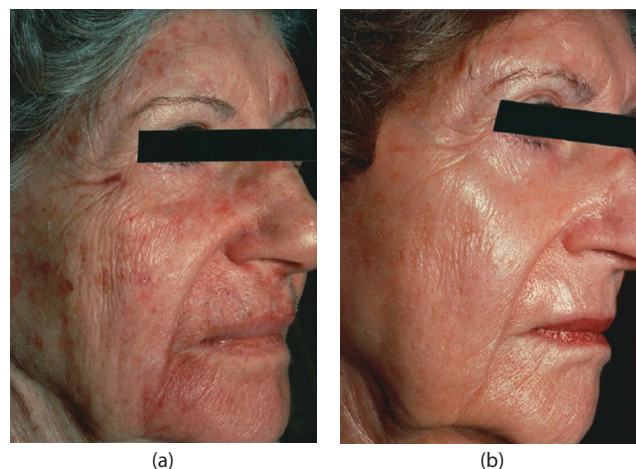
### Age Spot Keratoses

Aging-related macules and papules on the face and the back of the hands are pigmented lentigines, non-pigmented keratosis, and/or seborrheic keratoses. For rapid removal of keratoses, 100% pyruvic acid, 90% lactic acid, or 70% glycolic acid peel solutions may be used as an office procedure (Figure 6.4). After the area is degreased with 70% ethanol, the peel solution is applied with a fine camel-hair brush, and the skin is neutralized with 5% sodium bicarbonate solution when epidermolysis occurs. Among the above peel solutions, 70% glycolic acid is preferred. The skin peel may be repeated after an interval of several weeks to eradicate remaining lesions. Home treatment with a cream or gel containing 10% hydroxyacid with or without 2% hydroquinone may be continued to subdue re-emergence or new lesions. Age spots on the dorsa of the hands and forearms, more resistant to simple topical treatment, may need sustained treatment with 20% or higher concentration of hydroxyacid (7). The addition of hydroquinone seems more effective in the eradication of pigmented skin spots such as lentigines and freckles (Figure 6.5). The time required for their clinical resolution is variable, from a few months to a year or more.

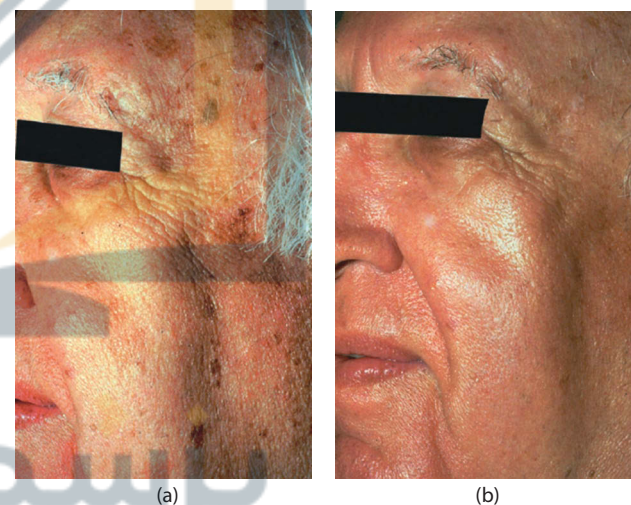
### Wrinkles and Aging Skin

AHAs are therapeutically effective for topical treatment of skin changes associated with aging, because they increase skin thickness by stimulating biosynthesis of GAGs and collagen fibers. An AHA such as glycolic acid at concentration of 10% can be used by a patient at home to treat fine wrinkles on the face (6,7). Substantial improvement may be perceived after several months of treatment. However, the time required for clinical improvement of coarse wrinkles may take years, depending on the degree of severity. Office procedures using 90% lactic acid or 70% glycolic acid peels seem to provide faster resolution of coarse wrinkles, combined with sustained home use of an AHA at or near 10% concentration (8,40,47,57,59).

Five parameters may be used to monitor the progress of therapeutic effects: improvement in dyspigmentation, skin texture, overt fine lines, overt wrinkles, and increased skin thickness. It is essential that "before" and "after" photos be taken for each patient to assess progress.



**Figure 6.4** Eighty-year-old woman with photodamaged skin, including coarse wrinkles and multiple lesions of actinic keratoses on her face, before (left) and after (right) 70% glycolic acid peels and home use of 10% glycolic acid cream for 4 years.



**Figure 6.5** Sixty-three-year-old man with age spots, including multiple lesions of seborrheic keratoses and lentigines on his left face, before (left) and after (right) twice daily topical application of 10% glycolic acid and 2% hydroquinone cream for 21 months.

### Antioxidants and Photoaging

The human body needs oxygen for energy and life itself. Therefore, oxidation is inherent to living. Reactive oxygen species (ROS) are produced by oxidation or peroxidation, and include superoxide, hydrogen peroxide, hydroxyl radicals, and peroxy radicals (48,49). Among these ROS, the hydroxyl radicals are most reactive and cause cellular and tissue stress by reacting with proteins, nucleic acids, lipids, and other biochemical entities. Under quiescent conditions, endogenous or available antioxidants and reductive enzymes in the body are capable of neutralizing these harmful ROS. Known antioxidants and enzymes include vitamin C, vitamin E, reduced  $\alpha$ -lipoic acid, reduced ubiquinones (coenzyme Q), reduced

glutathione (GSH), reduced nicotinamide adenine dinucleotide phosphate (NADPH), and superoxide dismutase, which converts superoxide to hydrogen peroxide and oxygen. Catalase and glutathione peroxidase in turn convert hydrogen peroxide to water and oxygen. Many other antioxidants probably have ancillary or primary roles.

Sunlight is essential for life on earth, but UV radiation is harmful to human skin. Human skin is equipped with antioxidant systems that counteract or dispose of some ROS induced by UVB (50). However, the amount of these endogenous or available antioxidants may not be sufficient to overcome the increased ROS produced by continued exposure to UVB. The skin reactions or damages caused by UVB include erythema, edema, exfoliation, tanning, abnormal thickening (elastosis) or thinning of the epidermis and dermis, and numerous other changes known as photoaging, including carcinogenesis.

Hydroxyacids have been shown to improve the appearance of photoaged skin by improving epidermal renewal and desquamation and by increasing dermal biosynthesis of GAGs and collagen fibers (5,10). Because PHAs and ABAs are antioxidants, they can be used to prevent or counteract ROS induced in the skin by UVB, in conjunction with sunscreens and sunblocks in cream, lotion, or gel form to prevent sun damage (34).

## SIMILARITIES AND DIFFERENCES OF HYDROXYACIDS

The chemical structure of a hydroxyacid determines whether the substance belongs to AHA, BHA, PHA, or ABA, and such classification is also based on its characteristics and usage. Different members of the same group may possess different physicochemical properties, e.g., hydrophilic or lipophilic, but they have similarities in their topical actions with different degrees of potency; these include glycolic acid, lactic acid, and mandelic acid. We have found that BHAs as a group are similar to AHAs in many ways, and the same is true between ABAs and PHAs. However, PHAs and ABAs are functionally different from AHAs and BHAs in certain aspects, as shown in Table 6.8. Because of multiple hydroxyl groups in the molecule, PHAs and ABAs are antioxidants, are gentle to the skin, and they do not increase sunburn cells following UV radiation. Certain members of PHAs, such as glucuronic acid and iduronic acid, are known constituents of GAGs. In the past, AHAs, especially glycolic acid, have been used quite extensively for cosmetic and dermatologic indications. More recently, PHAs

and ABAs, such as gluconolactone and lactobionic acid, are used as unique ingredients in cosmetic products.

## Antagonistic Acetoxyacids

Like some substances with antagonists in nature, hydroxyacids appear to have their own antagonistic counterparts. When the hydroxyl group at the  $\alpha$  position of an AHA is acetylated to an acetoxyl compound, the modulation on keratinization changes to reverse direction, causing hyperkeratinization. The antagonistic action is noticeably pronounced when an aralkyl AHA such as mandelic acid, benzilic acid, or phenyllactic acid is acetylated to O-acetyl-mandelic acid, O-acetyl-benzilic acid, or O-acetyl-phenyllactic acid, respectively. Aralkyl O-acetyl-AHA 5 to 10% creams have been shown to increase thickness and compactness of stratum corneum in hairless mouse and human forearms (3). The hyperkeratotic action of aralkyl O-acetyl-AHAs has been found to be useful and effective for topical treatment of brittle nails, psoriatic nails, and cheilitis caused by oral administration of 13-cis-retinoic acid.

## N-Acetylamino Acids and N-Acetyl Compounds

An amino acid is an organic acid having one or more than one alkaline radical such as amino, guanidino, imino, or hydrazine radical attached at any carbon atom other than carbon one, and NAA is obtained by N-acetylation of the amino group. In fact, the NAA can be considered as the organic acid in which one H is replaced by an acetamino group. There are 20 common amino acids present as L form in natural proteins, and there are also a number of related amino acids with different chemical structures and configurations. These common amino acids and related amino acids can form NAAs and are related N-acetyl compounds. N-Acetylglucosamine, N-acetylgalactosamine, and N-acetylmannosamine are N-acetylated derivatives of aminocarbohydrates glucosamine, galactosamine, and mannosamine, which are organic aldehydes instead of organic acids. Topical actions of N-acetylglucosamine and N-acetylgalactosamine have some similarities to that of NAA, as shown in Table 6.9. We have found that N-acetyl-L-proline and N-acetyl-D-glucosamine at 5% to 10% concentration are topically effective for ichthyosis and also for eradication of itch associated with eczema and xerosis.

Some NAAs and N-acetylaminocarbohydrates occur in nature as metabolites, biopeptides, glycoproteins, or glycosaminoglycans, e.g. N-acetyl-L-glutamic acid in liver (51),

**Table 6.8** Similarities And Differences In Characteristics And Use Of Hydroxyacids

Characteristics/Use	AHAs	BHAs	PHAs	ABAs
Physiological nutrients or natural substances	+	+	+	+
Antioxidants against superoxides, free radicals	*		+	+
Gentle to sensitive skin			+	+
Gel matrix formation/wound-healing				+
Constituents of GAGs			+	
Modulate keratinization, dry skin, acne, keratosis	+	+	+	+
Increase dermal components GAGs, collagen, elastin	+	+	+	+
Reduce wrinkles, photoaging	+	+	+	+
Synergistic effects of corticosteroids, antifungal agents	+	+	+	+

*Abbreviations:* AHAs,  $\alpha$ -hydroxyacids; BHAs,  $\beta$ -hydroxyacids; PHAs, polyhydroxy acids; ABAs, aldobionic acids; GAGs, glycosaminoglycans

\*Polycarboxy AHAs; malic acid, citric acid, tartaric acid are antioxidants

**Table 6.9** N-Acetylamino Acids and N-Acetyl Compounds

Chemical name	Chemical structure	Topical effects	
		Ichthyosis	Itch
N-Acetyl-L-alanine	$\text{CH}_3 \text{CH}(\text{NHCOCH}_3) \text{COOH}$		
N $\alpha$ -Acetyl-L-arginine	$\text{H}_2\text{NC}(=\text{NH})\text{NH}(\text{CH}_2)_3 \text{CH}(\text{NHCOCH}_3) \text{COOH}$		
N-Acetyl-L-aspartic acid	$\text{HOOC CH}_2 \text{CH}(\text{NHCOCH}_3) \text{COOH}$		
N-Acetyl-DL-asparagine	$\text{H}_2\text{NOC CH}_2 \text{CH}(\text{NHCOCH}_3) \text{COOH}$	4+	
N-Acetyl-L-cysteine	$\text{HSCH}_2 \text{CH}(\text{NHCOCH}_3) \text{COOH}$	3+	
N-Acetyl-glycine	$\text{CH}_2(\text{NHCOCH}_3) \text{COOH}$	3+	
N-Acetyl-L-glutamic acid	$\text{HOOC CH}_2 \text{CH}_2 \text{CH}(\text{NHCOCH}_3) \text{COOH}$	4+	
N-Acetyl-L-glutamine	$\text{H}_2\text{NOC CH}_2 \text{CH}_2 \text{CH}(\text{NHCOCH}_3) \text{COOH}$	4+	
N-Acetyl-L-histidine	$\text{C}_3\text{H}_3\text{N}_2 \text{CH}_2 \text{CH}(\text{NHCOCH}_3) \text{COOH}$		
N-Acetyl-L-isoleucine	$\text{CH}_3 \text{CH}_2 \text{CH}(\text{CH}_3) \text{CH}(\text{NHCOCH}_3) \text{COOH}$		
N-Acetyl-L-leucine	$(\text{H}_3\text{C})_2\text{CH CH}_2 \text{CH}(\text{NHCOCH}_3) \text{COOH}$		
N $\alpha$ -Acetyl-L-lysine	$\text{H}_2\text{NCH}_2 (\text{CH}_2)_3 \text{CH}(\text{NHCOCH}_3) \text{COOH}$	4+	
N-Acetyl-L-methionine	$(\text{H}_3\text{C})\text{SCH}_2 \text{CH}_2 \text{CH}(\text{NHCOCH}_3) \text{COOH}$		
N-Acetyl-L-phenylalanine	$\text{C}_6\text{H}_5 \text{CH}_2 \text{CH}(\text{NHCOCH}_3) \text{COOH}$		
N-Acetyl-L-proline	$\text{N}(\text{COCH}_3)\text{C}_4\text{H}_7 \text{COOH}$	4+ 4+	
N-Acetyl-L-serine	$\text{HOCH}_2 \text{CH}_2(\text{NHCOCH}_3) \text{COOH}$		
N-Acetyl-L-threonine	$\text{H}_3\text{C CHOCH CH}(\text{NHCOCH}_3) \text{COOH}$		
N-Acetyl-L-tryptophan	$\text{C}_8\text{H}_6\text{N CH}_2 \text{CH}(\text{NHCOCH}_3) \text{COOH}$		
N-Acetyl-L-tyrosine	$\text{HOC}_6\text{H}_4 \text{CH}_2 \text{CH}(\text{NHCOCH}_3) \text{COOH}$	4+	
N-Acetyl-L-tyrosinamide	$\text{HOC}_6\text{H}_4 \text{CH}_2 \text{CH}(\text{NHCOCH}_3) \text{CONH}_2$	4+	
N-Acetyl-L-valine	$(\text{H}_3\text{C})_2\text{CH CH}(\text{NHCOCH}_3) \text{COOH}$		
N-Acetyl- $\beta$ -alanine	$\text{CH}_2(\text{NHCOCH}_3) \text{CH}_2 \text{COOH}$	4+	
N-Acetyl- $\gamma$ -aminobutanoic acid	$\text{CH}_2(\text{NHCOCH}_3) \text{CH}_2 \text{CH}_2 \text{COOH}$	3+	
N $\alpha$ -Acetyl-L-ornithine	$\text{H}_2\text{NCH}_2 (\text{CH}_2)_2 \text{CH}(\text{NHCOCH}_3) \text{COOH}$		
N-Acetyl-L-citrulline	$\text{H}_2\text{NCONH}(\text{CH}_2)_3 \text{CH}(\text{NHCOCH}_3) \text{COOH}$		
N-Acetyl-creatine	$\text{H}_2\text{NC}(=\text{NCOCH}_3)\text{N}(\text{CH}_3) \text{CH}_2 \text{COOH}$		
N-Acetyl-creatinine	$-\text{HNC}(=\text{NCOCH}_3)\text{N}(\text{CH}_3) \text{CH}_2 \text{CO}-$		
N-Acetyl-phenylglycine	$\text{C}_6\text{H}_5 \text{CH}(\text{NHCOCH}_3) \text{COOH}$		
N-Acetyl-4-hydroxyphenylglycine	$\text{HOC}_6\text{H}_4 \text{CH}(\text{NHCOCH}_3) \text{COOH}$	3+	
N-Acetyl-D-glucosamine	$\text{HOH}_2\text{C} (\text{CHOH})_3 \text{CH}(\text{NHCOCH}_3) \text{CHO}$	4+ 4+	
N-Acetyl-D-galactosamine	$\text{HOH}_2\text{C} (\text{CHOH})_3 \text{CH}(\text{NHCOCH}_3) \text{CHO}$	4+	

Ichthyosis: 3+, 75%; 4+, 100% improvement  
 Itch: 4+, eradicate itch completely for 8 hours

N-acetyl-L-aspartic acid in brain (52), N-acetyl-L-serine in melanocyte-stimulating hormone ( $\alpha$ -MSH) (53), N-acetyl-L-tyrosine in N-acetyl- $\beta$ -endorphin (53), N-acetyl-D-glucosamine in hyaluronic acid and keratan sulfate (54), and N-acetyl-D-galactosamine in chondroitin sulfate and dermatan sulfate (54).

NAC is a potent antioxidant against free radicals such as hydroxyl radical (55) and is a good precursor for glutathione synthesis in the body. NAC is used as a mucolytic, detoxifying, and antiviral agent. We have found that NAC 8 % in hydrophilic ointment is topically effective for ichthyosis (Figure 6.6).

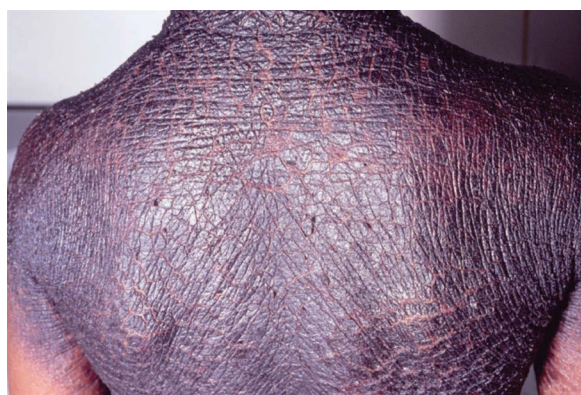
An amino acid can be in amide or hydrazide form, e.g. N-acetylaminoamide and N,N'-diacetylaminohydrazide. We have found that N-acetyl-L-tyrosinamide and N,N'-diacetyl-L-tyrosinhydrazide in oil-in-water emulsion on topical application to normal human skin can stimulate biosynthesis of hyaluronic acid, and increase skin thickness.

### N-Acylpeptides

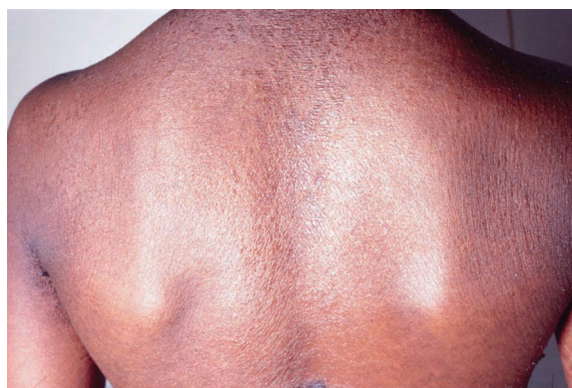
NAP is an acylated peptide derivative. A peptide is formed from two or more amino acids by a covalent amide bond,  $\text{C}(=\text{O})\text{NH}$ , when the carboxyl group on one amino acid reacts with the amino group of the other amino acid in a dehydration reaction. A dipeptide is formed from two amino acids, a tripeptide is formed from three amino acids, and a polypeptide is formed from multiple amino acids. The 20 common amino acids are represented by chemical names, such as "glycine," or abbreviated symbols such as three letters, "Gly," or one letter, "G." Except for glycine, all other common amino acids have stereoisomers, i.e., enantiomer, D, or L form. The amino acids in most natural peptides and proteins are all in L-form.

The three-letter symbols used for the 20 common amino acids are as follows: alanine (Ala), arginine (Arg), aspartic acid (Asp), asparagine (Asn), cysteine (Cys), glycine (Gly), glutamic acid (Glu), glutamine (Gln), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe),





(a)



(b)

**Figure 6.6** Six-year-old boy with lamellar ichthyosis before (a) and after (b) topical application of 8% N-acetyl-L-cysteine in hydrophilic ointment twice daily for 4 weeks.

proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val).

Common acyl groups are acetyl (Ac) and propanoyl (Pa) radicals, such as N-acetyldipeptide and N-propanoyldipeptide. The NAP derivatives can have different forms at carboxyl terminal group such as free acid (OH), amide (NH<sub>2</sub>), ethyl ester (OEt), hydrazide (NHNH<sub>2</sub>), and N-acetyl hydrazide (NHNHAc).

NAP derivatives at concentrations of 0.5% to 10% on topical application have been found to increase skin thickness. For example, N-acetyldipeptide amide, N-Ac-L-Tyr-L-Tyr-NH<sub>2</sub>, at 3% increased skin thickness 15% to 20% after 4 weeks; N-acetyltri-peptide amide, N-Ac-L-Tyr-L-Tyr-L-Tyr-NH<sub>2</sub>, at 0.5% increased skin thickness 5% to 10% after 7 days; N-acetyltri-peptide ethyl ester, N-Ac-L-Tyr-L-Tyr-L-Tyr-OEt, at 0.5% increased skin thickness 20% to 30% after 10 days. The increased skin thickness or plump was not due to increased water retention or edema of the skin, because the thickness was maintained for many months after discontinuation of the treatment. As shown earlier (5) increased skin thickness was due to increased biosynthesis of GAGs and collagen fibers, improved quality of elastic fibers, less clumping of melanin, and lighter appearance of age spots. Therefore, increased skin thickness is expected to improve aging-related skin changes, including fine lines, wrinkles, photoaging, age spots, blotches, hyperpigmented skin, mottled skin, and can be used for younger-looking skin and skin lightening (Table 6.10).

NAP derivatives at concentrations of 0.5% to 5% on topical application have been found to eradicate itch within a few minutes and improve eczema lesions within a few weeks with continuing topical application (Figure 6.7).

## CONCLUSION, DISCUSSION, AND PERSPECTIVES

Organic acids cover various organic compounds which include retinoic acid, salicylic acid,  $\alpha$ -hydroxyacids (AHAs), polyhydroxy acids (PHAs), aldobionic acids (ABAs), N-acetyl amino acids (NAAs), and N-Acylpeptides (NAPs). On topical administration, these organic acids exert distinctive pharmacological actions on keratinization and/or biosynthesis of dermal components; i.e. glycosaminoglycans, collagen, and elastic fibers. However, these organic acids also have certain similarities and

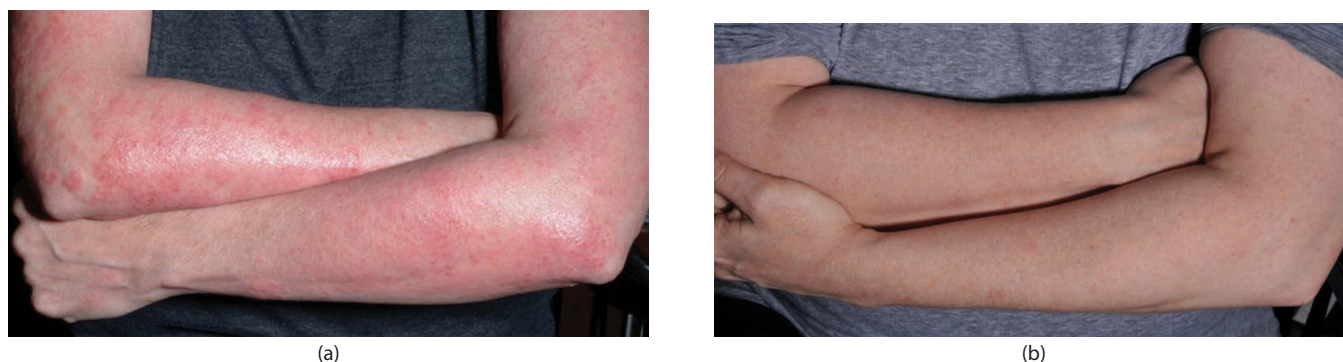
differences in topical actions. Both salicylic acid and glycolic acid have similar beneficial effects on disturbed keratinization, e.g. acne, ichthyosis, calluses, etc. Glycolic acid increases

**Table 6.10** N-Acylpeptide Derivatives

N-acylpeptide derivatives	Aging related skin changes	Itch, eczema
N-Ac-L-Ile-L-Ala-NH <sub>2</sub>	2+	4+
N-Ac-L-leu-L-Ala-NH <sub>2</sub>	3+	4+
N-Ac-L-leu-L-Ala-OH	2+	3+
N-Ac-L-Val-L-Ala-NH <sub>2</sub>	4+	4+
N-Ac-L-Val-L-Ala-OH	2+	4+
N-Pa-L-Val-L-Ala-OH	2+	4+
N-Ac-L-Cys-L-Cys-NH <sub>2</sub>	3+	2+
N-Ac-L-Cys-L-Cys-OH	3+	2+
N-Ac-L-Ile-Gly-NH <sub>2</sub>	3+	4+
N-Ac-L-Ile-Gly-OH	2+	4+
N-Ac-L-leu-Gly-NH <sub>2</sub>	3+	4+
N-Ac-L-leu-Gly-OH	2+	3+
N-Pa-L-leu-Gly-OH	2+	3+
N-Ac-L-Pro-Gly-NH <sub>2</sub>	2+	4+
N-Ac-L-Val-Gly-NH <sub>2</sub>	2+	4+
N-Ac-L-Val-Gly-OH	2+	4+
N-Ac-L-Ala-L-Ile-NH <sub>2</sub>	2+	3+
N-Ac-L-Ile-L-Ile-NH <sub>2</sub>	3+	4+
N-Ac-L-Cys-L-Tyr-NH <sub>2</sub>	2+	3+
N-Ac-L-Tyr-L-Tyr-NH <sub>2</sub>	4+	4+
N-Ac-L-Tyr-L-Tyr-OH	4+	4+
N-Ac-L-Tyr-L-Tyr-NHNH <sub>2</sub>	4+	2+
N-Ac-L-Tyr-L-Tyr-NHNHAc	4+	4+
N-Ac-L-Tyr-L-Tyr-L-Tyr-OH	2+	3+
N-Ac-L-Tyr-L-Tyr-L-Tyr-OEt	3+	4+
N-Ac-L-Tyr-L-Tyr-L-Tyr-NH <sub>2</sub>	4+	4+
N-Ac-L-Val-L-Val-L-Ala-NH <sub>2</sub>	2+	3+
N-Ac-L-Tyr-L-Val-L-Tyr-NH <sub>2</sub>	3+	4+

*Note:* Aging skin including age spots; itch and eczema: 2+: 50% efficacy; 3+: 75% efficacy; 4+: 95%–100% efficacy





**Figure 6.7** Forty-three-year-old woman with chronic eczema on both arms, before (a) and after (b) N-Ac-L-Val-L-Ala-NH<sub>2</sub> 0.5% in oil-in-water emulsion applied twice daily for 4 weeks.

while salicylic acid diminishes the biosynthesis of dermal components, i.e. glycolic acid but not salicylic acid is beneficial for wrinkles and aging skin. NAAs and NAPs have similar beneficial effects on disturbed keratinization, e.g. ichthyosis, and hyperkeratoses.

N-Acetyl-L-tyrosinamide is more effective than N-acetyl-L-tyrosine for increasing the skin thickness by stimulation of hyaluronic acid biosynthesis. N-Acetyl-L-glutamic acid diethyl ester is more effective than N-acetyl-L-glutamic acid for topical treatment of ichthyosis. Based on these observations, the active form of the NAA or NAP may not be in free acid or anion form.

Since 1974, many studies have shown that physiologic and nontoxic hydroxyacids can promote normal keratinization and increase synthesis of dermal components, including hyaluronic acid and collagen fibers. AHAs, specifically glycolic acid, have been widely used in cosmetic products and dermatologic practice for topical treatment of dry skin, acne, keratoses, wrinkles, photoaging, and photodamaged skin. Because of multiple hydroxyl groups in the molecules, gluconolactone (a PHA lactone) and lactobionic acid (an ABA) are gentle to the skin and are antioxidants.

Topical effects of most organic acids appear to be from the free acid form, and the amide or ester form seems ineffective or much less effective, e.g. retinoic acid, glycolic acid, salicylic acid. On the contrary, for NAAs and NAPs, the amide or ester form appears more effective for topical treatment of itch, eczema, and aging-related skin changes. N-Acetyl-L-proline ethyl ester appears more effective than N-acetyl-L-proline for topical treatment of itch associated with eczema.

The mechanisms for itch eradication by NAAs or NAPs are unknown. Acetylcholine, by injection into lesions, has been reported as a pruritogen to induce itch in eczema patients (60). Based on chemical structures, acetylcholine has O-acetyl radical, whereas NAAs and NAPs have N-acetyl or N-propanoyl radicals. Therefore, we can speculate that the anti-itch effect is due to the interference of acetylcholine action on free nerve endings in the epidermis by NAAs and NAPs. The relative potency of anti-itch, and the associated improvement of eczema lesions by NAAs and NAPs, may be due to relative affinity of the binding to receptor molecules. For example, N-Ac-L-Tyr-L-Tyr-NH<sub>2</sub> is more potent than N-Ac-L-Tyr-L-Tyr-OH in eradicating itch and improving eczema lesions.

We are fortunate at this time of general awareness and concern over aging, photoaging, photodamage, and environmental and disease impingements disfiguring the integument

that an entire category of physiologic and nontoxic substances can be drawn upon to repair or prevent such disfigurements. The hydroxyacids and other organic acids can be utilized to nudge skin form and function toward a more youthful state. They can be topically applied to decelerate the otherwise inexorable progression toward old-looking integument.

The antioxidant PHAs and ABAs are especially beneficial and can be utilized to repair and prevent damage caused by UV radiation. However, these latter substances are still new on the scene, and expanding use is projected. Dermatologists, cosmetologists, pharmacologists, pharmacists, and formulators need to be closely familiar with chemical attributes of the various hydroxyacids and other organic acids including PHAs and ABAs, their clinical performance, and how best to design and compound formulations to provide maximal achievement of intended objectives.

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# Retinyl Propionate and Related Retinoids

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## INTRODUCTION

Retinoids represent a class of lipophilic compounds that are comprised of natural derivatives of vitamin A (retinol) as well as synthetic analogues. Naturally-occurring retinoids are sourced via dietary intake of beta-carotene as well as retinyl esters, both of which are found in a broad range of foodstuffs and animal by-products. Structurally, beta-carotene exists essentially as a dimer form of retinol and the enzymatic conversion in the intestinal tract via retinal production is the initial sourcing of retinoids for usage by the human body. Most eukaryotic cells have the capability of enzymatically converting retinol and retinyl esters to various metabolites that are critical in maintaining cellular homeostasis, regulating proliferation and differentiation patterns, as well as embryonic development. Of the metabolites generated, the true “business end” of retinoids is retinoic acid, which is present in cells as various *cis* conformations as well as *trans*-retinoic acid (tRA). Retinoids play such a critical role in developmental biology and the metabolic pathways are tightly regulated. This includes not only enzymatic processes but transport via cellular retinoid binding proteins as well.

Topical usage of potent forms of natural and synthetic retinoids such as tRA has shown a high degree of efficacy against acne, psoriasis, ichthyosis, and actinic keratosis. Relative to photodamaged skin, tRA has clearly been established as having a robust effect (1,2) and is currently marketed as a prescription drug. These retinoid effects in diseased skin can be ascribed on some level as being a normalization of altered skin conditions. However, two of the key negatives associated with highly potent topical retinoids are irritation that, in some instances, does not mitigate itself completely even after long term chronic exposure and teratogenic side effects.

Less potent topical retinoids such as retinol and various retinyl esters (Figure 7.1) have been building a rich history of usage in the cosmetic marketplace. More important for the cosmetic marketplace is the ability of retinoids to positively impact changes in the appearance of photodamaged facial skin, particularly for fine lines, wrinkles, and pigmentation-related changes. Additionally, understanding the basic biochemical metabolic processes that control endogenous retinoid levels and synthesis has shown that cosmetic-type retinoids present an attractive compromise for less serious conditions such as photodamaged skin. This chapter focuses on cutaneously delivered retinyl esters. The topics covered include an overview of the metabolism, biochemistry, and molecular biology as well as human study findings. The principle focus in terms of treatment effects will be upon photodamaged skin.

## Biochemistry and Molecular Biology of Topical Retinol and Retinyl Esters

Of most relevance to the cosmetic industry was the confirmation that cosmetic forms of retinoids have the potential to be enzymatically converted to various retinoid metabolites including tRA when topically administered (3–8). The biochemical conversions are shown schematically in Figure 7.2a, where retinol is oxidized via small chain alcohol dehydrogenases to retinal and in turn to form tRA (5,9). This process is begun when free retinol associates with a specific cytoplasmic retinol-binding protein (CRBP). This family of proteins with high retinoid specificity includes CRBP as well as retinoic acid binding protein (CRABP), of which there are two isoforms, I and II (7). These binding proteins play a direct role in regulation of retinoid responses in cells by acting as chaperones in enzymatic conversions, sequestration, and stabilization. The retinol-CRBP complex is a substrate for retinol dehydrogenase, a microsomal enzyme uniquely capable of catalyzing the conversion of retinol to retinaldehyde. Retinaldehyde is then rapidly and quantitatively oxidized to retinoic acid by retinaldehyde oxidase (5,10). However, the primary reaction that occurs with CRBP-bound retinol is esterification of retinol via lecithin:retinol acyltransferase (LRAT) or acyl CoA:retinol acyltransferase (ARAT) to retinyl esters, the primary storage form in lipid bilayers and of which retinyl palmitate is the major species in human skin (11,12). Recent work has identified the neutral lipid synthesis enzyme acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) as a novel ARAT enzyme present in murine skin that regulates retinol oxidation to retinoic acid (13). Additionally, retinyl stearate has been identified as the major form that is present in circulating serum in mammalian systems (14). This multi-step processing of retinyl esters serves as a point of regulation to control the level of active retinoid in the skin and may thus contribute to the lower irritation potential of these derivatives.

The molecular mechanistic route that tRA follows in skin is via its function as an agonist for binding to a family of nuclear receptors called the retinoic acid receptors (RAR) and which also includes the retinoid X receptors (RXR). In each of these two families, three isoforms exist and interact to form heterodimers as the functionally active form. Significant research has gone into understanding these various dimer combinations, including agonist binding as related to various tissues and cells. Upon binding of tRA by RAR and dimerization with a member of RXR, this active complex becomes a transcriptional regulator that binds to select sequences in promoter regions of select genes, termed retinoic acid responsive elements, or RARE (15).

As with most biological processes that involve highly potent agents, the retinoid pathway has several regulatory



feedback loops that help maintain an optimal level of tRA. One of the most important ones is depicted in Figure 7.2b, which shows the degradation of tRA to the less active metabolites via hydroxylation to 4-hydroxy-retinoic acid and 4-oxo-retinoic acid, catalyzed by members of the CYP26 family (16). As tRA levels become temporarily elevated, increased hydroxylase activity via higher expression levels leads to a decrease to steady state levels.

In summary, the biochemical confirmation that topical retinol and retinyl esters can be metabolized to the more active form of tRA in skin cells has provided a richer understanding

of how cosmetic retinoids are capable of having subtle effects upon photodamaged skin. As we begin to understand deeper the molecular biology changes that are caused via tRA binding to RAR/RXR, novel mechanistic insights into the efficacy as well as the negative irritation side effects could allow for an improvement in current commercialized products.

### Potential to Break the Retinoid Efficacy to Irritation Correlation with Retinyl Esters

Over the past few decades, retinol has been used extensively in cosmetic products, with the levels of retinol ranging from barely detectable to upwards of 0.5%. The relative dosage levels vary in part due to intolerance among consumers of irritation side effects that is connected in part by a sensitivity to formulation differences. One approach to reduce retinoid-induced inflammation is to attempt to regulate skin penetration and delivery rates into the skin via usage of nanoparticles and sponges (17). It is unclear yet whether this provides an advantage versus merely reducing delivery loads and reducing efficacy as well. As highlighted above, it has been generally assumed that any efficacy from topically delivered retinol occurs via its sequential conversion via the intermediate retinal to tRA (8). For example, it has been shown that 0.4% retinol can significantly improve the appearance of wrinkles on the surface of skin but histological changes have also been measured in the extracellular matrix components of epidermal glycosaminoglycans and dermal collagen Type I (18). The authors also speculated that retinol-treated skin would be more resistant to future skin injury, including ulcer formation, among elderly consumers. These reported effects are hallmark responses associated with topical tRA.

Several natural and synthetic esters of retinol have been evaluated for retinoid-like effects, including acetate, propionate, palmitate, ascorbate, beta-glucuronide, and retinyl

Retinoid	Structure
retinol	
retinyl acetate	
retinyl propionate	
retinyl palmitate	

Figure 7.1 Chemical structures of retinol and retinyl esters.

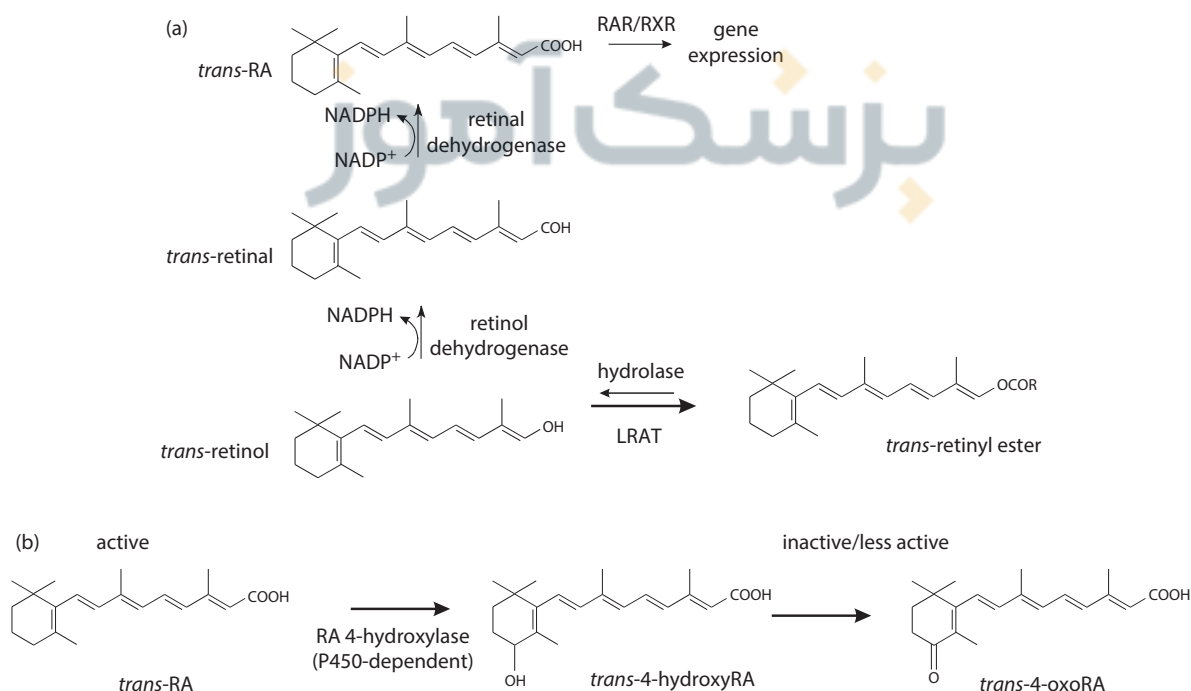


Figure 7.2 Retinoid metabolic pathways in skin.



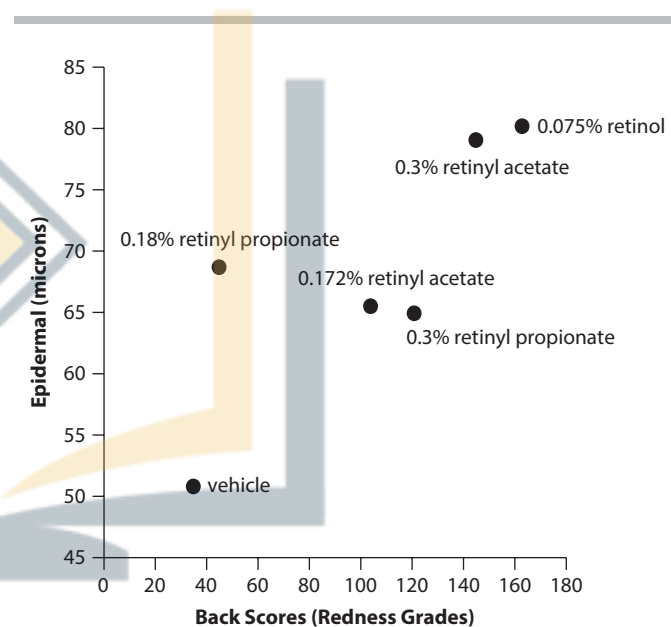
N-formyl aspartamate. While the chemical structure of retinol is conducive to being esterified via the alcohol group, the ability for retinyl esters to deliver efficacy that approaches retinol becomes limited by variables such as skin penetration, cellular uptake, hydrolase active site binding, re-esterification via acyl transferases, and general pharmacokinetics. As with most retinoid analogue work, the objective of evaluating retinyl esters is to be able to provide a retinoid-like response on facial skin but without the negatives of irritation associated with topical usage.

Since retinyl esters are dependent upon an additional biochemical processing step before releasing a free form of retinol (Figure 7.2a), it is anticipated that topical retinyl esters would have a weaker retinoid responsiveness range when compared with retinol. A human forearm biopsy study was performed that evaluated via histology the effect of tRA, retinol, and three retinyl esters (acetate, propionate, and palmitate) for the effects upon changes in epidermal and granular layer thickness compared to a placebo control (Table 7.1). Both retinyl acetate and retinyl propionate were able to show a significant increase in both thickness measures as compared to the placebo control. The results with retinyl propionate are similar to what has been previously reported (19). These results also highlight that there is a relative rank ordering based on the biochemical processing steps amongst the retinoids tested. In comparing the retinyl esters, there also appears a ranking in terms of chain length from shortest to longest, with shorter chained esters being more potent than longer chained esters. While less intuitive, the potential that shorter chained retinyl esters can be acted upon by other esterases could partially explain their enhanced retinoid response profile compared with retinyl palmitate, the endogenous storage form. Since the experimental data in the table was performed under semi-occlusive patch, the ability of the retinoids to penetrate into the viable cell layers is lessened as a variable for this comparison but may not explain the complete story for retinyl palmitate (20).

As would be suspected, this rank ordering of retinoid-based activity (efficacy) is also indirectly proportional to the level of irritation that some topical retinoids can induce on skin (20, Oblong, unpublished data). A comparison of human retinoid activity data from Table 7.1 with irritation data from a human back cumulative irritation protocol using nearly identical formulations, one can see that there is a general correlation among the esters and retinol between retinoid activity and

irritation with the one exception of retinyl propionate at 0.18% levels (Figure 7.3). This supports in-house clinical findings that retinyl propionate does not cause as much irritation as retinol and still is able to positively impact the appearance of fine lines and wrinkles in 12-week human facial studies (21). While additional data would be required around the doses tested to confirm, these results support the suggestion that retinyl propionate is capable of eliciting a retinoid response in human skin but with a weaker overall retinoid irritation profile.

In terms of application to photodamaged skin, retinyl propionate has recently become of interest since it is capable of affecting human skin much like retinol but with a lower irritation profile than other retinoids (21,22, and Figure 7.3). While the relative activity of retinyl propionate on photodamaged skin is weaker than retinol (23), it is clear that some of the effects are still significant (21,22). More recently, a retinyl



**Figure 7.3** Correlation between human irritation measures and epidermal thickening for retinyl acetate, retinyl propionate, and retinol.

**Table 7.1** Changes in Epidermal and Granular Layer Thicknesses in Forearm Skin due to Retinoid Treatment

Topical treatment	Code	Epidermal thickness (microns)	Significance vs. control and other treatments*	Granular layer thickness (microns)	Significance vs. control and other treatments*
Emulsion control	A	50.75	—	3.03	—
1.0% retinyl palmitate	B	52.07	ns	4.88	ns
0.172% retinyl acetate	C	65.45	ABGHI	10.73	ABH
0.30% retinyl acetate	D	79.00	ABCeF	12.34	ABH
0.18% retinyl propionate	E	68.83	ABGHI	10.61	ABH
0.30% retinyl propionate	F	64.87	ABGHI	10.73	ABH
0.075% retinol	G	80.13	ABCEF	14.06	AB
0.15% retinol	H	85.66	ABCEF	17.44	ABCDE
0.025% <i>t</i> -retinoic acid	I	80.64	ABCEF	14.72	AB

\*Upper case letters denote significant differences ( $p < 0.05$ ), while lower case letters denote directional differences ( $p < 0.10$ ); ns = not significant.

propionate formulation that also contained niacinamide and collagen fragment peptides showed an equivalent level of efficacy compared with 0.02% tRA (24). This combination of significant efficacy with an overall lower irritation profile has been noted in cross-comparison of human biopsy studies, back cumulative irritation, and human facial skin clinicals (21). While less critical than its overall efficacy and irritation profile, retinyl propionate has been reported to have a better chemical stability profile compared to other esters, thereby increasing half-life upon skin during topical delivery (25).

The more potent ester retinyl acetate has been shown in *in vivo* models as being able to induce a retinoid-like response as measured by epidermal thickening as well as indirect markers of epidermal proliferation (26). Additionally, the kinetics of the accommodation response to retinyl acetate as measured by erythema has a similar time curve over a 20-day period as has been observed in human studies for accommodation to retinol (Oblong, unpublished results). Since retinyl acetate has a similar pharmacologic activity and irritation profile as retinol, there appears to be no advantage of this ester over retinol in cosmetic products.

Of the retinyl esters currently practiced in the cosmetic marketplace, retinyl palmitate appears to be one of the weaker retinoids in terms of efficacy for generating a retinoid response in human skin, including effects on photodamaged skin. This is more than likely due to the primary storage role of endogenous retinyl palmitate, which is a key regulatory point. Although some level of topically applied retinyl palmitate can be converted to retinol, the small amount of retinyl palmitate that actually penetrates the skin would be expected to become accumulated into endogenous storage pools (4). Based on published information and historical cosmetic usage, it is accepted that retinyl palmitate has at best an overall weak activity profile and is non-irritating (20). Evaluation of epidermal and granular layer thickening also shows the weak retinoid effect in human biopsy studies (Table 7.1).

Finally, it has been observed that there may be a plateau of responsiveness among retinyl esters as observed in various retinoid-sensitive models (Oblong, unpublished data). This suggests a threshold level in which excess retinol derived from shorter chain retinyl esters could be competitively targeted by acyl transferases over retinol dehydrogenases and incorporated into retinyl palmitate storage pools. Thus, the ability to deliver greater efficacy from retinyl esters in general may become dependent more upon optimal R-groups or combination therapies rather than attempting to elevate the topical dose used. Examples include the published clinical findings that showed the photostable ester retinyl N-formyl aspartate was able to deliver retinoid-like effects on photodamaged skin without significant irritation (27).

## SUMMARY

Retinoids are a broad family of molecules that can be metabolized via endogenous enzymatic pathways to generate agonists for members of the RAR and RXR nuclear receptor family. In turn, this denotes their critical role in regulating gene expression profiles via RAR/RXR binding to RAREs. In dermatology, prescription forms of natural and synthetic retinoids have been shown to have beneficial effects upon acne, psoriasis, ichthyosis, actinic keratosis, and photodamaged/aging skin attributes. Relative to photodamaged skin, the cosmetic industry has been using retinol and various retinyl esters for several decades. This class of molecules continues to be one of the more efficacious materials available to consumers to help

combat photoaged skin appearance. While the overall efficacy provided by these cosmetic forms of retinoid are generally less potent than the prescription forms such as tRA, the knowledge that these forms can be metabolized to more active forms supports the indirect mechanism of action that they utilize. Of the retinyl esters currently practiced in the marketplace, there appear to be advantages for the usage of retinyl propionate over retinyl acetate in terms of having a similar efficacy response range but with an improved irritation profile. In contrast, retinyl palmitate has a very low efficacy response range, rendering its low irritation profile as moot in terms of being able to have any positive benefits for affecting the appearance of photodamaged skin.

Future research in better understanding the connections between efficacy and retinoid-induced irritation should allow for the identification of novel retinyl ester that decouple these two phenomena. The results presented with retinyl propionate suggest the potential exists. Additionally, there would appear to be an opportunity to identify a final solution that combines optimized levels, formulations, and potentially additional materials to maintain or elevate retinoid-like efficacy but with a reduced overall irritation profile.

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# Idebenone (Hydroxydecyl Ubiquinone)

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## INTRODUCTION

Idebenone is a unique new antioxidant in dermatology used to treat the visible and cellular signs of skin aging. This chapter reviews the description, uses, functionality, cell biological and clinical research, safety, and new improvements of this respiratory chain analog of coenzyme Q10.

## DESCRIPTION

Idebenone is a bioengineered analog, but not a derivative of ubiquinone (coenzyme Q10). It belongs in the family of molecules known as quinones; in particular it is a 1,4-benzoquinone molecule, as is hydroquinone. Its molecular weight is 338, compared with coenzyme Q10 with a molecular weight of 860, and therefore in theory has much greater skin penetration potential. Idebenone is a potent antioxidant, with the ability to operate under low oxygen tension situations, and not showing prooxidative metabolites (1,2). Because of its ability to inhibit lipid peroxidation, idebenone protects cell membranes and mitochondria from oxidative damage caused by free radicals generated during cell metabolism, processes which are also summarized as intrinsic aging (2–4). Ultraviolet radiation (UVR)-generated reactive oxygen species (ROS) play a critical role in the process of photocarcinogenesis and environmentally induced aging, also termed as photoaging or extrinsic aging (5–9). Some studies claim that infrared radiation and visible light also contribute to a major extend to such kind of damage (10,11). Although the skin possesses a complex antioxidant network, both intrinsic and extrinsic pathways can provoke an imbalance of the fragile prooxidant-antioxidant equilibrium (12–17) eventually leading to alteration of cells and structural macromolecules of the dermal connective tissue with its clinical appearance of wrinkle formation, laxity, and pigment disorders. In comparison with other antioxidants, idebenone shows in vitro studies as well as in vivo studies unique properties providing protection regarding both intrinsic and extrinsic processes of aging (18–20).

## HISTORY AND USES

Idebenone has a history in medical applications dating back to 1982, with its initial introduction to the scientific community in papers published by Shimamoto et al. (21–23) citing its potential applications in the energy metabolism of red blood cells and myocardial tissue. Additional research over the next few years lead to the first phase I study to determine the tolerance, safety, and pharmacokinetics of idebenone (24) followed by clinical studies in the early 1990s in subjects with Alzheimer's disease (25,26). It has since been researched extensively for a variety of applications related to the treatment of various age-related disorders of the human body, including cerebrovascular and Alzheimer's disease, Friedreich's ataxia, and showed

protective properties in organ preservation solutions (2). Until 2009 over 270 articles have been published, mostly related to the health benefits of idebenone as an antiaging modality (27–32). The potential benefits of idebenone fall into five categories: antiaging, energy enhancement, cognition enhancement, organ protection, and protection against excitatory amino acid neurotoxicity. Its introduction in the field of dermatology in 2003 as a compound purported to be beneficial in the treatment of many skin changes associated with acute and chronic skin aging.

As with other antioxidants, idebenone exists in a reduced and an oxidized state. In a study of its effect on astroglial cells, idebenone, in either redox state, significantly inhibited the enzymatic metabolism of arachidonic acid by cyclooxygenase and lipoxygenase. This effect was stronger with the reduced form, and showed potential central nervous system anti-inflammatory activity (27).

In another study, synaptosomes isolated from rat brain cortex were treated with iron and ascorbate, establishing experimental cellular oxidant injury. Idebenone prevented both the formation of ROS in the cytosol and mitochondria, as well as a decrease in protein sulfhydryl content (an indicator of protein oxidation), compared with controls (28).

In addition to its function as an antioxidant, idebenone works as an electron carrier in the electron transfer chain, similar to coenzyme Q10. Idebenone was introduced into a canine coenzyme Q10-depleted brain mitochondrial preparation, which prevented the loss of electron chain transfer activity normally seen with coenzyme Q10 depletion (3).

Idebenone also inhibited mitochondrial lipid peroxidation (4), which can be interpreted as protecting against mitochondrial damage. Other animal studies confirm the mitochondrial membrane protective effects of idebenone (29,30).

## FUNCTIONALITY

According to the mitochondrial theory of aging, nonrepaired damage of mitochondrial DNA and unstable electron transfer cause an important loss of mitochondrial function in correlation with progression of age (6). Mitochondria are the sites of cellular metabolic energy production where oxygen is used to convert carbohydrates into ATP to power and enable all cellular metabolic activity. The process itself produces toxic free radicals as the result of electron transfer, which can result in cellular damage if uncontrolled (33,34). Therefore cells have developed defense mechanisms, including essential enzymes and coenzymes such as coenzyme Q10, SOD, and catalase that are capable of reducing radical by-products of cellular energy production eventually into water (12,35). Unfortunately, these defense mechanisms are efficient only to a certain degree, and liberated free radicals such as superoxide radicals can react with cellular organelles and



molecules as, for example, DNA, lipids, sugars, and proteins to cause cellular damage, which expresses itself as aging (6,8,9,12). Mitochondria possess the ability to replicate containing their own DNA but have far fewer repair mechanisms than nuclear DNA despite the fact that they are the sites where most internal free radicals are formed. The result is cellular aging directly associated with metabolic energy production (33). For this reason, mitochondria require potent free radical scavenging antioxidants to prevent the early onset of cellular aging. Coenzyme Q10 protects mitochondria from oxidant decay. As mentioned above, the function of idebenone as an electron transfer respiratory chain antioxidant is closely related to that of coenzyme Q10, which itself is a vital biochemical found in cellular and mitochondrial membranes and plays a critical role in the electron transport chain during the production of energy in the mitochondria. However, unlike coenzyme Q10, idebenone lacks the potential downside of a degradative prooxidative effect, especially under hypoxic cellular conditions, such as those found after stroke, heart attack, excessive exercise, or other conditions that lead to poor tissue oxygenation. Idebenone appears to be able to tightly couple oxygen to the electron transport chain and thus prevent toxic oxygen radical production far better than coenzyme Q10 (3).

## DERMATOLOGY: CELL BIOLOGICAL AND CLINICAL RESEARCH

It is important not to confuse idebenone with other commonly used antioxidants in skincare such as vitamin antioxidants like vitamin C and vitamin E or botanical antioxidants such as polyphenols, flavonoids, and proanthocyanidins. Idebenone differs from these other types of antioxidants in that it is a respiratory chain antioxidant that targets aging at the mitochondria. In 2003 and 2005, the first cell biological *in vitro* and *in vivo* clinical studies were published (18–20) that demonstrated that idebenone had a potent ability to protect cellular lipids, cell membranes, and DNA from oxidative stress and prevent sunburn cell (SBC) formation post ultraviolet (UV) exposure, and thus could have a significant protective and corrective effect on skin aging. In a multistep protocol, a series of five different cell biological *in vitro* and clinical *in vivo* methods were combined to compare the antioxidative capacities of the following antioxidants: vitamin C, vitamin E, α-lipoic acid, coenzyme Q10, kinetin, and idebenone. The studies included measurement of the ability of the antioxidant to

1. Scavenge free radicals produced in a reaction chamber using instrumental analysis (Photochem<sup>1</sup>),

2. Protect against low-density lipoprotein (LDL) oxidation as a marker of lipid peroxidation,
3. Protect against cell membrane oxidation,
4. Protect against DNA cross-linking post UV exposure, and
5. Protect human skin from SBC formation post UV exposure.

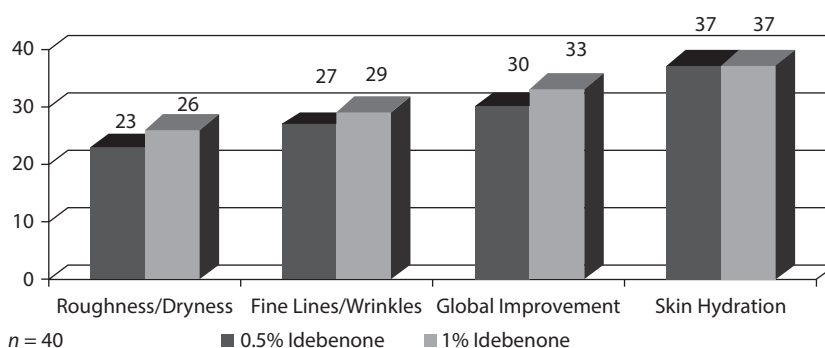
In a scoring system, which was based on a maximum score of 100, idebenone was found to be the most efficient antioxidant tested at the time. Since that time idebenone has been researched in further clinical studies measuring the effects at various concentrations to reduce the overall appearance of fine lines, wrinkles, hyperpigmentation, and other skin changes associated with photo-damage and global skin aging. The results of these studies (unpublished) are outlined below.

Forty subjects, 6 weeks, b.i.d., double blind, global assessment of 0.5% (20 subjects) and 1.0% (20 subjects) idebenone in the treatment of photodamaged skin with measurements for skin roughness/dryness, fine lines/wrinkles, skin hydration and global assessment by expert dermatologist grader. The results are given in Figure 8.1. In this study, punch biopsies of several patients were taken and stained for collagen 1, MMP-1, IL-1, and IL-6. The results demonstrated that idebenone was able to suppress formation of MMP, IL-1, and IL-6, and increase deposition of collagen.

Low concentrations of idebenone have been shown to upregulate certain important genes to suppress MMPs and interleukins and increase expression of certain structural proteins for collagen (personal communication with Dr D. McDaniel). Additionally, lifespan extension studies using *Drosophila* have demonstrated the ability of idebenone to extend the lifespan of fruit flies under oxidative stress conditions (36). Similar results were found in knockout mouse models of Friedreich's ataxia (37).

## SAFETY

As previously reviewed, sufficient preclinical and clinical data is available to demonstrate both cell biological and clinical antiaging benefits of idebenone. Consultations with several companies that market idebenone in topical skincare formulations also indicate that premarket safety HIRPT studies did not indicate any skin sensitivity concerns. However, post-market surveillance (various formulations have been on the market since 2004, including Prevege MD, Prevege, and PRIORI) suggests that there have been a few reported cases of skin sensitivity to the molecule, in most cases immediate and cumulative irritation reactions that can include skin redness, itching, and



**Figure 8.1** Percent increase/decrease after 6-week use.

folliculitis, but in more severe cases, what appear to be classic allergic type reactions (38–40). Since the use of idebenone is new in skincare so it is in the general population, consumers reporting allergic-type reactions on first use can only be explained via a cross-sensitivity type model. Because the compound is a 1,4-benzoquinone, consumers who exhibit allergy to para-phenylenediamine, hydroquinone, evening primrose oil, and other similar compounds may exhibit the same sensitivity to idebenone. For this reason, products should carry a caution to “patch test” products with idebenone and to wait at least 24 hours prior to commencing regular use. If sensitivity occurs, consumers should not use products containing idebenone. It is worthy of note here that there has never been a recorded allergic reaction to the internal consumption of idebenone in spite of its wide availability as a dietary supplement in many markets around the world.

## NEW IDEBENONE DERIVATIVE MOLECULES

Improvements in the topical skincare delivery of idebenone include the development of new synthetic molecular derivatives, which seem to have the capability to enhance efficacy and safety of this new antioxidant technology. Specifically, various water and oil soluble esters of idebenone were synthesized and tested for their ability to inhibit SBC formation post-UV irradiation, and the first clinical studies were conducted with assessments for known antiaging parameters including red and brown pigmentation, fine lines and wrinkles, and global improvement in photodamage. Finally, skin maximization studies were conducted to assess skin tolerance. In these pilot studies the dipalmitic glyceric acid ester of idebenone (hydroxydecyl ubiquinoyl dipalmitoyl glycerate) was found to be the most efficient new idebenone derivative molecule across all test parameters (unpublished study, personal communication with Dr. D. McDaniel).

It is believed that the improved antioxidant and anti-aging efficiency of the new derivative is a result of increased skin and cell permeability, improved time-released action of idebenone based on rate constant enzymatic hydrolysis in the skin, leading to broader skin compatibility, and more efficient antioxidant capacity.

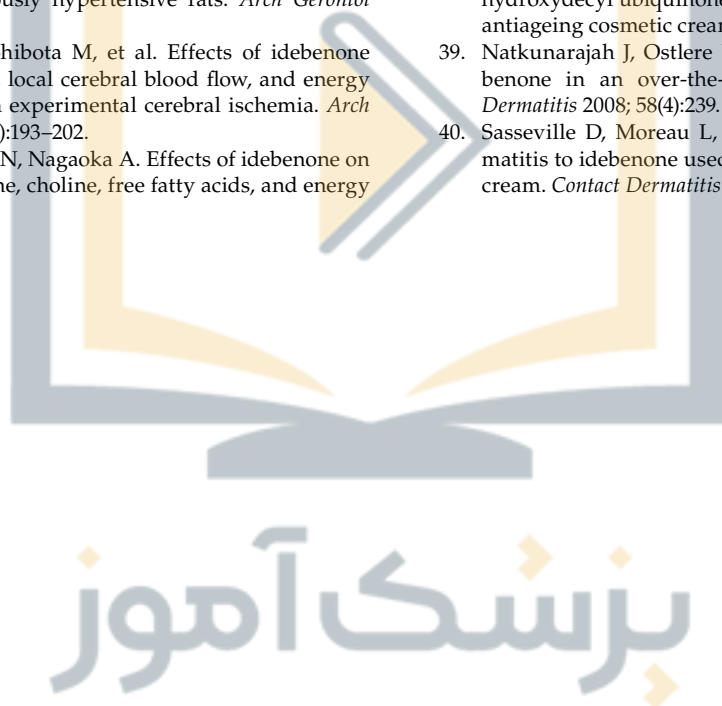
## CONCLUSION

Idebenone is a very promising new unique respiratory chain antioxidant for topical skincare use in treating various signs of skin aging. In cell biological studies it has been shown to protect cellular lipoproteins, cell membranes, and DNA from damage caused by oxidative stress. Clinically it has an anti-inflammatory effect, the ability to reduce SBCs post UV exposure, and the ability to diminish the visible signs of skin aging including fine lines, wrinkles, hyperpigmentation, and overall improvement in photodamaged skin. Improvements in the technology have produced new idebenone derivative molecules; in particular, dipalmitic glyceric acid, which seems to improve the delivery of the molecule and its efficacy but also significantly decreases the potential for skin sensitivity.

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# Antioxidants

Frank Dreher

## INTRODUCTION

The skin forms an efficient barrier limiting chemicals from entering our body and protects from the harmful environment encompassing exposure to solar ultraviolet radiation (UVR) and air pollutants. Such an exposure results in the formation of reactive oxygen (ROS) and reactive nitrogen species (RNS). As oxidative stress-promoting chemicals, those species can react with skin biomolecules what may ultimately lead to skin damage (1,2). To counteract ROS- and RNS-induced oxidative stress, the skin is equipped with a variety of antioxidants forming an antioxidant network. The antioxidants intervene at different levels of oxidative processes by scavenging and removing free radicals or oxidatively damaged biomolecules. However, the antioxidant defense in skin can be overwhelmed by an increased exposure to oxidative stress, or may be compromised due to malnutrition or certain health conditions.

Well documented solar UVR-induced skin damage includes acute reactions including sunburn. Premature skin aging (photoaging) and photocarcinogenesis are the consequences of chronic UVR exposure. Terrestrial solar UVR consists of UVB (290–320 nm) and UVA (UVA II: 320–340 nm, UVA I: 340–400 nm). Radiation less than 290 nm (UVC) does not reach the earth's surface, since these wavelengths are absorbed by stratospheric ozone. While ozone in the upper atmosphere occurs naturally and protects skin by filtering out harmful solar UVR, ozone at ground level (troposphere) is a noxious, highly reactive oxidant pollutant. Besides UVR and air pollutants such as tropospheric ozone, the presence of chemically unstable and ROS/RNS-forming drugs as well as the exposure of skin to photoreactive chemicals may be other sources of cutaneous oxidative stress. Recently, it was found that also the visible light and infrared parts of the sun spectrum appear to contribute to oxidative stress in skin.

## REACTIVE OXYGEN SPECIES

Several steps lead to the formation of ROS during UVR exposure, which represents a well characterized source of oxidative stress in skin (1,2). The cascade of ROS formation is initiated by UVA absorption of endogenous or exogenous chromophores present in the skin. Of the many skin constituents capable of absorbing UVA, trans-urocanic acid, melanins, flavins, porphyrins, protein-bound tryptophan, or advanced glycation end products are believed to be relevant chromophores initiating the ROS formation cascade. Following UVR absorption, the activated chromophore may react in two ways. In type I photoreactions, the excited chromophore directly reacts with a substrate molecule via electron or hydrogen atom transfer and gives rise to free radical formation. In the presence of molecular oxygen (minor type II reaction), this reaction may lead to the formation of superoxide anion radical  $\bullet\text{O}_2^-$ . Subsequently,

$\bullet\text{O}_2^-$  gives hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) by a dismutation reaction either spontaneously or catalyzed by cutaneous superoxide dismutase (SOD). Further, in the presence of metal ions such as Fe(II) or Cu(II),  $\text{H}_2\text{O}_2$  can be converted to the highly reactive hydroxyl radical  $\bullet\text{OH}$ . Otherwise (major type II reaction), electronically excited and reactive singlet oxygen  $^1\text{O}_2$  is formed by photoenergy transfer from UVR-excited chromophores in the presence of triplet oxygen  $^3\text{O}_2$  (molecular oxygen in its ground state). Following their formation, ROS species including  $^1\text{O}_2$ ,  $\bullet\text{O}_2^-$ ,  $\bullet\text{OH}$ , and  $\text{H}_2\text{O}_2$  react with an array of skin biomolecules including lipids, proteins, DNA, and carbohydrates. For instance, (poly)unsaturated lipids (LH) may react with ROS forming lipid peroxyl ( $\text{LOO}\bullet$ ) and alkoxyl radicals ( $\text{LO}\bullet$ ), which may initiate a chain-propagating autocatalytic reaction. ROS can also cause modifications of amino acids of proteins resulting in functional changes of structural or enzymatic proteins. Besides a multitude of ROS-mediated DNA damages, reaction of singlet oxygen with DNA results in the formation of 8-hydroxy-deoxyguanosine. Since DNA absorbs strongly in the UVB region and is only a weak chromophore in the UVA region, UVB is largely considered as a direct, ROS-independent inducer of DNA damage. UVB absorption of DNA leads to major base modifications such as pyrimidine dimer or (6–4) photo-adduct formation. These modifications together with indirect DNA damage induced by ROS are involved in solar genotoxicity.

Additionally, oxidative damage to mitochondrial DNA (mtDNA) has been described to play a major role in photoaging (3). Studies have shown that a common deletion of mtDNA is increased about 10-fold in photoaged skin as compared to sun-protected skin in the same individual (4). Although normal ATP production in the mitochondria results in some level of oxidative stress, it is thought that UVA exposure increases oxidative stress in the mitochondria what leads to mutations of mtDNA and consequently to a defective respiratory chain. The defective respiratory chain results in reduced energy production by the mitochondria and increased production of ROS (5–8). This increase in ROS leads to more mtDNA mutations which further perpetuate the production of ROS. As a consequence, mtDNA mutations will increase even in the absence of UV exposure (3,9). This hypothesis has been termed “the defective powerhouse model of premature skin aging” (3).

## CONSTITUTIVE SKIN ANTIOXIDANT NETWORK

To protect against oxidative stress, skin is equipped with a complex network of enzymatic and nonenzymatic antioxidants (1,2). Antioxidant enzymes such as SOD, catalase, glutathione reductase and peroxidase, glutathion-S-transferase and thioredoxin reductase, and peroxidase interact with low molecular weight lipophilic antioxidants including vitamin E



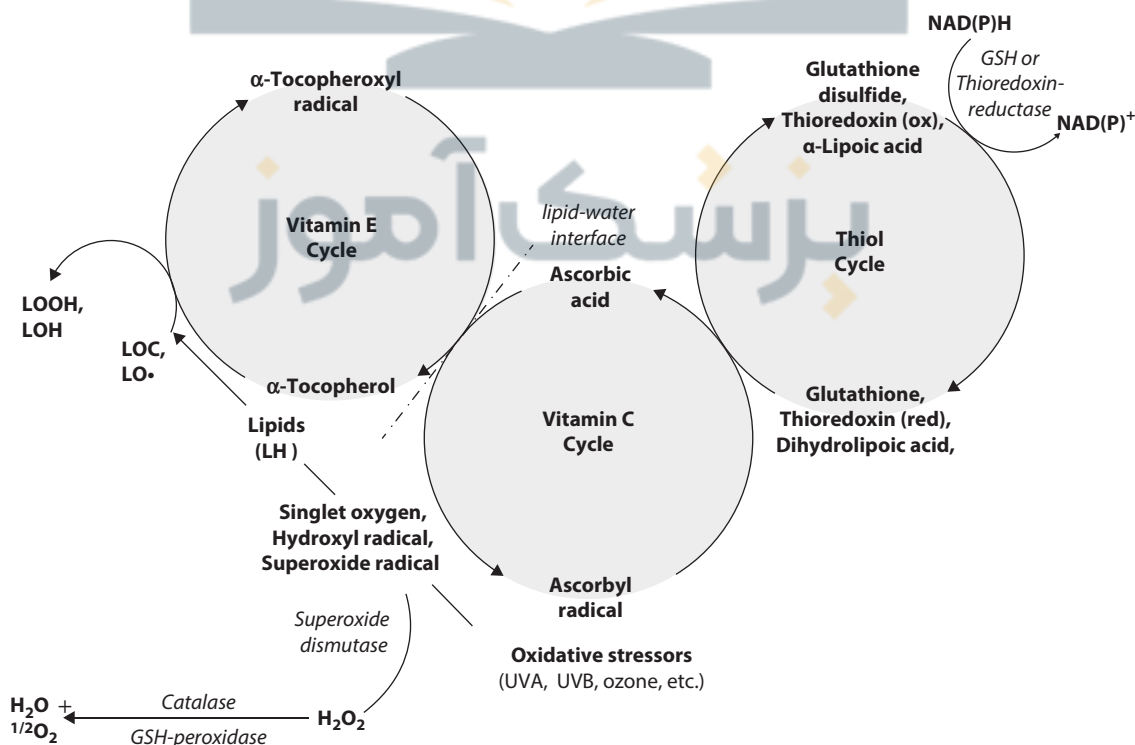
homologues (tocopherols and tocotrienols) and ubiquinols (coenzyme Q) as well as hydrophilic antioxidants such as vitamin C (ascorbic acid or ascorbate) and glutathione (GSH) (Figure 9.1). Carotenoids, retinoids, and uric acid, also possessing antioxidant activity, were also found in skin. Their role within the cutaneous antioxidant network is, however, less well understood.

$\alpha$ -Tocopherol, the predominant vitamin E homologue in skin, is known to efficiently scavenge lipid peroxyl and alkoxyl radicals by intercepting lipid chain propagation, which results in the formation of the metastable tocopheroxyl radical. This radical then either reacts with another lipid radical, leading to  $\alpha$ -tocopherol consumption, or abstracts a hydrogen atom from polyunsaturated lipids to give  $\alpha$ -tocopherol and lipid radical. In the latter case, occurring preferentially at low lipid radical concentration, the lipid radical may later react with oxygen to form a lipid peroxyl radical. This reaction consequently induces the  $\alpha$ -tocopherol-mediated lipid peroxidation chain reaction. Formation of one molecule of  $\alpha$ -tocopherol radical results in the formation of many lipid hydroperoxides. However, as demonstrated *in vitro* in lipid and cellular systems, when ascorbic acid or ubiquinol are present, the tocopheroxyl radical is rapidly reduced, regenerating  $\alpha$ -tocopherol. The  $\alpha$ -tocopherol-mediated lipid peroxidation chain reaction is thereby terminated. In addition, because of its high reduction potential, ascorbic acid is an efficient scavenger of a series of ROS such as superoxide anion radicals, hydroxyl radicals, singlet oxygen, as well as water-soluble peroxyl radicals. The resulting ascorbic acid radical can be either recycled to ascorbic acid by co-antioxidants such as glutathione or, respectively, is further oxidized

to dehydroascorbic acid and then irreversibly decomposed. Glutathione also reacts with singlet oxygen, superoxide anion radicals, and hydroxyl radicals resulting in the formation of the thiyl radical GS• and subsequently glutathione disulfide GSSG. The latter can be recycled to GSH by the NAD(P)H-dependent enzyme glutathione reductase.

GSH is also a cofactor for a few reducing enzymes, among them glutathione peroxidases. Glutathione peroxidase utilizes lipid peroxides as substrate and converts them into hydroxy fatty acids. Glutathione peroxidase also catalyzes the conversion of  $\text{H}_2\text{O}_2$  into water and oxygen. As mentioned, less reactive  $\text{H}_2\text{O}_2$  is produced by SOD catalyzing the dismutation reaction of superoxide anion radicals. SOD is present in skin as Cu/Zn- and Mn-SOD. GSH is likewise used by glutathion-S-transferases, which catalyze the conjugation of GSH to a variety of electrophils, including oxidized lipids, DNA, and other molecules. Glutathion-S-transferases therefore play an important role in removing products of oxidative stress.

Skin also contains catalase, which is similar to glutathione peroxidase and eliminates  $\text{H}_2\text{O}_2$ . However, catalase contributes to scavenging  $\text{H}_2\text{O}_2$  differently than glutathione peroxidase with respect to its cellular distribution, enzyme stability, and reaction rate. The enzymatic activity of catalase is much higher than that of glutathione peroxidase in human epidermis (10). Besides GSH peroxidase, skin contains another selenium-dependent enzyme, thioredoxin reductase (11). Thioredoxin reductase, together with its electron acceptor thioredoxin and thioredoxin peroxidase, participates in the cutaneous  $\text{H}_2\text{O}_2$  turnover similarly to the enzymic thiol redox couple GSH reductase/peroxidase.



**Figure 9.1** Postulated activation of interactive network of antioxidants and antioxidant enzymes by oxidative stress in skin; note that some of the depicted antioxidant recycling mechanisms have been found *in vitro* and in other than cutaneous systems.

Along with skin's "interceptive" antioxidant network that scavenges ROS and RNS, skin also possesses a mechanism of "antioxidant repair" that is able to reverse oxidatively damaged proteins (12). In general, nonenzymic antioxidant concentrations as well as enzymic antioxidant activities are significantly higher in the epidermis as compared to the dermis. This probably reflects the fact that the epidermis is directly exposed to various exogenous sources of oxidative stress and might have therefore evolved to possess a more pronounced antioxidant defense capacity than the dermis. On a molar basis, hydrophilic nonenzymatic antioxidants including L-ascorbic acid, GSH, and uric acid appear to be the predominant antioxidants in human skin. Their dermal and epidermal overall concentrations are more than approximately 10- to 100-fold greater than found for vitamin E or ubiquinol/ubiquinone. Ascorbic acid, GSH, uric acid (13), and vitamin E (14) as well as catalase and SOD (15,16) were also detected in the outermost epidermal layer, the human stratum corneum. On the other hand, glutathione peroxidase activity seems not to be detectable in human stratum corneum (16). It was found that the distribution of antioxidants in stratum corneum is not homogeneous, but follows a gradient with lower concentrations toward the skin surface (14). Such a gradient may be explained by the fact that the outer skin layers are more directly exposed to environmental sources of ROS. A second reason may be related to the longer exposure of the superficial stratum corneum layers to oxidative stress as compared to the lower layers, as a consequence of the physiological turnover of keratinocytes during their differentiation process. Interestingly, while human stratum corneum concentrations of vitamin E are as high as in lower epidermal layers, concentrations of hydrophilic antioxidants ascorbic acid and uric acid are in the range of one to two orders of magnitude lower. This is most likely due to the decreased water content of the human stratum corneum as compared with less keratinized epidermal layers. In contrast to uric acid, GSH, and ubiquinol, ascorbic acid and the vitamin E homologues cannot be synthesized by humans and must be taken up by the diet. Consequently, the skin's antioxidant defense is dependent on nutritive factors. Knowledge of the physiological regulation of ascorbic acid and vitamin E in skin is emerging. For instance, once ascorbic acid reaches skin via dermal blood vessels, it eventually enters the dermis where it is taken up by fibroblasts using a specific, sodium-dependent vitamin C transporter (SVCT) 2, or further diffuses through the dermis, finally reaching the epidermis and supplying keratinocytes mainly via SVCT1 (17).

$\alpha$ -Tocopherol is known to be a significant constituent of human sebum and is continuously secreted to the skin surface (18). Similarly as for carotenoids (19), sebaceous gland secretion is believed to be a relevant physiological delivery pathway of  $\alpha$ -tocopherol to sebaceous gland-rich skin regions, such as the well-exposed facial skin. This may explain the increased level of  $\alpha$ -tocopherol detected in the upper stratum corneum of facial skin as compared to upper arm skin. The physiological role of vitamin E in human sebum may be to particularly limit the formation of toxic skin surface lipid photooxidation products, such as squalene mono-hydroperoxides (20). In addition to its antioxidant activity, L-ascorbic acid acts as cofactor in a multitude of metabolic processes involved in skin formation. For example, it is required in hydroxylation reactions during collagen synthesis to form connective tissue (21) and participates in biosynthesis of epidermal barrier lipids (22).

## EFFECTS OF ENVIRONMENTAL STRESSORS ON SKIN ANTIOXIDANTS

Numerous studies have documented the effects of UVR on cutaneous antioxidants after acute or chronic exposure using different animal models, but fewer studies exist that investigate the mechanisms and consequences of such effects in humans (1,2). The antioxidants contained in the stratum corneum have been demonstrated to be susceptible to UVR. For example, a single suberythral dose of solar-simulated UVR depleted human stratum corneum  $\alpha$ -tocopherol by almost half, while dermal and epidermal  $\alpha$ -tocopherol were only depleted at much higher doses (14). The high susceptibility of stratum corneum vitamin E to UVR may be, at least in part, due to a lack of co-antioxidants in the outermost skin layer. The lipophilic antioxidant ubiquinol-10 (oxidized form of ubiquinol-10), the most abundant ubiquinol/ubiquinone found in human skin, was undetectable in human stratum corneum.

Additionally, ascorbic acid, the major hydrophilic co-antioxidant that is also capable of recycling photooxidized  $\alpha$ -tocopherol, is present at lower levels in human stratum corneum than in other skin tissues. Because stratum corneum represents a compartmentalized structure, the antioxidants are probably not homogeneously distributed. This may further affect their interactions and thus limit the recycling capacity of  $\alpha$ -tocopherol. The hydrophilic antioxidants were also shown to be sensitive to UVR. Direct depletion of  $\alpha$ -tocopherol and formation of its radical may further affect these endogenous antioxidant pools. However, it seems that ascorbic and uric acid are less susceptible to solar-simulated UVR than  $\alpha$ -tocopherol or ubiquinol-10, as shown with cultured human skin models (23). In full thickness epidermis of hairless mice, however, ascorbic acid was depleted at lower solar-simulated UV doses than those needed to deplete lipophilic antioxidants or GSH (24). In another study, murine epidermal GSH levels were significantly depleted within minutes after UVB exposure but returned to normal levels after half an hour (25). Moreover, exposures of hairless mice to solar simulated UVR demonstrated that dermal and epidermal catalase is more susceptible to photoinactivation than SOD, and far more than GSH peroxidase and GSSG reductase (26,27).

Effects of the air pollutant ozone on skin antioxidants have also been reported (1,2). Similarly, as found for UVR exposure, the stratum corneum is the most susceptible skin layer for ozone-induced depletion of lipophilic and hydrophilic antioxidants, as was demonstrated using hairless mice. Ozone itself is too reactive to penetrate deeply into the skin and reacts therefore predominantly with the skin barrier lipids and proteins in the outermost epidermis. Based on transepidermal water loss changes measured in hairless mice after exposure to either solar-simulated UVR or repetitive high doses of ozone, UVR appears a more damaging source of oxidative stress than ozone for skin (28).

A complex regulation in the antioxidant system of human skin was revealed during aging processes (15,29).  $\alpha$ -Tocopherol concentrations were significantly lower in the epidermis of aged skin but not for the dermis. Ascorbic acid levels were lower in both epidermis and dermis of aged skin. Total glutathione levels were also lower, whereas uric acid concentrations were constant in the epidermis and dermis. Moreover, protein oxidation is increased in intrinsically aged, and, most significantly, in photoaged human skin. Here, the oxidative damage is most pronounced in the papillary dermis and correlates well with solar elastosis. Remarkably, both protein oxidation as well

a sharp decline in catalase protein levels were also found in the stratum corneum; however, not in the lower epidermal layers, where antioxidant protection is overall higher than in dermal and stratum corneum layers (15). Accordingly, an age- and UVR-dependent decline of stratum corneum catalase enzyme activity was later demonstrated (16).

## PHOTOPROTECTION OF HUMAN SKIN BY TOPICAL ANTIOXIDANTS

Apart from using sunscreens to diminish the intensity of UVR reaching the deeper skin layers, supplementation of the skin with topically applied antioxidants and thereby strengthening its antioxidative capacity is an established approach in limiting oxidative stress-induced skin damage (1,2,30). Oral supplementation of antioxidants, which is another strategy to prevent photodamage of skin, is not the subject of this chapter and has been reviewed elsewhere (31–33). Topical application of antioxidants provides an efficient means of increasing antioxidant tissue levels in human skin. As the most susceptible skin layer for UVR- and ozone-induced depletion of cutaneous antioxidants, the stratum corneum may particularly benefit from an enhanced antioxidant capacity after topical supplementation.

### Vitamin E

The photoprotective effects of vitamin E ( $\alpha$ -tocopherol) have been studied extensively. Most studies were performed in animals, but several studies also exist investigating the photoprotective effects of topically applied vitamin E in humans (1,2,34). Significantly reduced acute skin responses such as erythema and edema, sunburn cell formation, lipid peroxidation, DNA adduct formation, immunosuppression, as well as UVA-induced binding of photosensitizers was demonstrated when vitamin E was applied before UVR exposure. As shown in animal studies, skin wrinkling and skin tumor incidence due to chronic UVR exposure seem also to be diminished by topical vitamin E. A human study proved that an alcoholic lotion containing 2%  $\alpha$ -tocopherol significantly diminished the erythema responses when applied 30 minutes before UVR exposure (35). While this lotion had no sunscreens properties, some  $\alpha$ -tocopherol preparations may also act as sunscreens (36).

Diverse vitamin E esters, in particular vitamin E acetate, were also shown to reduce UVR-induced skin damage. However, their photoprotective effects are less pronounced as compared to vitamin E. Vitamin E esters need to be hydrolyzed during skin absorption to show antioxidant activity. For instance, bioconversion of vitamin E acetate into  $\alpha$ -tocopherol, its active antioxidant form, seems slow and occurs only to some extent. There is evidence that vitamin E acetate is not hydrolyzed in the stratum corneum and that its bioconversion into  $\alpha$ -tocopherol only occurs after penetration into the nucleated epidermis (37). Consequently, the controversial observations of photoprotective effects of topically applied vitamin E acetate may be explained by a limited bioavailability of the active, ester-cleaved form during oxidative stress at the site of action. Intriguingly, the bioconversion of vitamin E acetate into its active form can be enhanced when skin is exposed to sun, possibly by an UVB dependent increase in esterase activity as demonstrated in murine epidermis (38).

### Vitamin C

Several studies investigated the photoprotective effects of topical vitamin C (L-ascorbic acid). Using a porcine skin model, topically applied vitamin C protects from UVB-induced erythema

and sunburn cell formation when formulated at higher concentrations (e.g., 15%) in an appropriate vehicle at low pH (e.g., pH 3.2) (39,40). In a human study, however, a hydroalcoholic lotion with 5% vitamin C was unable to induce any significant photoprotective effects when applied once 30 minutes before irradiation at a dose of 2mg/cm<sup>2</sup> (35). Besides differences between pig and human skin responses, differences in vitamin C concentration, mode of application including applied dose and vehicle composition, as well as other experimental parameters may explain this difference in the reported photoprotective efficacy of the vitamin C formulations.

The development of a stable formulation containing vitamin C is challenging since vitamin C is easily oxidized. Vitamin C can be stabilized in an aqueous formulation at low pH when kept under oxygen exclusion (41). Newly, special water-free, silicon-based preparations allow stabilizing vitamin C over a prolonged period of time (42). Additionally, esterified vitamin C derivatives such as magnesium or sodium ascorbyl phosphate, aminopropyl ascorbyl phosphate, and tetrahexyldecyl ascorbate (tetra-isopalmitate ascorbate) are stable alternatives to vitamin C (43,44). However, as described for vitamin E esters, these esters must be hydrolyzed to vitamin C to reveal antioxidant properties. This will take time and may occur only to a limited extent. Furthermore, some of those derivatives (e.g., tetrahexyldecyl ascorbate) are of significantly higher molecular weight as compared to vitamin C, that can lower their skin permeability and consequently also their antioxidant efficacy after topical application.

Vitamin C does not act as sunscreen, nor does it absorb UVA. In addition to its antioxidant properties, vitamin C participates in the formation of collagen fibers as a cofactor of prolyl and lysyl hydroxylase, enzymes essential for the stabilizing and cross-linking of newly synthesized collagen molecules. A human placebo-controlled study demonstrated that the use of a 5% vitamin C cream resulted in significantly improved skin relief and a decrease in deep furrows after 6 months of use (45).

### Polyphenols

Compounds from dietary and medical plants and vegetables have gained considerable attention as promising agents in protecting skin from UVR-induced photodamage after topical application (46–49). Extracts from green tea, wine grapes, coffee berry, feverfew, milk thistle, pomegranate, tropical ferns, and turmeric were particularly well studied. They contain a wide variety of polyphenols known as flavonoids, which are divided into flavonols, flavones, catechins, flavanones, anthocyanidins, and isoflavonoids. They are synthesized conjointly with ascorbic acid, vitamin E, and GSH by plants as a response to mitigate cellular damage under oxidative conditions. Their antioxidant properties arise from their high reactivity as hydrogen or electron donors, from the ability of the polyphenol-derived radical to stabilize the unpaired electron, as well as from their ability to chelate transition metal ions such as Fe(II), thereby interfering with hydroxyl radical production. Besides hydroxyl radicals, polyphenols are believed to quench singlet oxygen, superoxide anion radicals, and peroxy radicals. Polyphenolic compounds also possess anti-inflammatory and other properties beneficial for skin.

Green tea (*Camellia sinensis*) extracts are the best studied plant-derived antioxidants for skin (50). In contrast to black tea, which is fermented, green tea leaves contain a high concentrations of polyphenols such as epigallocatechin gallate (EGCG). Green tea polyphenols act as antioxidants by scavenging ROS/



RNS, by sequestering metal ions, and act indirectly as antioxidants through inhibition of “pro-oxidant” enzymes such as inducible nitric oxide synthase, lipoxygenases, and cyclooxygenases, and further induce the antioxidant enzymes GSH-S-transferases and SOD (51). Protective effects of green tea extract and its major polyphenolic constituent EGCG on UVR-induced skin damage after topical application were first observed in animals (50). These effects were later confirmed in humans, where topical application of green tea extracts or EGCG significantly decreased erythema responses, lipid peroxidation, and DNA damage (52,53). More recently, a placebo-controlled study demonstrated that the combined use of a 10% green tea cream and oral green tea supplementation for 8 weeks resulted in a significant improvement in elastic tissue (54). A trend toward improvement but no significant differences in clinical grading were found between the green tea-treated and the placebo group, indicating that a longer treatment period may be required for clinically relevant improvements. In another placebo-controlled study, topical application of a green tea protected human skin from solar-simulated ultraviolet light when applied 15 minutes prior to exposure and reapplied immediately after exposure to two minimum erythema doses (55). A further study showed that three-time daily use of a lotion containing 0.4% of a green tea extract with 40% to 50% total polyphenol content helped to reduce UVB-mediated increase in sunburn cell formation and p53 expression in keratinocytes, but did not reduce erythema or formation of thymidine dimers (56). This study indicated that formulations with relatively low concentrations of green tea extracts can be sufficient for providing photoprotection. Green tea extracts and EGCG were further shown to have chemopreventive effects in rodents, and limit cancer formation. However, epidemiological studies with green tea are so far not conclusive (50,57).

Numerous other polyphenols have also been studied. For instance, a milk thistle extract containing silibinin as predominant polyphenol was shown to inhibit UVB-induced immunosuppression, reduce UVB-induced sunburn cell formation, prevent DNA adduct formation, and prevent photocarcinogenesis after topical application in mice (58,59). In another study, topical administration of genistein substantially inhibited UVR-induced hydrogen peroxide formation, lipid peroxidation, and DNA damage in mice, and protected human skin against UVB-induced erythema (60). Furthermore, topical application of a tropical fern extract reduced erythema when applied before UVR exposure as shown in humans (61). In a clinical study, 1% coffee berry extract (containing diverse (poly) phenolic compounds including chlorogenic acid, quinic acid, and ferulic acid) resulted in a significant improvement in signs of skin aging when compared to vehicle (62). Pomegranate fruit extract, comprising the polyphenol ellagic acid, possesses strong antioxidant and anti-inflammatory properties, and limited UVB-mediated damage in a human reconstituted skin model (63). Another natural extract, a parthenolide-depleted extract of feverfew, significantly reduced UV-induced erythema in humans (64).

Additional studies are warranted to further clarify whether the observed beneficial effects of those extracts or their constituents cannot be at least partially attributed to their potential sun-screening properties after topical application.

### Thiol Antioxidants

Thiol antioxidants, such as GSH, N-acetylcysteine, lipoic acid, and their derivatives are another important group of radical scavengers (1,2). Topical administration of GSH, GSH-ethyl

ester, and N-acetylcysteine, respectively, efficiently protected against UVB radiation-induced epidermal lipid peroxidation, cytotoxicity, and apoptosis using pig skin *ex vivo* as a skin model (65). However, their photoprotective effects have been reported in only a few clinical studies. Topical treatment with N-acetylcysteine under occlusion resulted in an increased GSH level and eliminated its oxidized form (GSSG) in humans (66). Thus, the additional stimulation of GSH biosynthesis might be a key mechanism accounting for the observed photoprotective effects of N-acetylcysteine. Dihydrolipoic acid, the reduced and primarily active antioxidant form of  $\alpha$ -lipoic acid, is another promising thiol antioxidant (67).

### Other Antioxidants

The pineal hormone melatonin has antioxidant properties. It has been shown to significantly reduce UVR-induced erythema in humans, although its potential sunscreens properties as well as its immunomodulatory function may have contributed to the observed photoprotective effects (35). In addition, L-ergothioneine, a thiourea derivative of histidine found in food plants and mushrooms, is another potent antioxidant suitable for topical application (68). Idebenone, a synthetic analogue of coenzyme Q, is another interesting agent with antioxidant properties (69). Whereas a clinical study with a 1% idebenone formulation demonstrated a reduction in wrinkles in females with moderate photodamage (70), a study in pigs revealed that idebenone seems to offer little to no photoprotective effects (71).

### Antioxidant Combinations

Antioxidants interact when combined and emanating radical or oxidized forms of antioxidants after ROS scavenging may be quickly regenerated in the presence of appropriate co-antioxidants. Accordingly, an enhanced photoprotective effect may be obtained by applying distinct combinations of antioxidants. Ample evidence exists about the interactive dependence of vitamins C and E in diminishing photodamage. A single topical application of a combination of 2% vitamin E and 5% vitamin C resulted in a significantly more pronounced photoprotective effect as compared to the application of either antioxidant alone in the identical vehicle (35). As demonstrated in the same clinical study, the most dramatic improvement resulted from the combination of melatonin with  $\alpha$ -tocopherol and ascorbic acid. Other mixtures of topically applied antioxidants were also shown to be more effective in reducing photodamage than single antioxidants. Adding 0.5% ferulic acid (a phenolic antioxidant found in plants) to a solution of 1%  $\alpha$ -tocopherol and 15% ascorbic acid doubled photoprotection to solar-simulated irradiation as measured by both erythema and sunburn cell formation in pigs (72). Another combination consisting of ferulic acid with tocopheryl acetate and  $\alpha$ -glycosylrutin limited the severity of experimentally induced polymorphous light eruptions when applied 1 week prior to photoprovocation with UVA in humans (73). However, since ferulic acid significantly absorbs in the UVB/A-range, the photoprotection observed in those studies can likely not be attributed solely to its antioxidant properties (74). A significantly enhanced antioxidative efficacy was also found for the combination of  $\alpha$ -tocopherol, ascorbic acid, and green tea polyphenols (75). Studies revealed that the antioxidant synergism of this combination might be due to the regeneration of  $\alpha$ -tocopherol by the green tea polyphenols, while the latter are regenerated by ascorbic acid.



Using an electron spin resonance spectroscopy-based antioxidant assay (see next paragraph), a novel silicon-oil based preparation comprising 15% L-ascorbic acid and 1%  $\alpha$ -tocopherol combined with EGCG, the synthetic vitamin E analogue dimethylmethoxy chromanol, and creatine was shown to be significantly more effective in neutralizing free radicals in vitro as compared to a solution of 15% L-ascorbic acid, 1%  $\alpha$ -tocopherol, and 0.5% ferulic acid (76).

## EVALUATING ANTIOXIDANTS

How to determine the capacity of antioxidants or antioxidant combinations to eliminate ROS/RNS is an important question when studying antioxidants. Accurate quantification of antioxidant capacity is, however, challenging (77). Currently, there is no single assay available that can sufficiently meet this task. Oxygen radical absorbance capacity (ORAC) is a frequently used antioxidant assay to determine the strength of antioxidants in skin care products. The assay uses a fluorescence probe that is susceptible to oxidation by free radicals. Despite being widely used, this method has significant limitations. In contrast to ORAC and other assays, electron spin resonance (ESR) spectroscopy based assays have several important advantages (77,78). For instance, ESR allows directly detecting and quantifying free radicals. ORAC requires the addition of a fluorescent probe and therefore quantifies radicals only indirectly (similarly to other decolorization assays). ESR methods therefore provide more accurate measures of the antioxidant capacity of antioxidants or antioxidant-containing formulations. In addition, only ESR methods can be accurately performed with colored or opaque formulations and in skin (77,78).

## CONCLUDING COMMENTS

Animal and human studies have convincingly demonstrated that topical antioxidants can limit UV-induced skin damage. In humans, the protective effects were well demonstrated for ascorbic acid, tocopherol, ferulic acid, and a few natural polyphenolic antioxidant mixtures including green tea extract, which is rich in EGCG. The efficacy of those antioxidants can be significantly increased when they are combined.

However, formulating products with antioxidants is not simple and a number of technical requirements must be fulfilled for an efficient product. First, antioxidants should be present in a product at high concentrations. Second, antioxidants need to be kept stable in the formulation. Third, antioxidants should be able to penetrate into and through the stratum corneum to provide adequate concentrations in lower skin layers for biologically relevant antioxidant effects. Furthermore, efficacy determination of antioxidant products should be preferentially based on clinical data and ESR-based methods because most other tests, including ORAC, provide only limited and possibly inaccurate information.

Since sunlight-induced skin damage occurs also through nonoxidative mechanisms, antioxidant supplementation will not provide complete photoprotection. In fact, photoprotective effects of antioxidants are relatively modest as compared to broadband sunscreens. Therefore, sunscreens are indispensable in the effective prevention of skin photodamage, while the antioxidants will provide additional benefits to sunscreen products (79,80).

Finally, it is important to keep in mind that antioxidants are of protective nature (i.e., from oxidative stress) and generally have no effect in reversing existing skin damage, including

wrinkles. Only agents which promote collagen, elastin, and hyaluronic acid formation such as retinoic acid, human growth factors, and a few distinct peptides, have been shown to help reverse the signs of skin aging.

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# Topical Retinol: An Efficacious Solution for Improvement of Main Photodamage Signs

Christiane Bertin and Thierry Oddos

## INTRODUCTION

Clinically, skin aging is associated with a variety of signs such as wrinkles, uneven pigmentation, skin roughness, skin color and laxity. These clinical features are consecutive to the structural and metabolic changes that occur during the passage of time (chronological aging). However, external factors such as repeated skin exposure to solar ultraviolet (UV) radiation can induce premature or photoaging of the skin. These changes are consecutive to a decrease in fibroblast number as well as collagen synthesis and an increase in UV-induced collagen degradation by matrix metalloproteinases (MMPs). An accumulation of non-functional elastin is also observed in the dermis, leading to a loss of skin elasticity and firmness. In addition, a constant hallmark of skin aging and photoaging is epidermal thinning, which is triggered by a decrease in keratinocyte turnover rate (1). Photoaging is also associated with a dysregulation in melanin synthesis and distribution and a general increase in the inflammatory status of the skin leading to the appearance of brown spots, a global increase in skin redness, and telangiectasia.

Retinoids have shown beneficial effects in reducing skin photoaging. For instance, it has been demonstrated that all-trans retinoic acid (ATRA) improves skin photoaging signs (2). This clinical efficacy was mainly attributed to the effect of ATRA on collagen metabolism by stimulation of the collagen synthesis that ultimately accumulates in the upper part of the papillary dermis. Moreover ATRA downregulates UV-induced MMP1 and MMP9 expression (3), thereby replenishing collagen levels. Although ATRA is recognized as an effective therapy for the treatment of photoaged skin through its regulatory effect on collagen metabolism, it has been suggested that retinol (ROL), known as vitamin A, may also alleviate some major signs of photoaging with a better irritation profile.

Retinoids are all derived from the naturally occurring all-trans retinol. This molecule is composed of 15 carbons. Three parts of the molecule can be distinguished: a cyclic group, a polyene group, and a polar group (primary alcohol for retinol  $-CH_2OH$ , and aldehyde  $-CHO$  for retinaldehyde and a carboxylic function  $-COOH$  for retinoic acid, the two latter compounds being obtained from the previous by oxidation [Figure 10.1]). Natural isomers carrying specific biological activities have also been described as 11-cis retinaldehyde and 9-cis retinoic acid.

## Metabolism of Retinol

Diet is the normal source of retinol in the body. It originates from carotenoids in plants and of the long-chain fatty acid esters of retinol (as retinyl palmitate) in animal tissue. Carotenoids are split, in the intestine epithelium, in the middle

of the molecule to form retinal, which is in turn reduced to retinol and esterified into retinyl ester. Retinyl esters are transported and stored in the liver, which is the main storage site in the body. The retinol is then delivered in the bloodstream associated with a specific transporter, the retinol-binding protein (RBP). In normal conditions, most of the circulating retinol is associated with RBP and the level of retinol in healthy volunteers fluctuates above 30  $\mu g/mL$  (4).

In the skin, at the keratinocyte level, retinol uptake is mediated through the binding of RBP-retinol complex to specific receptor(s), such as the 61 Kd RPE protein (retinal pigment epithelium protein) and internalization (5,6,7).

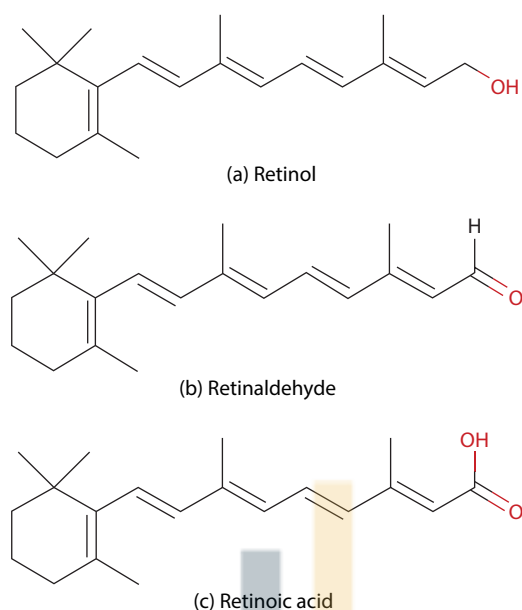
In keratinocytes, retinol is stored in the form of retinyl esters (i.e., mainly palmitate, oleate, and acetate esters) (8,9) primarily through the action of lecithin-retinolacyl transferase (10). It is then oxidized into retinal and retinoic acid by different alcohol dehydrogenases (11). Two other proteins responsible for retinol transportation play an important role in retinol metabolism. The first is the cellular retinol-binding protein (CRBP), which shows a high affinity for all-trans retinol. The second is the cellular retinoic acid binding proteins (CRABP I and CRABP II) that bind specifically retinoic acid. In skin, CRABP II is the predominant retinoic acid binding protein and its expression is enhanced by keratinocyte differentiation and treatment with retinoids. The role of these proteins is not completely clarified. CRBP could play a role in presenting the retinol in the adequate conformation to the dehydrogenase. They are also believed to control the level of free retinol and free retinoic acid and the translocation of these two molecules in specific cellular compartments such as translocation of retinoic acid in the nucleus for CRABP II (12).

## Biological Effect on Skin Including on Different Cell Types

Retinol plays a major role in the skin, as it is essential for regulating keratinocyte differentiation as suggested by the early observation of abnormal keratinization in vitamin A-deficient rats (13) and humans (14). Topically, retinol stimulates the proliferation of the basal keratinocytes through the release of heparin-binding-epidermal growth factor (HB-EGF) from suprabasal keratinocytes (15,16). The influence of retinol on keratinocyte differentiation is shown by the increase of some differentiation markers such as keratins (keratin 4, keratin 10, keratin 13, and cytokeratin 19) while others like fillagrine, transglutaminase, or lorincrine are decreased.

The overall effect on the epidermis is a stimulation of the cell proliferation in basal and suprabasal layers and an inhibition of the terminal differentiation of the keratinocytes. Taken together, this leads to a general thickening of the epidermis (8).





**Figure 10.1** Structure of most common retinoids (all trans conformation).

On dermal fibroblasts, retinol showed a potent stimulation of the growth of fibroblasts from aged donors and a stimulation of the synthesis of procollagen I and procollagen III (17). A suggested mechanism for the improvement of collagen synthesis by retinol involves the negative regulator of collagen homeostasis, the cysteine-rich protein 61 (CCN1). Topical treatment with retinol for 7 days significantly reduced CCN1 mRNA and protein expression in both chronologically and photoaged human skin in vivo (18). It has been shown that retinol could act also on elastin synthesis and elastin fiber formation (19). Indeed, retinol treatment on dermal fibroblasts cultures as well as topical treatment on human skin explants resulted in tropoelastin and fibrillin-1 gene expression and elastin fiber formation as measured by qPCR and immunohistochemistry.

Retinol represses the overexpression of UV-induced MMP1 and MMP9, two enzymes involved in the degradation of collagen molecules. The underlying mechanism is the repression by activated retinoic receptor of the of AP1 transcriptional activity that drives the expression of MMP1, MMP3, and MMP9. Skin exposure to UV light leads to the stimulation of the MAP kinases such as ERK, JNK, or P38 and then to the overexpression of the Jun protein which controls the level of AP1 in the cell. Thus retinol is able to decrease the UV-induced overexpression of MMPs and increase collagen synthesis, two mechanisms that could explain in part its potential to protect the skin against photodamage (3).

## CLINICAL ASPECTS

Retinoids have been widely studied during the past 20 years for their activity on human skin. In 1969, Kligman proved the therapeutic efficacy of topical tretinoin (all-trans retinoic acid) in acne vulgaris and later in 1986 pioneered the use of retinoids in cosmetic dermatology by demonstrating its effects on photoaged skin. He conducted an open study on eight elderly patients receiving 0.05% tretinoin cream on the face for 6 to 12 months, while six other patients received the vehicle alone.

Although only slight clinical differences were observed, histology showed a thickening of the previously atrophic epidermis, decrease of keratinocyte dysplasia and atypia, more even dispersion of melanin, and formation of new dermal collagen and blood vessels.

Since then, several clinical studies have been performed to assess the activity of tretinoin on humans, especially in the treatment of photoaging signs. Weiss and Voorhees in 1988 performed the first double-blind randomized placebo-controlled study by applying 0.1% tretinoin on the forearm and face of 30 patients for 16 weeks. They demonstrated a significant improvement of photoaging in the tretinoin-treated areas. The greatest improvement was in the reduction of fine wrinkles, with a statistically significant difference occurring 8 weeks after beginning the study. Other features such as coarse wrinkles, roughness, and sallowness also showed an improvement, but to a lesser degree. Lever, in 1990, using a preparation containing 0.05% tretinoin during 12 weeks, observed an improvement in clinical grading of photoaging (fine lines and wrinkles). Grove again in 1991, assessing different concentrations of tretinoin (from 0.05 to 0.001%) over 24 weeks, demonstrated a better efficacy of the dose activity with the highest concentration (0.05%) of the ingredient on the Rz parameter (local mean amplitude) on skin replicas (20). Olsen in 1992 on 296 subjects, with the same ranking of concentrations as Grove, over a 24-week period showed a decrease of skin roughness, hyperpigmentation, and fine wrinkling (21).

Unfortunately, tretinoin tends to be irritating, with many patients experiencing redness, flaking, and increased skin sensitivity. Some patients also reported increased sun sensitivity during tretinoin therapy (22).

Retinol, an alternative to tretinoin, has fewer side effects and is a better tolerated additive in cosmetic preparations for the improvement of photodamage signs. In 1995, Kang et al. demonstrated that the application of retinol up to 1.6%, on human skin under an occlusive patch, was unable to induce significant erythematous reaction versus vehicle (8). It also evokes the same physiological response as the 0.025% erythemato-genic concentration of retinoic acid as demonstrated by epidermal thickening and the enhancement of CRABP II mRNA and protein expression. This retinoid activity of retinol was obtained without a detectable increase of retinoic acid in the epidermis. Duell et al. (23) showed that topical retinol penetrated the epidermis more and produced less irritation than the acid form. Finally, Goffin in 1997 tested 0.075% of retinol for 8 weeks with corneografmetry and demonstrated its better tolerance versus tretinoin (24).

## Clinical Efficacy of Retinol

Although retinol was demonstrated to induce in human skin a physiological response typical of retinoids, only a few clinical studies were published demonstrating its efficacy in improving the signs of skin aging. In 2007, Kafi et al. reported a clinical study to demonstrate the beneficial effect of retinol on the skin signs associated with chronological aging (25). This randomized study versus vehicle was performed on 23 elderly subjects over 80 years old (average 87 years old). Retinol 0.4% and vehicle were topically applied on the upper inner arm for 6 months. The results showed a significant improvement in fine wrinkles versus vehicle after 2 months of application. This improvement increased with the continued application of retinol and was associated with a significant better tactile roughness and overall severity

in chronologically aged skin. Skin biopsies demonstrated a thickening of the epidermis and a significant increase in GAG expression and procollagen I accumulation in the dermis compared to the vehicle-treated arm. In this study, although rated as mild, adverse reactions were reported by most subjects including erythema, peeling, pruritus, dryness, and burning or stinging, with the withdrawal of three subjects following severe enough symptoms (25).

We explored the action of retinol at a lower concentration (0.1%) on a photoexposed area in order to investigate the anti-aging effect of retinol at more tolerated doses on the photoaging signs. In a first blind randomized vehicle-controlled study, 48 volunteers (41–60 years old) topically applied to the face either the retinol 0.1% containing product or the vehicle, every day for 56 weeks. The clinical evaluation, performed by an expert grader after 12, 24, and 56 weeks of application, showed that wrinkles under the eyes, fine lines at the crow's feet area, and skin tone evenness significantly improved versus both baseline and vehicle. The improvement of the fine lines in the crow's feet area was also demonstrated with digital imaging and with surface profilometry. A progressive improvement was seen between baseline and after 24 and 36 weeks of treatment (Figure 10.2). The improvement in fine lines was also documented by surface profilometry, and a progressive disappearance of the fine lines in the crow's feet area was observed. Moreover, we demonstrated that retinol stimulates epidermal cell proliferation along with the treatment. The epidermal cell turnover rate was evaluated using in vivo fluorescence spectroscopy by measuring the fluorescence maximum attributed to tryptophan moieties. The placebo-treated group did not show any significant change from baseline for any time point. In contrast, the intensity of tryptophan fluorescence increased for the actively treated group evident from the first time point (12

weeks vs. baseline) and then it appeared to reach a plateau. Most importantly, the change in the fluorescence intensity from baseline was significantly higher for the active-treated group than for the placebo-treated group at the 36-week time point (26). These anti-aging effects of retinol were confirmed in two other shorter term double-blind randomized studies performed with moisturizing product containing 0.1% retinol. In the first study the retinol-containing product was topically applied on 40 subjects (40–60 years old) once a day for 8 weeks. The effect of the product was compared to the effect of its vehicle on 41 subjects. The results presented in Table 10.1 showed that the application of the retinol 0.1% significantly improved the facial features of photodamaged areas as early as 4 weeks of use. Results from the second study have been published. In this 8-week, split-face, double-blind randomized clinical study, a 0.1% retinol-containing moisturizer was tested (36 subjects) versus placebo (28 subjects) in women with moderate facial photodamage. Clinical evaluation of photodamage signs by a dermatologist demonstrated significant improvement versus placebo of fine lines, wrinkles, elasticity, firmness, and overall photodamage after 4 weeks. Thus, using a low level of retinol (e.g. 0.1%) could still deliver a significant and rapid anti-aging efficacy while maintaining a good tolerance profile, as only a few subjects experienced a slight adverse skin reaction without any withdrawal with the retinol product. In the two studies, the number of adverse reactions observed with the retinol product was not significantly higher than with the vehicle. These clinical results were later confirmed by two other clinical studies performed on middle-aged Japanese females (27). Both studies included 57 volunteers and were performed with 0.075% and 0.04% retinol creams during 26 and 13 weeks, respectively. Both trials revealed significant improvement, especially on fine wrinkles, with



**Figure 10.2** Example of improvement of crow's feet area by retinol 0.1% product treatment.

**Table 10.1** Clinical Assessment of Retinol Anti-Aging Efficacy

Parameter	Vehicle		Retinol 0.1%	
	4 weeks	8 weeks	4 weeks	8 weeks
Under-eye wrinkles	0%	2.2%	4.3%*†	6.5%*†
Crow's feet fine lines	8.1%*	10.8%*	29.7%*†	35.1%*†
Crow's feet wrinkles	0%	0%	2.8%*	8.3%*†
Forehead wrinkles	0%	0%	6.4%*†	6.4%*†
Skin radiance	16.7%*	20.8%*	34.8%*†	43.5%*†
Cheek wrinkles	0%	0%	7.3%*†	9.8%*†
Overall photodamage	3.8%*	3.8%*	17.3%*†	19.2%*†

*Note:* Values represent the percentage of improvement versus baseline.

\*Statistical significance versus baseline ( $p < 0.05$ ).

†Statistical significance versus vehicle ( $p < 0.05$ ).

minimal irritation. These results are very promising as they demonstrate that retinol could be efficient at lower concentration, thus decreasing irritation risks.

Recently, we also demonstrated that prolonged use of topical retinol (0.1%) significantly improved clinical signs of photoaging on two 52-week, double-blind vehicle-controlled studies (28). In the main study, 62 volunteers applied either a stabilized retinol formulation or its vehicle to the full face, while the second study evaluated histological/histochemical markers in 12 subjects after 52 weeks of either retinol or vehicle use on contralateral dorsal forearms. The retinol group showed significant photodamage improvement over vehicle at all time-points during the study. After 52 weeks, retinol had improved crow's feet fine lines by 44% and mottled pigmentation by 84%, with over 50% of subjects showing +2 grades of improvement in many parameters. Additionally, at week 52, histochemical data confirmed the clinical results, showing increased expression of type I procollagen, hyaluronan, and Ki67 expression (a marker of cell proliferation) in basal keratinocytes as compared to vehicle.

## CONCLUSION

Skin aging is a complex process involving numerous different mechanisms such as loss of cell proliferation, decreased potential of extracellular matrix synthesis, and overexpression of matrix-degrading proteinases. Retinoids are naturally present in the skin and regulate epidermal cell differentiation, activate collagen synthesis, and attenuate the UV-induced overexpression of MMPs. The potential of regulating several pathways involved in skin chronological and photoaging makes them good candidates as active ingredients to alleviate facial aging signs. Although retinoic acid has been the first retinoid to demonstrate an efficacious wrinkle improvement, the severe side effects induced by its topical application warrant the identification of other retinoids for this benefit. Therefore retinol was identified as a good alternative to retinoic acid, as its topical application induces a low level of skin adverse reaction while delivering physiological skin responses similar to those induced by retinoic acid. Moreover, the retinoic acid level in the epidermis observed after topical application of retinol is extremely low or undetectable and thus provides a substantial margin of safety with respect to possible systemic effect. Results presented here focus preferentially on the clinical efficacy of well-tolerated doses of retinol. Indeed, in two double-blind randomized placebo-controlled studies, retinol at 0.1% demonstrated a significant visible improvement of facial aging features such as fine lines and wrinkles within 1 month's time.

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# Applications of Non-Denatured Soy in Skin Care

Jue-Chen Liu, Jeff Wu, and Miri Seiberg

## INTRODUCTION

The soybean plant belongs to the pea family, Leguminosae, subfamily Papilionoideae, and the genus *Glycine*. The cultivated form is named *Glycine max* (L.) Merr. It is one of the oldest annual crops of the Far East. Dietary soy has been known for centuries to provide nutritional, medicinal, and health benefits. Because of the nutritional value of soybeans, there is an inherited value for skin care applications. In fact, the use of soy to treat skin conditions has a long history. It was first reported in Traditional Chinese Medicine Encyclopedia (1) that soybeans provided therapeutic efficacy against skin conditions including hyperpigmented lesions and dry skin. Additionally, Chinese folklore has it that women workers in tofu industries have the most beautiful skin, i.e., fair, smooth and porcelain-like fine skin. For a very long time, soy or components of soy for topical applications have been limited to functional applications such as emollient and skin conditioning agents. The scientific fundamentals and clinical efficacy of soy for skin benefits have not been proven until recent years. It was reported that soy isoflavones possess phytoestrogenic properties which may play a role in the skin of menopausal women (2). Specific protein components in soybeans were identified for inhibition of melanosome transfer resulting in pigment reduction (3–5). Other soybean components such as lipids, oligosaccharides, and saponins can also provide skin benefits. Such new knowledge and findings have been implemented into dermatological and cosmeceutical products for broader skin applications. This chapter reviews the components of soy and their clinical uses in improving skin conditions.

## SOY IN HUMAN HEALTH

Soybeans originate from China. In 2853 BC, Emperor Sheng-Nung of China named five sacred plants—soybeans, rice, wheat, barley, and millet. Soybean plants were then domesticated and cultivated into a food crop for everyday diet (6–7). The soybean has blossomed from legendary Chinese origins to the “miracle crop” vastly produced on modern-day American farms since its first introduction into the United States in the early 1800s (7).

From about the first century AC to the Age of Discovery (15th to 16th century), soybeans were introduced into several countries such as Japan, Indonesia, the Philippines, Vietnam, Thailand, Malaysia, Burma, Nepal, and India. The first soybeans arrived in America in the early 1800s and since then have played an increasingly important role for human nutrition and health.

Soybeans with their valuable proteins and oil are an important source of nutrition. The soybean is rich in minerals including calcium, iron, and potassium, amino acids, and vitamins. It is also a good fiber source (8–9). They can be used

as meat and dairy substitutes in various items. Today, most people are aware of the use of soy proteins in baby formula, weight-loss drinks, sport drinks, and as a low-fat substitute for hamburger. Soybeans are also valued for their medicinal qualities. Epidemiological data indicates that people from Asian cultures have lower rates of certain cancers, including cancers of the breast, prostate, and colon, and lower rates of cardiovascular disease, osteoporosis, and postmenopausal symptoms (10–16). In the past decade, studies on soy's health benefits have dramatically increased in these areas, and more systematic and controlled clinical studies have been presented on its health benefits (17–18). In 1999, the U.S. Food and Drug Administration (FDA) approved a healthy claim for soy protein that associated with reduced risk of coronary heart disease (19). This ruling is based on the FDA's decision that foods containing soy protein included in a diet low in saturated fat and cholesterol may reduce the risk of coronary heart disease by lowering blood cholesterol levels.

## EARLY UTILIZATION OF SOY IN TOPICAL APPLICATION

For many years, due to the lack of basic research in understanding the fundamental mechanisms of soy on skin, soy's nutritional components were used mainly as functional ingredients in the cosmetic industry (20) and mostly have remained so until today. The most frequently used soy components include soybean oil, soy protein isolates, and soy lecithin as shown in Table 11.1. Soybean oil is one of the valuable materials used to adjust the rheology of cosmetic emulsions. Examples of soy oil as an emollient and moisturizer include baby oil and moisturizing lotion. Soy protein isolate/hydrolysate has been formulated as an emulsifier and foaming enhancer into a wide variety of products, including laundry detergent, bath products, shampoos, and skin cleansers (21). The lecithin fraction of crude soybean oil has found application as an emulsifying, surface active agent, and a stabilizing agent in industrial products such as paint, ink, soap, and cosmetics (22). Soy sterols are employed as skin conditioners for lotion, creams, and cleansers (23).

In the early 1990s, soy extracts or soy germ extracts that claimed to provide specific biological activities started to appear in skin care products. These soy extracts, although defined vaguely as soy (soja) concentrates or soy proteins, were actually produced using different chemical extraction conditions and could result in dissimilar compositions and skin effects. Such soy extracts often had too small a fraction of the original soy components to provide the desired biological activities to keep the bioactivities for skin efficacy, or were processed excessively under harsh extraction conditions such as extreme pH, strong solvents, and elevated temperatures.

**Table 11.1** Traditional Use of Soy in Skin and Hair Care

Soy components	Functionality	Applications	Reference
Soybean oil	Skin conditioner/ emollient	Baby oil, moisturizer	(22)
Soy protein Isolate/hydrolyzate	Emulsifier	Bath products, cleansers, shampoos	(21)
Soy lecithin /phospholipids	Surface active, emulsifier, stabilizing agent	Liposome	(60)
Soy sterol	Skin conditioner	Lotion /cream/cleanser	(20)

Few clinical reports or publications were available for cosmetic products spiked with such soy extracts. The use of these soy extracts has been limited to product marketing claims.

### SCIENTIFIC ADVANCES IN THE USE OF SOYBEANS FOR SKIN CARE

#### Soy Proteins, Non-Denatured Soybean Extracts, and Depigmentation

There is an increasing global desire for skin care products to induce fair skin and even skin tone. In addition, hyperpigmented spots including mottled hyperpigmentation, solar lentigines, and melasma are key concerns for the aging population and pregnant women. The most frequently used topical treatment agents for depigmentation are tyrosinase inhibitors, hydroxyacids, retinoids, hydroquinones, anti-inflammatory agents, and their combinations. Treatment with these agents is not always satisfactory and may result in irritation, sun sensitivity, or toxicity (24). In its proposal published in the U.S. government's Federal Register, U.S. FDA notes: "The actual risk to humans from use of hydroquinone has yet to be fully determined.... We're acting for safety reasons. There is a potential for hydroquinone to be a carcinogen in humans." In December 2009, U.S. FDA nominated hydroquinone for further study by the National Toxicology Program (NTP). FDA believed that the NTP studies are necessary before it can determine whether hydroquinone is generally recognized as safe (GRAS) when used in over-the-counter (OTC) skin bleaching drug products. Therefore, safer and more effective depigmenting agents are needed to address consumer needs for fair skin, even tone, and spot reduction. Moreover, a move toward "green" therapies has led to a demand for natural, safe, and efficacious skin-lightening agents.

Soybeans contain two proteins, which are reported to inhibit the protease-activated receptor-2 (PAR-2) pathway, leading to reduced melanosome transfer from melanocyte to keratinocyte and resulting in pigmentation reduction. These two proteins are serine proteinase inhibitors: the Kunitz-type trypsin inhibitor (soybean trypsin inhibitor [STI]), and the Bowman-Birk protease inhibitor (BBI) (25–26). STI has a molecular weight of 20 k daltons (k-Da), consists of 181 amino acid residues with two disulfide bridges, and is roughly spherically shaped (27). BBI is an 8 k-Da protein that inhibits the proteases trypsin and chymotrypsin at separate reactive sites (28). PAR-2 is a G-protein-coupled receptor activated by a serine protease cleavage (29–31). Trypsin, mast cell tryptase, and kallikrein are the only known natural serine proteases that activate PAR-2 (32–34). PAR-2 is expressed in keratinocytes (35–36), but not in melanocytes (3) and is involved in the regulation of pigmentation (3,37–38). It was reported that the inhibition of PAR-2 activation by serine protease inhibitors reduces pigment

deposition. This effect is possible only when keratinocyte-melanocyte contact is established (see Figure 11.1, top panel). Melanocytes alone do not respond to PAR-2 modulating agents with pigmentary changes (3). When serine protease inhibitors interfere with PAR-2 activation, melanosome transfer from the melanocytes to the keratinocytes is inhibited. The reduced melanosome ingestion by keratinocytes results in depigmentation both in vitro and in vivo (3,38). This depigmenting effect caused by serine protease inhibitors is reversible in vivo (3,38), therefore excluding the possibility of melanocyte death following such treatments.

In soy foods, STI and BBI are inactivated and denatured by heat and pressure or by fermentation to prevent the blocking of trypsin activity in the digestive system for human, which would cause gastrointestinal adverse effects.

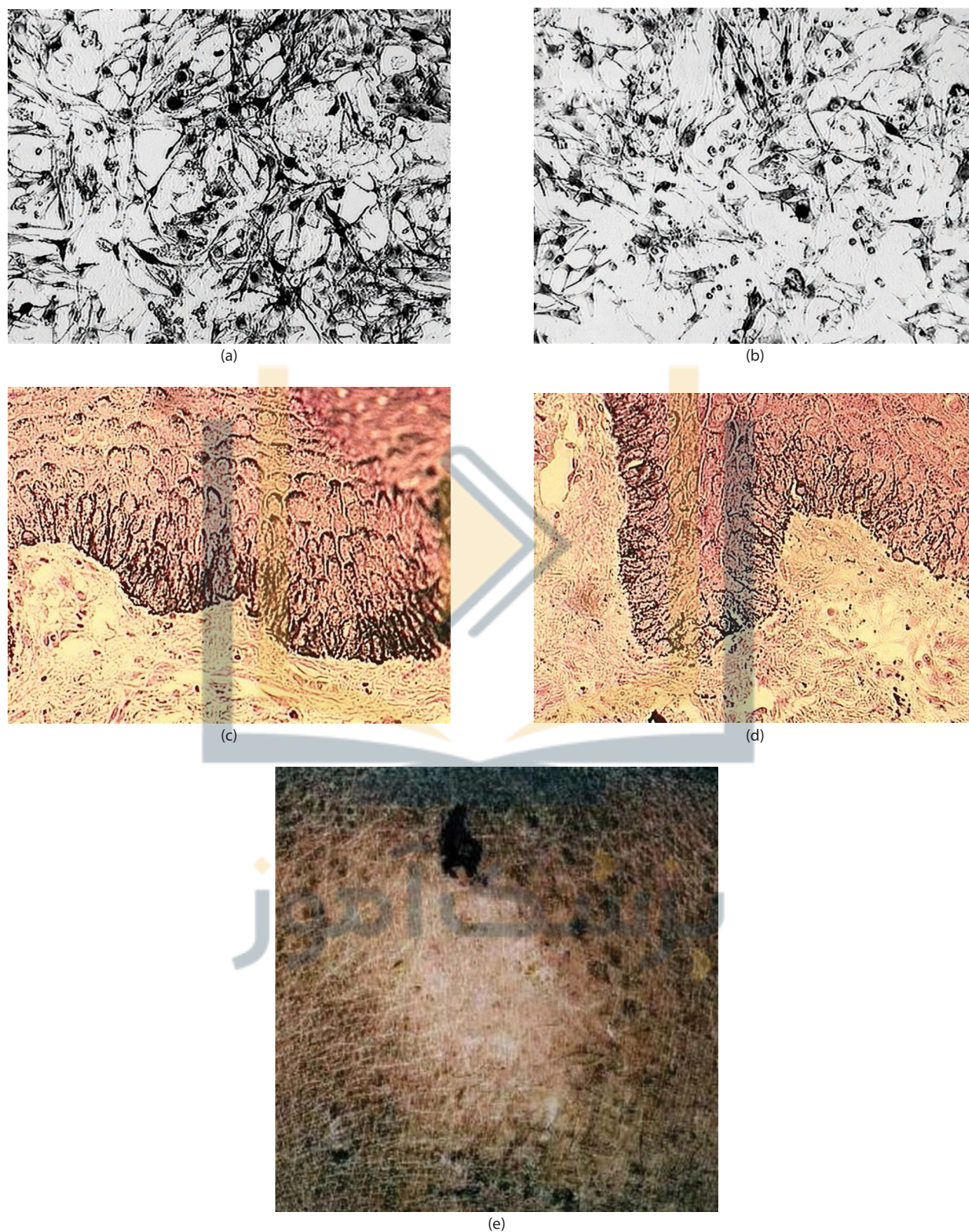
It was found that non-denatured soybean extracts, non-denatured soy, and the isolated soy-derived proteins STI and BBI inhibit PAR-2 activation, resulting in skin depigmentation (5). As shown in Figure 11.1, keratinocyte-melanocyte co-cultures treated with the soy protein STI have reduced tyrosinase activity, documented by DOPA staining (upper panel). Darkly-pigmented human skin grafted on immunodeficient mice and treated with STI showed reduced pigment deposition, as demonstrated by Fontana-Mason (F&M) staining of histological sections after five weeks of topical treatment (middle panel). F&M staining identifies silver nitrate-reducing molecules. In skin, this nonspecific stain identifies primarily melanin. Dark-skinned swine treated with the non-denatured soy extract displayed visible skin lightening following 8 weeks of treatment (lower panel). Therefore, agents such as STI, BBI, or non-denatured soybean extracts may serve as alternative, natural treatments for skin lightening or hyperpigmentation.

### Non-Denatured Soybean Extracts and Delayed Hair Growth

Mammalian hair provides environmental protection, but this function has been lost in humans, who keep or remove their hair at different body locations for social and cosmetic purposes. Many procedures are used to remove unwanted hair, ranging from simple and inexpensive treatments like shaving to costly and time-consuming methods such as electrolysis and laser therapies. Hair removal methods differ in the duration of the effect, pain and discomfort levels, and possible undesired side effects (39).

The hair follicle undergoes cycles of active growth (anagen), regression (catagen) and rest (telogen) (40). While the morphological changes throughout the hair cycle are well documented (41), the regulation of the different phases of this cycle is not completely understood. It was recently demonstrated that non-denatured soybean extracts and the soybean-derived





**Figure 11.1** The inhibition of PAR-2 activation by serine protease inhibitors results in reduced pigment deposition. Keratinocyte-melanocyte co-cultures treated with the soy protein STI have reduced tyrosinase activity, documented by DOPA staining (Top panels (a) Control vs. (b) Treated). Hyperpigmented human skin grafted on immunodeficient mice and treated with STI showed reduced pigment deposition, demonstrated by F&M staining of histological sections after 5 weeks of treatment [(c) control vs. (d) treated]. Dark-skinned swine treated with a non-denatured soy extract demonstrates visible skin lightening following 8 weeks of treatment (e).



proteins STI and BBI reduce not only hair pigmentation but also the rate of hair growth and the final dimensions of the hair shaft (5). Following non-denatured soy treatment, the onset of anagen was delayed, the duration of the hair cycle was reduced, and the resulting hair shafts were shorter, thinner, and softer. As shown in Figure 11.2, mouse hair treated with non-denatured soy showed reduction in the length of the hair shafts (upper panel). Hair follicle development (anagen) was delayed as seen following 7 days of treatment (middle panel). While the untreated follicles were completely developed, at this time point the soy-treated follicles were smaller and contained reduced levels of pigment. At day 15 of treatment, when all follicles had reached their final dimensions, the soy-treated follicles were significantly smaller (lower panel). While the soy isoflavones may play some role in the delayed hair growth by the non-denatured soy lotion, the ability of the non-denatured soy product to thin the hair and facilitate less-frequent hair removal seems to result primarily from the actions of two of the soy-derived proteins STI and BBI (5). These results indicate that agents such as non-denatured soy extracts may serve as an inexpensive, natural alternative treatment for undesired hair growth.

### Non-Denatured Soybean Extracts and Dermal Matrix Enhancement

Skin elasticity and elastin fiber production are reduced with aging (42–44) as elastin synthesis decreases and the secretion of elastases increases. UV exposure induces extensive elastin synthesis and crosslinking (“solar elastosis”), which add to skin’s rigidity. Sunscreens, antioxidants, and retinoids are promoted as “antiaging” agents, however they are not specific for repairing or enhancing skin elasticity. Inhibiting UV-induced fibroblast-derived elastases protected the dermal elastic network and reduced wrinkle formation in rodents (45–46). The non-denatured soybean extracts were found to have elastase-inhibitory activities (47), to induce the synthesis of both collagen and elastin, and to promote the correct assembly of newly-synthesized elastin fibers, suggesting both dermal protection and restoration of the dermal extracellular matrix.

Human skin, transplanted onto immunodeficient mice and topically treated with these extracts, showed an increase in the expression of elastin, elastin-accessory proteins, and collagen (47). Human skin explants treated ex-vivo with non-denatured soybean extracts showed an increase in collagen, elastin, and fibrillin-1 production by histological staining and immunohistochemistry (see Figure 11.3). These data suggest that non-denatured soybean extracts could induce collagen and elastin synthesis in human skin and provide cosmetic antiaging effects.

### Non-Denatured Soybean Extracts May Reduce the Risk of Non-Melanoma Skin Cancer Development

#### *Non-Denatured Soybean Extracts Inhibit the COX-2 Pathway*

Chronic inflammation is associated with numerous skin conditions and diseases, ranging from skin aging to epithelial skin tumors (reviewed in 48, 49). Cyclooxygenase-2 (COX-2) is involved in UV-induced skin inflammation and carcinogenesis (reviewed in 50, 51), and in UV-induced edema, epidermal hyperplasia, and the generation of oxidative DNA damage. Repeated UV exposure results in chronic upregulation of COX-2 expression and chronic inflammation, which accelerate skin aging and increase skin cancer risk. Indeed, aged human

skin produces higher amounts of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>, the product of the COX-2 pathway) relative to young skin (52). In animal models, oral and topical COX-2 inhibitors have chemopreventive activity against UV-induced skin cancer (reviewed in 50, 51). COX-2 expression is reduced in mouse skins upon the topical applications of soy isoflavones (53).

Non-denatured soybean extracts inhibit the COX-2 pathway (54). Cultured keratinocytes or epidermal equivalents were pretreated with non-denatured soybean extracts, washed, and then UV-exposed. As shown in Figure 11.4, UV irradiation induced COX-2 expression and PGE<sub>2</sub> secretion, which were reduced upon pretreatment with the soy extracts (54). When mice were topically treated with non-denatured soybean extracts, then washed and then UV-exposed, they had reduced levels of COX-2 expression in their skins as compared to mice exposed to UVB without soy pre-treatment (54). These data confirm the COX-2 inhibitory activity of the non-denatured soybean extracts in vivo and suggest their possible activity in delaying photoaging symptoms and skin cancer processes in human skin.

#### *Non-Denatured Soybean Extracts Reduce Skin Cancer Progression in High-Risk Mice*

Polyphenols, including green tea polyphenols, resveratrol, and soy isoflavones like genistein, have a photoprotective effect on UV-induced skin inflammation, oxidative stress, and DNA damage (55). The soy protein BBI is chemoprotective, and an organic extract of soybeans enriched in BBI has been evaluated in clinical studies (26). Mice fed with a non-denatured soymilk protein supplement had a reduction in the numbers and volumes of experimentally-induced skin tumors (56), however this was accompanied with undesired gastrointestinal effects. Since non-denatured soybean extracts contain isoflavones and BBI, and since they possess COX-2 inhibitory activity, the use of these extracts as topical chemopreventive agents was evaluated.

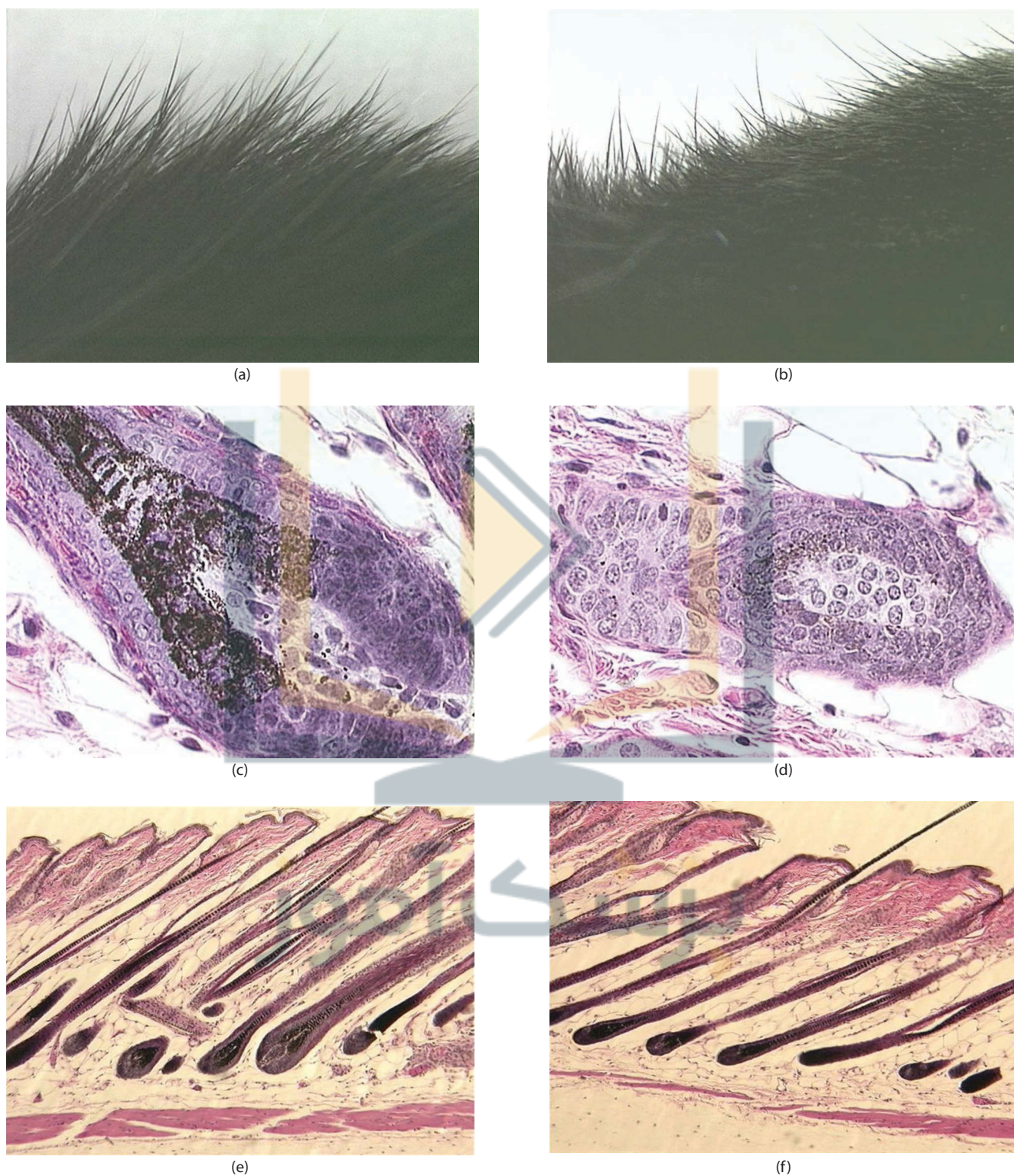
Hairless mice exposed to chronic low UV levels become “high-risk mice.” They are tumor-free, but with a high risk of developing skin tumors in the absence of additional UV exposure. High-risk mice were topically treated with non-denatured soymilk, heat-denatured soymilk, STI, or BBI, or with water or BSA as controls (57). Non-denatured soybean extracts, but not heat-denatured soybean extracts, reduced the incidence and slowed the growth and progression of skin tumors (see Figure 11.5). STI and BBI had a similar but reduced effect, suggesting that other components of the non-denatured extract provide additional protection. The number of tumor-bearing mice, tumors per mouse, and the volume of the visible part of the tumors were all markedly reduced upon the topical treatment of non-denatured soybean extracts (57). Histopathological examination documented numerous squamous cell carcinoma (SCC) lesions in the UV-exposed mice. The non-denatured soybean extracts-treated skins, in contrast, had very small lesions, with no dysplasia or carcinoma, which were not classified as SCC (Figure 11.5). These data might suggest that non-denatured soybean extracts could be topically applied to humans to reduce or prevent UV-induced skin damage and to reduce the risk of skin tumor formation and progression.

## NON-DENATURED SOY FOR COSMETIC DERMATOLOGY APPLICATIONS

### Non-Denatured Soy Composition

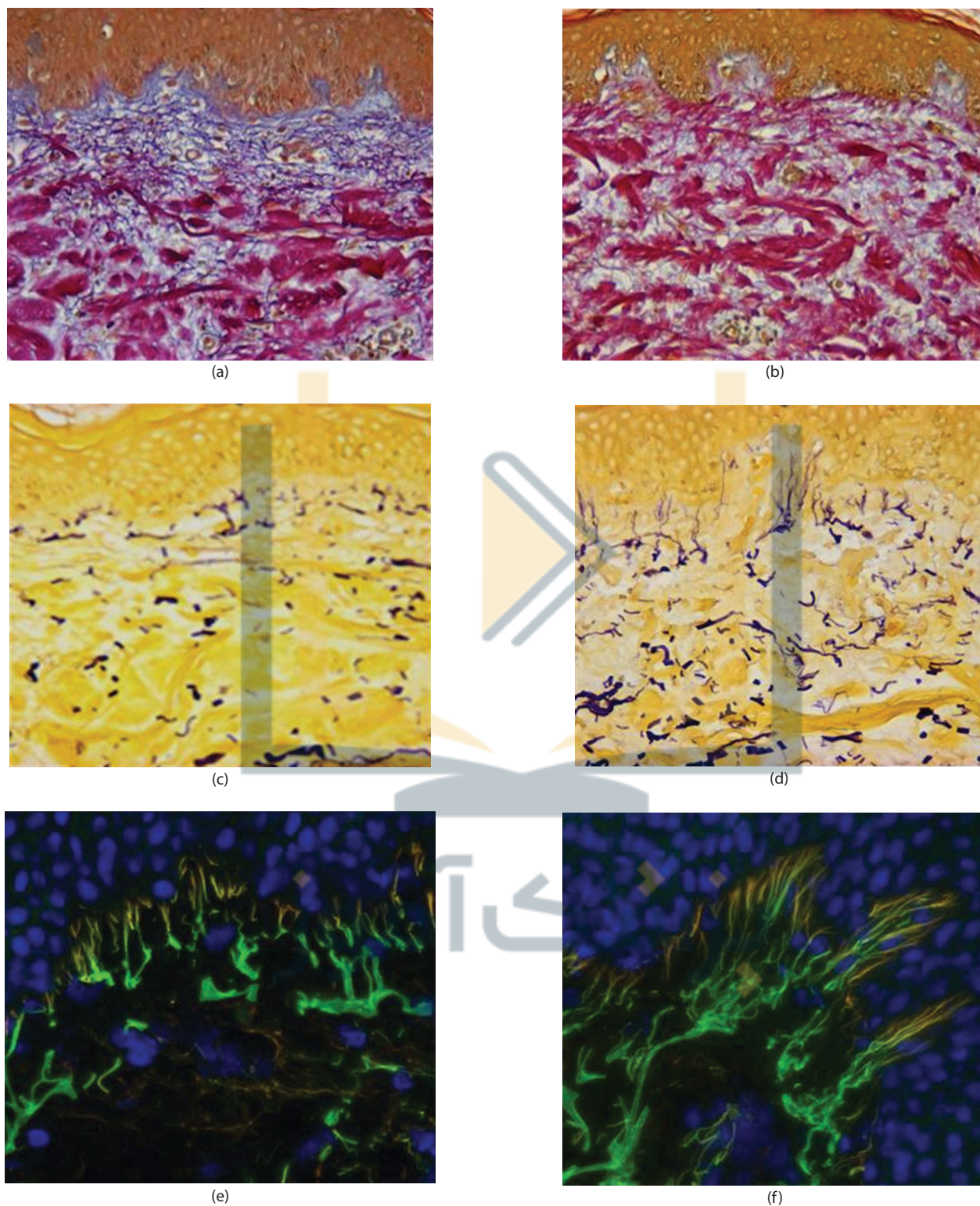
While the compositions of natural soybeans can inevitably be subject to variations, depending on the particular harvest, soil/region, and species variation, the basic chemical composition is similar, as illustrated in Table 11.2 (58). Mature soybeans



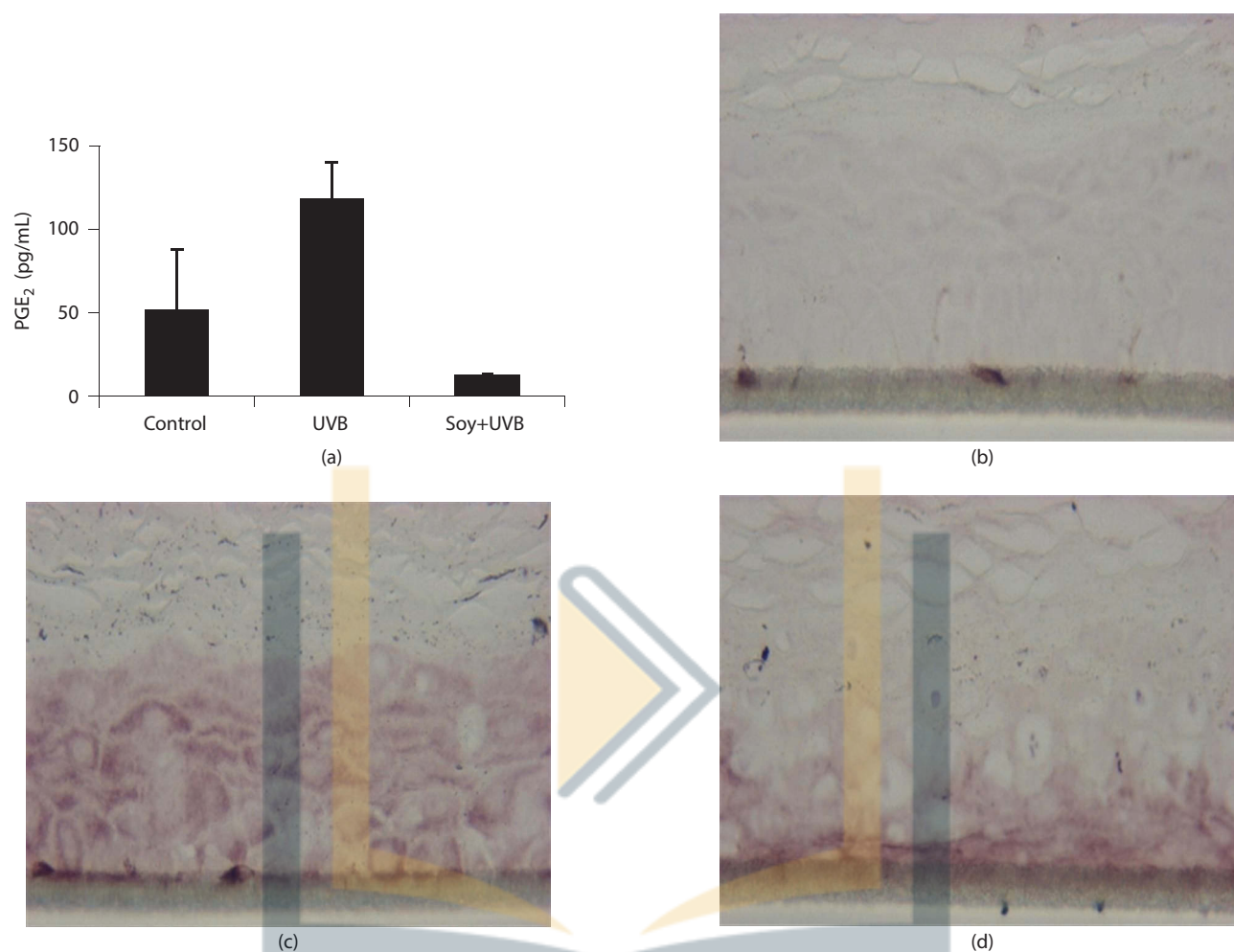


**Figure 11.2** Images of hair growth patterns under different conditions. (a) Untreated control and (b) treated illustrate reduction in the length of the mouse hair shafts treated with a non-denatured soy extract. (c) and (d) show delayed hair follicle development (anagen) as seen following 7 days of treatment. While the untreated follicles (c) were completely developed, at this time point the soy-treated follicles (d) were smaller and contained reduced levels of pigment. At day 15 of treatment, when all follicles had reached their final dimensions, the soy-treated follicles were significantly smaller (e and f).





**Figure 11.3** Non-denatured soybean extracts induce collagen and elastin synthesis. Human skin explants (obtained from surgical procedures with informed consent and institutional board approval) were maintained in culture for 8 days, with or without topical daily treatment, 5 days/week, using a base formulation with 2.5% non-denatured soybean extract. (a) Untreated control versus (b) treated Herovici staining documents mature collagen fibers (magenta-red). Topical treatment with non-denatured soybean extracts induces the production of collagen fibers. (c, d) Luna elastin staining documents mature elastin fibers (purple-brown). Topical treatment with non-denatured soybean extracts enhanced the elastic fiber network. (e, f) Immunohistochemical staining documents tropoelastin (the elastin fiber monomer, green) and fibrillin-1 (an elastin-accessory protein, red). Non-denatured soybean extracts induce tropoelastin and fibrillin-1 synthesis, and their co-localization (yellow) documents the site of elastin fiber formation. ([a,b] Images courtesy of Dr. CB Lin and D. Rossetti.)



**Figure 11.4** Non-denatured soybean extracts inhibit the COX-2 pathway. Epidermal equivalents were topically treated for 24 hours with 2% non-denatured soybean extracts in PBS or with PBS alone, and then UVB-exposed (100 mJ/cm<sup>2</sup>) as in (ref. 54). Samples were collected at 24 hours post UVB exposure for analysis. (a) Culture media were analyzed for PGE<sub>2</sub> secretion. Non-denatured soybean extracts inhibited the UV-induced secretion of the inflammatory mediator PGE<sub>2</sub>. (b–d) COX-2 staining of the treated equivalents: (b) PBS, (c) PBS followed by UVB, (d) 2% non-denatured soybean extracts followed by UVB. Non-denatured soybean extracts led to reduced COX-2 protein levels after UV exposure.

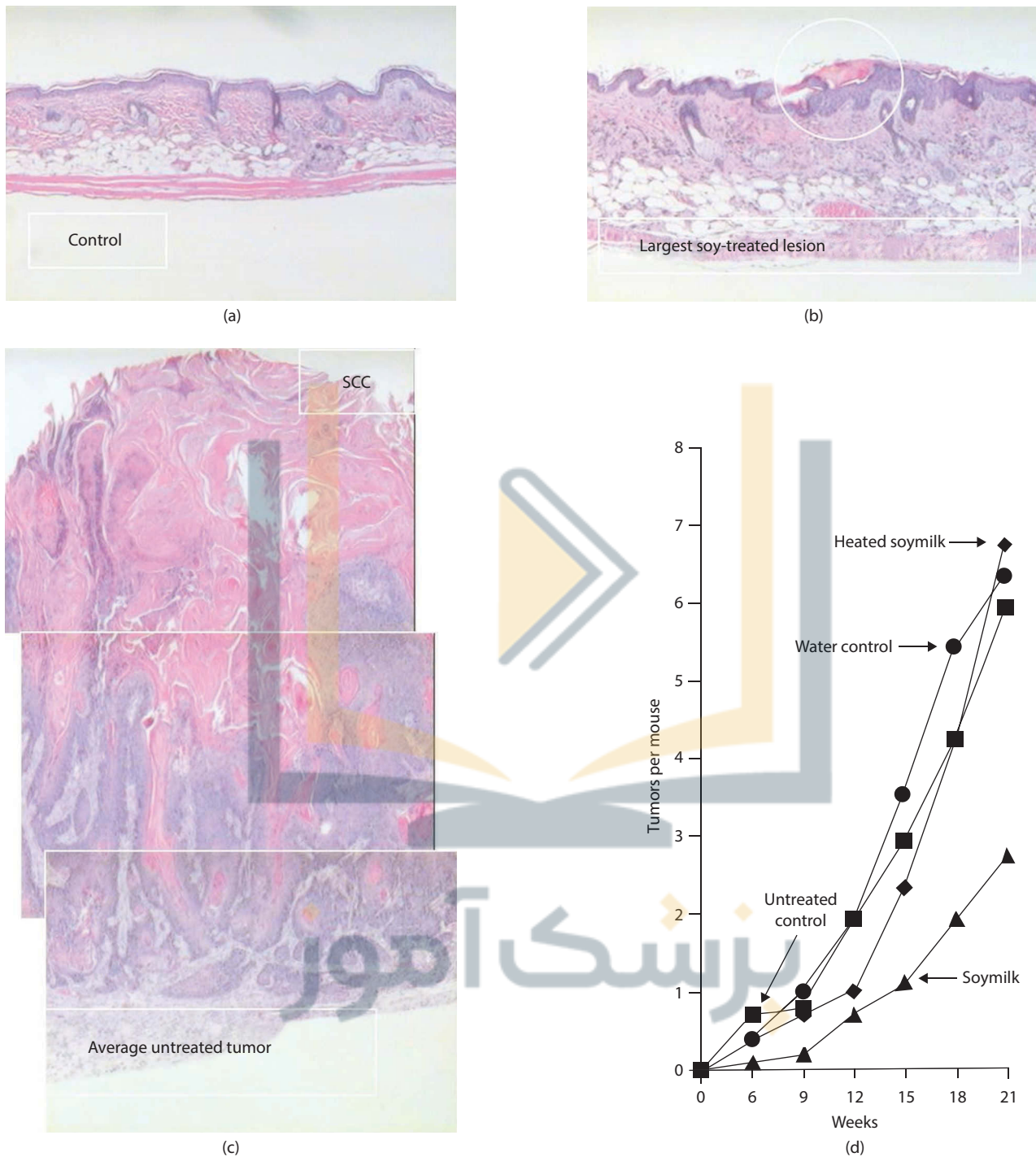
are composed of up to 36% proteins, 30% carbohydrates, and 20% lipids. The highly innovative non-denatured soy technology was created based on scientific findings. The non-denatured soy raw material was created from whole soybeans without using either chemicals or chemical solvents. The novel standardization of the manufacturing process results in a blend of balanced non-denatured components including active proteins, essential lipids, oligosaccharides, and other soy ingredients, which are in a similar proportion to that naturally present in the soybeans. The standardization process includes quality control such as measurements and lower limits of biochemical activities, of serine protease inhibitory activities.

It is well known that due to its natural origin, high levels of microorganisms are commonly associated with soybeans and soy products. Consequently, decontamination processes such as heat, organic/aqueous solvent extraction, and high shear purification are used to reduce microorganism concentrations in soy products, to allow soy ingredients to be safe

for human skin care applications. However, these processes frequently denature the active proteins in the soy, resulting in a compromised biological efficacy, e.g., a reduction in protease inhibitory activity. Furthermore, such processes also can lead to instability of the soy product as well as to an undesirable odor and color generation. A novel manufacturing process was developed for the non-denatured soy technology to reduce the levels of microbial contamination in non-denatured soy and maintain its protease (trypsin) inhibitory activity and preserve stability. This proprietary gamma irradiation process with optimized intensity and duration enabled reducing bioburden to attain safe levels while maintaining a stable non-denatured composition with full protease inhibitory activity, and with no color or odor changes.

Table 11.3 illustrates the non-denatured soy components and their potential applications in skin care. Non-denatured soy contains both non-denatured small and large soy proteins. The small soy proteins include STI, BBI, and lunasin, with their





**Figure 11.5** Non-denatured soybean extracts slow the progression of skin cancer in high-risk mice. SKH-1 mice were irradiated with UVB (30 mJ/cm<sup>2</sup>) twice weekly for 20 weeks and then UVB irradiation was stopped (as in ref. 57). Three weeks later, the mice were topically treated once a day, 5 days/week, for 21 weeks, with non-denatured soybean, heat-denatured soybean, or water control. (a) H&E staining of visible skin lesions and visually unaffected skins (same magnification): Control skin (not exposed to UV and not treated). (b) Largest visible lesion of a non-denatured soybean-treated skins. (c) An average lesion in the high-risk, UV-exposed and untreated group shows a well-differentiated SCC. Topical treatment with non-denatured soybean significantly reduced the severity of the developing skin lesions. (d) The number of visible lesions per mouse was determined every 3 weeks. Topical treatment with non-denatured soybean, but not with heat-denatured soybean, led to a marked reduction in the number of tumors per mouse. (Images courtesy of C. Paine.)



**Table 11.2** Soybean Composition Characteristics

Major components	Green/Raw % wt/wt	Mature seeds % wt/wt
Water	67	8
Proteins	12	36
Lipids	6	19
Carbohydrates	11	29
Fiber	4	8

Source: USDA National Nutrient Database for Standard Reference, 1999. Available at: <http://www.nal.usda.gov/fnic/foodcomp/search/>.

**Table 11.3** Non-Denatured Soy Components with Potential Applications in Skin Care

Soy components	Skin care applications	Reference(s)
Proteins 30%–50%	Small soy proteins (STI, BBI, Lunasin) Large soy proteins (e.g. glycinin 360 k-Da, $\beta$ -conglycinin 180 k-Da)	Depigmenting and delay hair growth Skin softening and smoothing (3–5, 37–38, 63) (60)
Lipids 10%–30%	Essential fatty acids (linoleic, linolenic, oleic acids)	Antioxidant protection, skin lightening, restore barrier function (60–62)
Carbohydrates	Lecithins/ phospholipids Di- and oligosaccharides and polysaccharides	Skin moisture, cleansing Skin hydration (60) (60)
Minor components	Soy flavones (e.g. Daidzein, genistein, daidzin, genistin) Phytosterols	Weak antioxidant and anti-inflammatory, inhibition of tyrosine Skin moisture, anti-inflammatory (64–67) (68–69)
	Vitamins (tocopherols) Minerals	Antioxidant Deficient can cause untoward effects (60) (71)
Others	Saponins Phytic acid	Cleansing Depigmentating (60, 72) (60)

depigmenting activity retained and quantified (3–4). Unlike trypsin inhibitors of soy protein nature, the non-protein trypsin inhibitors lack specificity and are very susceptible to cationic suppression (59). The large proteins of the non-denatured soy extract include hundreds of proteins with varying molecular weight up to millions Daltons. The most abundant proteins are glycinin and conglycinin, with molecular weight around 180 k-Da and 360 k-Da (60). Each protein group precipitates at different pH values, which may affect topical formulation preparations. Non-denatured soy contains essential fatty acids, including linoleic acid and linolenic acids, which are essential to restore stratum corneum barrier functions and may potentially help acne resolution. These unsaturated fatty acids may also provide antioxidant properties (61). In addition, linoleic acid is known to enhance skin penetration of various compounds (62). Crude soybean oil contains 1%–3% phospholipids, which are the major components of cell membranes (63). Phospholipids can also form liposome, a vesicle delivery system that could enhance skin penetration for both hydrophilic and lipophilic large molecules such as proteins, and small such as alpha-hydroxy acids. Non-denatured soy contains about 30% carbohydrates, which can provide skin hydration properties.

Non-denatured soy also contains minor components like isoflavones, vitamins, and minerals, which could offer important skin care benefits. Isoflavones have been reported to provide a number of skin care benefits. They have weak antioxidant and anti-inflammatory activities, which are important to combat oxidative stresses induced by the sun. A number of studies have indicated isoflavones can prevent and treat sun-induced cancer (64–65). Isoflavones such as genistein and daidzein, normally classified as phytoestrogens, originate in plants, unlike estrogens, which are of animal origin. The isoflavone structures are nonsteroidal, while all estrogenic hormones are steroidal (66–67). Soybean oil can contain as much as 0.37% of phytosterols, which are mostly in unsaponifiable forms (68). The most significant phytosterols found in oils and fats are campesterol, stigmasterol, beta-sitosterol, delta 5-avenasterol, and delta7-stigmasterol. Phytosterols are reported to provide anti-inflammatory effects via the arachidonic cascade. Clinical applications can be found for antipruritic effects, reducing sun-induced erythema, and diaper dermatitis (68–69). Such characteristics combined with the conditioned use make them possible alternatives to cortisone and corticosteroids, as well as silicone replacement. Soybeans contain both water-soluble and oil-soluble vitamins. The water-soluble vitamins present in

soybeans mainly include thiamin, riboflavin, niacin, pantothenic acid, and folic acid. The oil-soluble vitamins found in soybeans are vitamin A and E. Soybean oil contains a substantial amount of vitamin E, which provides effective natural antioxidant and nutrition values (70). Soy contains relatively high levels of desired metal ions such as iron, zinc, manganese, magnesium, and calcium that are important in skin health. Metal ions play an important role in skin health. Deficiency in certain metal ions in the skin will result in unwanted effects such as dermatitis, discoloration of hair, and retardation of hair growth. Optimal metal ions such as manganese, magnesium, calcium, and zinc are needed to maintain skin health (71). Soy saponins (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-one) contain carboxyl groups, which have surface activity for foaming and emulsifying power in cosmetic formulation (72), and are also reported to provide an active oxygen-scavenging effect in the skin and maintain healthy epidermal conditions (73).

### Safety of Non-Denatured Soy for Human Topical Applications

It is known that some naturally derived ingredients can elicit irritant or allergic responses. Soy has been used for centuries and is well known to be safe for human use. A systematical safety evaluation has been completed to determine the clinical topical profiles of the non-denatured soy raw material and the non-denatured soy-containing skin care formulations (74). Clinical cumulative irritation evaluations utilized two different methodologies that consisted of patching the subject with the formulation over a 2–3 week time period. Method A was conducted to assess irritancy, in which a minimum of 25 subjects was patched continuously for a total of six applications over a 2-week period. Method B was conducted to assess comparative irritation potential of the formulations, in which approximately 200 subjects were patched intermittently with the formulation three times a week for a total of nine applications over a 3-week period. All test sites were graded three times a week after patch removal. Using a scale of potential total irritation score of 660, both non-denatured soy and all skin care formulations with soy resulted in negligible cumulative irritation score <10. Comparative cumulative irritation of non-denatured soy and vitamin formulas also demonstrated negligible irritation. Non-denatured soy formulations were clinically evaluated with the repeated insult patch test (RIPT). These studies demonstrated that specific topical formulations containing the non-denatured soy alone, soy extract, or soy extract in combination with vitamins A and C did not induce contact dermal sensitization. Photosensitivity was also assessed by photoallergy and phototoxicity tests, which did not find any photosensitivity when non-denatured soy was applied. Recently, soy isoflavones have drawn attention for their potential phytoestrogenic effects. Soy isoflavones differ significantly in terms of their molecular structure from estrogens, such as estradiol, and they are not metabolized to these estrogens. Experimental data suggest that isoflavones exhibit an estrogenic-like potency of between 1000 and 100,000 times lower than estradiol, depending on the nature of the assay used. (75–77). Largely as a result of research in in-vitro or in animal models, concerns have been voiced regarding isoflavone use in soy products and soy infant formulas in relation to nutritional adequacy, sexual development, neurobehavioral development, immune function, and thyroid disease. Available evidence from adult human and infant populations indicates that dietary isoflavone consumption in soy infant

formulas does not adversely affect human growth, development, or reproduction (78).

The non-denatured soy products contain much lower concentrations of isoflavones, typically less than 0.01% soy isoflavones, which is negligible compared to the level of phytoestrogen intake by average consumers in most Western countries. By way of example, following European guidelines, the estimated maximum exaggerated topical exposure from non-denatured soy products (0.96 mg/day) is considerably lower than the measured dietary intake for mid-life Australian women (~ 17 mg/day) (79), which is, in turn, less than the exposure by infants fed soy-based formula milk (22–45 mg/day) (80). Moreover, the potential systemic isoflavone exposure from the non-denatured soy products (assuming 60 kg body weight, 0.00208 mg/kg/day = 2.08 ng/g/day) is insignificant when compared to typical systemic concentrations. For example, the endogenous systemic plasma baseline concentration for U.S. adult women is 7.94–34.54 ng/mL (81) and for UK adult women consuming 20 g roasted soybean products, the systemic concentration is 540 ng/mL genistein (82).

Broadly speaking, however, a rank order may be determined of daily isoflavone intake: soy formula fed infant (approximately 40 mg/day), average Japanese consumer (approximately 25–100 mg/day), vegetarian consumer (approximately 3 mg/day), average consumer (approximately 1 mg/day) (109). There is no data documenting that soy extract or soy oil has estrogenic effects when applied to the skin as it might when taken orally at high doses (83).

A new longer-term (2 years), multicenter, randomized, double-blind, placebo-controlled clinical trial assessed the effects of daily high level of supplementation with 80 or 120 mg aglycone equivalent soy hypocotyl isoflavones plus calcium and vitamin D on the health of 403 postmenopausal women (84). At baseline and after 1 and 2 y, clinical blood chemistry values were measured and a well-woman examination was conducted, which included a mammogram and a Papanicolaou test. A cohort also underwent transvaginal ultrasound measurements to assess endometrial thickness and fibroids. The baseline characteristics of the groups were similar. After 2 y of daily isoflavone exposure, all clinical chemistry values remained within the normal range. Isoflavone supplementation did not affect blood lymphocyte or serum free thyroxine concentrations. No significant differences in endometrial thickness or fibroids were observed between the groups. It concluded that daily supplementation for 2 y with 80–120 mg soy hypocotyl isoflavones has minimal risk in healthy menopausal women.

A placebo controlled in-vitro dermal absorption study using human cadaver skin found that the potential systemic soy isoflavones absorption from topical applications of non-denatured soy products is below the detection limits of the current state-of-the-art analytical instrumentation and negligible compared to any endogenous levels of isoflavones resulting from dietary soy consumptions or supplement consumptions of infant soy milk. The potential local epidermal and dermal accumulations for soy isoflavones are estimated to be at least 2500 times lower than the no-observed adverse effect level (NOAEL) limit. In summary, soy isoflavones are naturally occurring in non-denatured soy at trace levels, and their potential systemic absorption is negligible and presents no harmful risks to human health.

Council of Europe guidance on the use of plants in cosmetics specifically endorses the use of soy extracts. With particular reference to phytoestrogens, guidance from 1994

provides that “the use of a natural ingredient in cosmetic products is recommended only if the finished products are free of estrogenic activity” (85). More recent guidance explicitly confirms that glycine soja (soy) ingredients including soy extracts containing isoflavones and phytoestrogens, can be used in cosmetics. It classifies them as “category A” ingredients (i.e. ingredients that are considered to be safe) and specifically envisages their use in concentrations up to 5%.

The official International Nomenclature of Cosmetic Ingredients (INCI) confirms that soy extracts may be used in cosmetics (<http://www.cosmeticsinfo.org/ingredient/glycine-soja-soybean-protein-and-related-ingredients>). The entry for glycine soja covers the following: “Soybean oil. Extractives and their physically modified derivatives. It consists primarily of the glycerides of the fatty acids linoleic, oleic, palmitic and stearic. (*Soja hispida*, Leguminosae).” The official INCI list also lists other permitted plants and their extracts that are known to contain phytoestrogens, such as *Daucus carota*, *Hedera helix*, and *Humulus lupulus*. All four plants are also referred to by the Council of Europe as containing phytoestrogens (85).

There is an increasing public concern that genetically modified (GMO) plants may be harmful, and some scientific publications claiming danger have received attention in the media. However, there is a broad scientific consensus that GMO plants on the market poses no greater risk to human health than conventional plants. Nevertheless, consumer prefers non-GMO soybean or conventional harvested soybean-derived products. Non-denatured soy uses high quality non-GMO soybean to address consumer’s needs.

### Preclinical Results of Non-Denatured Soy

Non-denatured soy has been found to show multiple bioactivities including trypsin-inhibitory activity, stimulating collagen and elastin production, anti-inflammatory, antioxidant, anti-stress/thiol retention, and anti-UVB damage activity. A summary of the biological activities of non-denatured soybean extracts and their relevance to skin health and beauty can be found in reference 86.

#### Trypsin Inhibitory Activity of Non-Denatured Soy

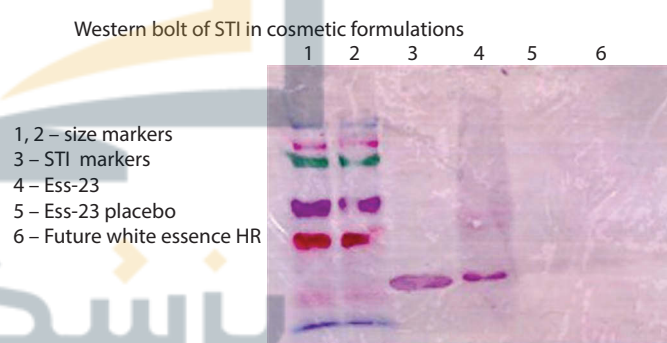
Soy preparations containing various levels of non-denatured soymilk resulted in a dose-dependent inhibition of trypsin activity, measured by an in-vitro fluorescence assay. A stabilized soy formulation containing non-denatured soy protein STI was developed (87). The stability of the STI was monitored by enzyme inhibition assay and Western blot. The results illustrate that the soy formulation retained the serine protease inhibitory activity. The STI protein was shown to be intact by Western blot analysis after 1 year at room temperature (Figure 11.6).

#### Collagen Production

Preclinical studies of a non-denatured soy composition were investigated for in-vitro collagen production via monitoring normal human dermal fibroblast synthesis method. Table 11.4 shows that non-denatured soy could stimulate collagen synthesis (88–89). For a non-denatured soy concentration as low as 0.01 mg/mL, the increase rate in the fibroblasts was found to be 33% after 72 hours of non-denatured soy action at 37°C in a humidified atmosphere.

#### Elastin Enhancement

The effect of non-denatured soy on elastin production was evaluated preclinically on swine skin (88). The histological analysis from these elastin evaluations demonstrates an increase in fine and highly branched elastin fibers after non-denatured soy composition applications, as shown in Figure 11.7, suggesting the capability of non-denatured soy to enhance skin elasticity. Swine studies with the application of non-denatured soy composition, twice a day, 5 days/week, for 9 weeks showed no visual irritation, and histological analyses revealed no markers of irritation or other pathological signs. Sections from biopsies were stained with Luna stain, to document elastin fibers. At least three sections per biopsy, two sites per swine were processed. Each experiment was repeated at least three times. Histological analysis of Luna-stained sections demonstrates an increase in fine and highly branched elastin fibers at the upper part of the dermis following soy treatments as shown in Figure 11.7. This increase in elastin



**Figure 11.6** Western blots comparing a non-denaturing soy (Total Soy) product to a soy product using a single soy component. According to Western blot analysis, the STI protein was compromised for soy products using a single soy component. The STI protein was intact after 1 year at room temperature for the non-denatured soy preparation.

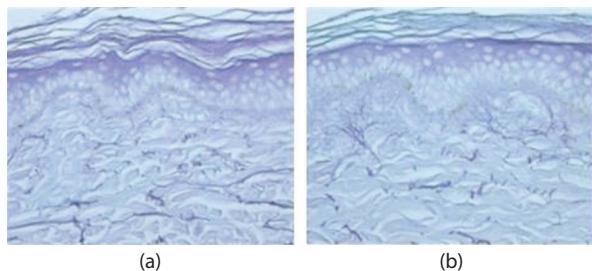
**Table 11.4** Effect of Non-Denatured Soy Complex on Extracellular Collagen Synthesis in Normal Human Dermal Fibroblasts

	Control	Non-denatured soy (µg/mL)		
		0.01	0.1	1
Collagen synthesis (dpm/cell) ×1000*1000	11.26 ± 1.62	15.0 ± 1.46	13.68 ± 2.51	17.80 ± 2.01
Stimulation		NS	NS	P < 0.05
		+ 33 %	+ 21 %	+ 58 %

Abbreviation: NS: nonsignificant

\* dpm = disintegration per minute, a unit measuring radioactivity level. The collagen synthesis assay uses a radioactive precursor and measures radioactivity of the collagen product.





**Figure 11.7** (a, b) Histological analysis of Luna-stained sections demonstrates an increase in fine and highly branched elastin fibers at the upper part of the dermis following soy treatments as displayed.

staining resembles a “repair zone,” as documented for the effect of retinoids on UV-damaged skin (90).

#### *Anti-Inflammatory and Antioxidant Activities*

The non-denatured soy extract was found to be active against the acute oxazolone application on mouse ear edema (91). Oxazolone was used to induce contact hypersensitivity or edema in mouse ear and the inhibition of edema was used to determine the degree of anti-inflammation activity. The studies showed that a 2% non-denatured soy lotion induced about 56% edema inhibition vs. 8% for its placebo control. As a comparison, 0.1% hydrocortisone induced an inhibition of 86%. The non-denatured soy extract was also found to have antioxidant activity.

#### *Anti-Stress/Thiol Retention Activity*

Non-denatured soy was found to maintain normal cell metabolism, even when exposed to a harsh environments such as environmental pollution, e.g. smoke-induced loss of thiols (92). The ability of non-denatured soy to prevent smoke-induced loss of thiols was evaluated in normal human dermal fibroblasts (Clonetics, San Diego, CA). Thiols, chiefly glutathione (GSH), are part of the endogenous cellular antioxidant defense system. GSH serves as a redox buffer, thereby maintaining the balance between oxidants and antioxidants (93). GSH is also the preferred substrate for several enzymes such as the GSH peroxidases (decomposing peroxides) and the glutathione-S-transferases (a major group of detoxification enzymes) (94).

Cutaneous antioxidants (both enzymatic and non-enzymatic), including GSH, are depleted after UV or ozone exposure (95–96). In cell culture models, low intracellular GSH levels lead to a higher UV radiation sensitivity. Glutathione is a major endogenous antioxidant, highly responsive against environmental challenges, able to regulate the tone and the wrinkling of skin, as well as treat external aggression.

The effects of non-denatured soy in preventing smoke-induced stress are displayed in Table 11.5. The results indicate that non-denatured soy afforded protection against smoke-induced loss of thiols or thiol retention activity. Data represent the mean  $\pm$  standard of the mean of replicates from three independent experiments. Thiol retention activity is the ability of the non-denatured soy at a concentration of 1% (w/v) to inhibit smoke-induced loss of thiols, as measured by the above assay.

#### *Reduction in UVB-Induced Skin Damage*

The incidence of non-melanoma skin cancers is increasing, and agents that can prevent or reduce UVB-induced skin cancer are

**Table 11.5** Thiol Retention Activity Measurement for Non-Denatured Soy

Environmental stress	Non-denatured soy complex concentration (weight %)	Thiol retention activity %*
No Smoke	0	100 $\pm$ 6.71
Smoke (10 min.)	0	65.38 $\pm$ 7.16
	0.5	91.24 $\pm$ 14.25
	1	95.39 $\pm$ 4.52
	2	106.92 $\pm$ 17.06

\*Thiol retention activity % (% thiols contained in No Smoke Group; mean  $\pm$  S.E.M.)

desired. The BBI protein is a known cancer suppressive agent that is effective in many different species, in different organs and tissues, and when given via different routes of administration (26). Recent studies showed that pretreatment with non-denatured soy extracts, BBI, and STI proteins reduced UVB-induced skin tumor formation and progression in high-risk hairless mice with low dose of UVB pretreatment for a long time (58). In contrast, denatured soy extracts were found to have no effects on skin tumor formation and progression.

Multiple mechanisms of action were identified for non-denatured soybean extracts. In vitro, non-denatured soybean extracts enhanced UVB-induced checkpoint kinase-1 (Ck1) activation, suggesting a delay in cell cycle progression that enables longer time for DNA repair. Non-denatured soybean extracts display anti-inflammatory activity via reduced UVB-induced COX-2 expression and prostaglandin E2 secretion, and inhibited p-38 MAP kinase activation. Mice pretreated topically with non-denatured soybean extracts had reduced levels of UVB-induced TT dimers and COX-2 expression in their skins compared with UVB alone. Non-denatured soybean extracts also inhibited vascular endothelial growth factor (VEGF)-induced endothelial tube formation in Matrigel, suggesting a possible inhibitory effect on angiogenesis and tumor progression. These findings suggest that topical application of non-denatured soybean extracts could potentially reduce the incidence of skin cancer, via multiple molecular mechanisms, at both the tumor initiation and tumor progression stages (54).

#### *Non-Denatured Soybean Extracts Reduce Skin Cancer Risk by Multiple Mechanisms*

Non-denatured soybean extracts contain multiple agents and are expected therefore to act by multiple mechanisms. Mechanism-of-action studies identified effects of these extracts both at the initiation stage and during the progression of skin tumors, with multiple mechanisms affecting the tumor and the microenvironment (54). Hairless mice and Yucatan swine were topically treated with non-denatured soybean extracts for several days prior to UVB exposure. Histochemical staining documented reduced formation of UVB-induced DNA damage (T-T dimers) and reduced numbers of apoptotic cells. Non-denatured soybean extracts inhibited matrix metalloproteinase (MMP) expression in vivo, suggesting the inhibition of dermal ECM degradation and remodeling, which are required for tumor progression. In vitro, these extracts inhibited VEGF-induced endothelial tube formation in Matrigel,



suggesting a possible inhibitory effect on angiogenesis and tumor progression. The ability to inhibit multiple proteases and to enhance elastin and collagen production (47), the ability to inhibit the COX-2 pathway (54), and the content of intact STI and BBI suggest that non-denatured soybean extracts could affect the tumor and the microenvironment at multiple stages of skin cancer initiation and development. Topical treatment with non-denatured soybean extracts could therefore reduce UVB-induced DNA and cellular damage, and reduce microenvironment processes affecting tumor progression, suggesting that daily topical treatment could reduce the risk of skin cancer development.

#### *Reduction in Visible Light–Induced Skin Oxidative Stress*

A new study (97) demonstrated that irradiation of human skin equivalents with visible light (400–700 nm) could induce the production of reactive oxygen species (ROS), pro-inflammatory cytokines, and MMP-1 expression. Human subjects were exposed to visible light, and chemiluminescence was measured as a marker of ROS. A 50 Jcm<sup>-2</sup> dose of visible light at 150 mWcm<sup>-2</sup> significantly increased free-radical production ( $p < 0.05$ ), compared with untreated skin (N = 40). Commercially available sunscreens were found to have minimal effects on reducing visible light-induced ROS, suggesting that UVA/UVB sunscreens do not protect the skin from visible light-induced responses. However, pretreatment with a photo-stable UVA/UVB sunscreen containing an antioxidant combination of non-denatured soy extract, feverfew (*Tanacetum parthenium*) extract, and Gamma tocopherol, compared to the sunscreen alone (N = 12 per group of sunscreens), significantly reduced the production of ROS, cytokines, and MMP expression *in vitro*, and decreased oxidative stress in human subjects after visible light irradiation at 65 J/cm<sup>2</sup>.

### **Clinical Efficacy of Non-Denatured Soy**

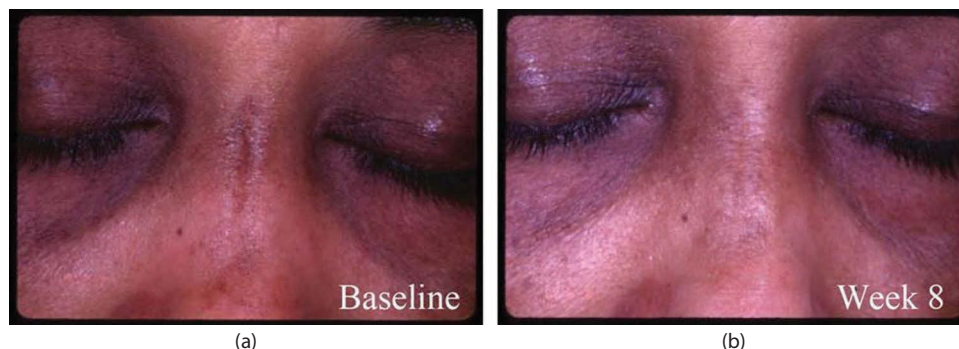
#### *Reduction of Hyperpigmented Spots*

A stabilized non-denatured soy formulation was tested on a Caucasian male population with solar lentigine hyperpigmented lesions (98). The effect of stabilized non-denatured soy formulation was compared with that of 15% azelaic acid or 12% glycolic acid. After 3 weeks of once-daily treatment, the pigmented lesions were significantly lightened. The effect of stabilized non-denatured soy formulation is comparable to or better than either to 15% azelaic acid or 12% glycolic acid according

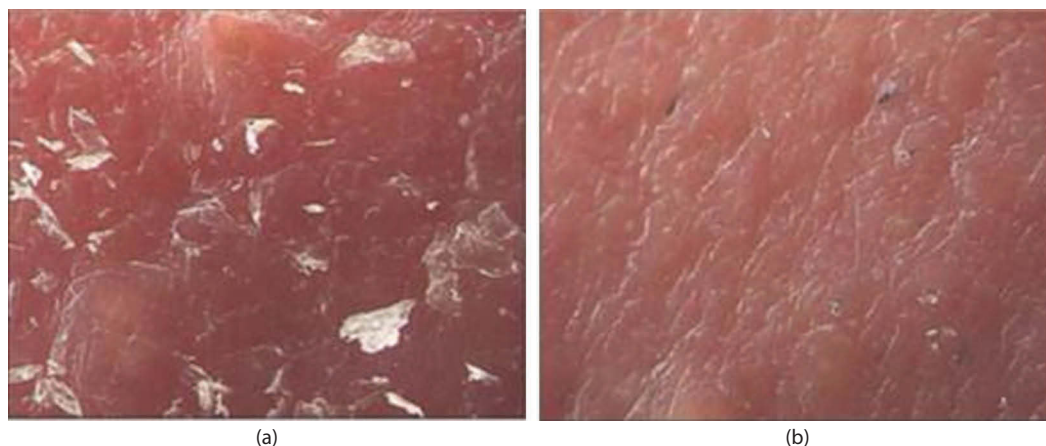
to dermatological grading. In another open-label clinical study on a Caucasian population with various types of pigmented lesions, the stabilized non-denatured soy formulation was found to improve 78% tested subjects in dermatological grading (Figure 11.8).

#### *Reduction of Facial Lentigines (Age Spots)*

Manifestations of photodamage are multidimensional, including facial lentigines, mottled hyperpigmentation, skin tone deterioration (sallowness), and skin texture deterioration with the appearance of fine lines, coarse and fine wrinkles, and skin roughness. Overall, photodamage has many visible characteristics. There are numerous prescription and OTC cosmetics that target photodamage. Certain prescription products such as hydroquinone are effective in targeting hyperpigmented lesions but result in unknown side effects. There is a strong need for products which are effective over a broader range of photodamage symptoms as well as being gentle to the photodamaged skin. The non-denatured soy preparation was proven to reduce the appearance of solar lentigines. A 12-week randomized, blind, half-face benchmark-controlled clinical study was conducted on N = 52 skin Type I-III healthy subjects with solar lentigines/mild-moderate photodamage (2–5 on a 9 scale) to evaluate the clinical efficacy of a moisturizer lotion containing 2% non-denatured soy in reducing the appearance of solar lentigines (99). Melanex (3% hydroquinone) was used as a benchmark. Negligible signs of irritation (rash, erythema, edema, stinging, burning, and itching) or sensitization were reported in the non-denatured soy-treated population. Non-denatured soy lotion had significant ( $p < 0.01$ ) improvement in facial lentigines, overall photodamage, mottled hyperpigmentation, and skin sallowness from week 4 vs. baseline. The non-denatured soy lotion had no significant difference in overall photodamage ( $p > 0.71$ ) vs. Melanex. At 8 weeks, facial age spot and brown spot reduction was observed by 48% of the subjects for non-denatured soy lotion. In the same clinical study, non-denatured soy also had a significant improvement in coarse wrinkles from week 8 ( $p = 0.057$ ) and significant improvement in laxity ( $p = 0.057$ ) at week 12 vs. baseline. Non-denatured soy was significantly superior to Melanex in surface roughness reduction ( $p < 0.056$ ) and in facial sallowness reduction ( $p < 0.02$ ) at the end of the study (week 12). These results suggest that the non-denatured soy formulation may be considered for reducing lentigines or age spots.



**Figure 11.8** (a) A non-denatured soy formulation containing STI was tested on a female population with hyperpigmented lesions ( $n = 42$ ). (b) After 8 weeks of once-daily treatment on the selected lesions, the pigmented lesions were significantly lightened, thereby evening the appearance of skin tone.



**Figure 11.9** (a) A Caucasian male volunteer treated half of his face with soy once daily for 6 weeks followed by an 8-hour exposure (b). The measurement was conducted at 24 hours post exposure.

#### *Reduction of Ultraviolet-Induced Damage*

Other proven benefits include reducing UV-induced erythema and flakiness (100–101) (Figure 11.9). Based upon the preclinical learning of non-denatured soy in preventing UV-induced pigmentation, a topical non-denatured soy formulation was tested in a placebo controlled clinical study on Type I and III skins with 0.8–2 MED UV irradiation. Both pretreatment and post treatment were performed on six different subjects, each for 5 consecutive days. Evaluation techniques employed to determine the effect of non-denatured soy on the irradiation included visual clinical assessment, diffused reflectance spectroscopy, photo imaging, and desquamation taping. Observations were made at 24 hours and 7 days post treatment. Dermatologists' clinical assessment displayed that an immediate application of non-denatured soy reduced redness induced by UV irradiation at 0.8–1.5 MED. Clinical assessment also indicated that a consecutive 5-day application of non-denatured soy protected skin from UV-induced redness. Diffuse reflectance spectroscopy further illustrated that reduction of redness occurred at the highest irradiation dose (2 MEDs) in this clinical study. In follow-up measurements at 7 days post irradiation, it was found that flakiness existed for some of the control sites (placebo or untreated), however none was found in both preventive treatment and post treatment. The study clearly demonstrates the usefulness and potential of non-denatured soy for daily defense against sun irradiation.

#### *Non-Denatured Soy and UVA/UVB Protection*

A double-blind, placebo-controlled clinical study was performed to determine the benefits of using a daily non-denatured soy facial preparation with broad spectrum SPF 30 in improving various skin tone and textural parameters (102–104). Sixty-three panelists between the ages of 30 and 50 exhibiting moderate levels of skin roughness, blotchiness, and mottled hyperpigmentation were enrolled into the 12-week study. Dermatologist evaluations, self-assessments, and instrumental analysis were completed at various time points during the study. Dermatologist evaluations demonstrated significant improvements ( $p < 0.05$ ) in skin roughness, clarity, and mottled hyperpigmentation after 2 weeks' use of the non-denatured soy facial preparation containing SPF 30. Significant improvements ( $p < 0.05$ ) in mottled

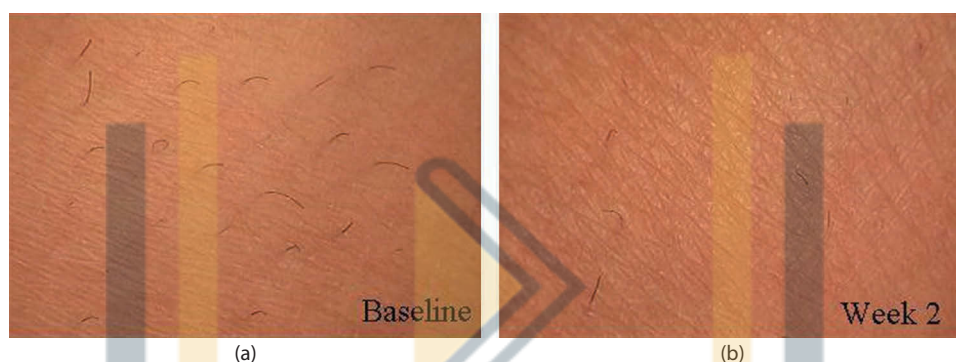
hyperpigmentation, blotchiness, appearance of fine lines, and overall skin tone and texture were observed vs. the placebo control group after only 2 weeks of use. Non-denatured soy showed improvements in mottled hyperpigmentation, blotchiness, and fine lines when compared to the placebo control group and baseline mean values. After 4 weeks of use there was over a 35% mean improvement in skin blotchiness and clarity of the skin. Colorimetry showed a significant increase ( $p < 0.05$ ) in skin luminosity with a significant decrease ( $p < 0.05$ ) in yellowness, correlating to an improvement in skin brightness and overall skin tone. Self-assessments showed that subjects began to perceive significant improvements ( $p < 0.05$ ) in various skin tone, texture, and brightness parameters as early as 1 week of using the non-denatured soy and SPF 30 facial moisturizer. Additional studies showed that the non-denatured soy and SPF 30 moisturizer was noncomedogenic, gentle to the skin, and did not induce dermal sensitization. The sunscreens used in this non-denatured soy moisturizer have been shown to be compatible with non-denatured soy and photostable in both clinical and scientific studies (102–103).

#### *Delay in Hair Regrowth*

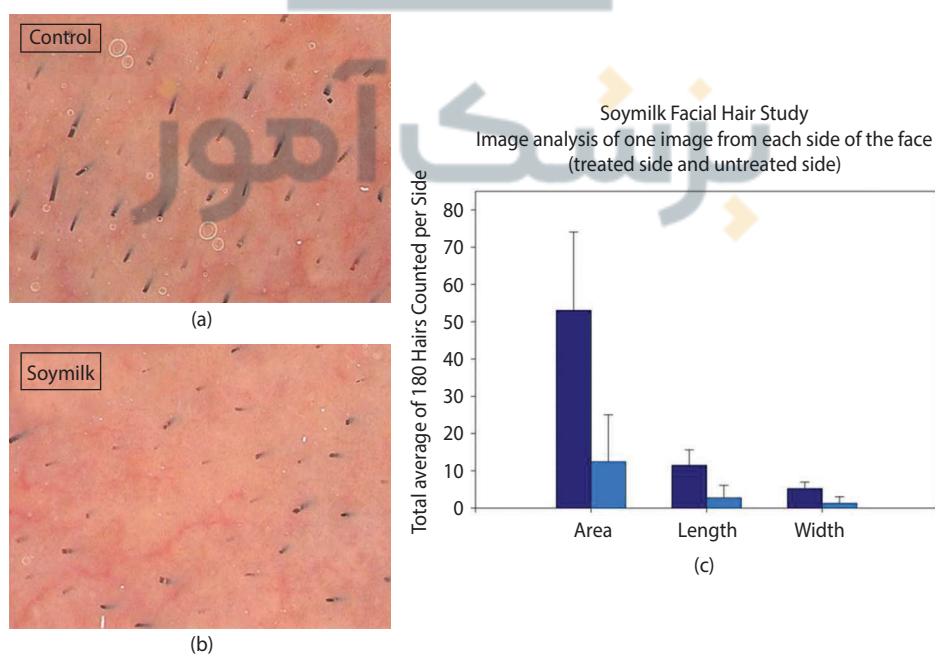
A double-blind, placebo controlled study examining leg hair regrowth enrolled 20 women aged 29 to 55. All subjects shaved immediately before the baseline randomization visit and then just once a week prior to returning for the follow-up assessments. Evaluations consisted of patient and investigator ratings of hair growth and adverse events. In addition, the treatment effects were quantified in terms of hair counts and hair growth rates through digital analysis of video microscope images taken of two areas of each leg—one toward the knee and the other close to the ankle. All of the women completed the study, and the only adverse effect reported was mild dryness. The digital analyses showed that mean hair regrowth rate was unchanged on the placebo-treated leg but progressively decreased with application of the non-denatured soy-based gel (Figure 11.10). As a result, the mean regrowth rate was consistently reduced on the treated leg for both areas measured at each weekly visit. By study end, the between-group difference for the effects on the lower portion of the leg achieved statistical significance.

Hair counts, which included any stubble, were consistently lower on the soy-treated leg compared with placebo beginning by week 2. However, there were large standard deviations in the means and the differences between treatments did not achieve statistical significance. The subjective evaluations from the study participants indicated the treatment caused the hair to regrow more slowly and that the hair present was softer. The proportion of women noting both of those outcomes increased over time was significant. At week 1, about 40% of women noted the hair on the soy-treated leg was softer and about 35% noticed the growth rate was reduced. At week 4, about two-thirds of the participants indicated that the hair on the soy-treated leg was softer and showed a reduced

growth rate. For the weekly assessments of the placebo-treated leg, between 5% and 22% of women considered the hair that regrew to feel softer, while 12% to 28% remarked that the hair growth rate was reduced. The results of self-assessments were consistent with investigator ratings that noted beneficial differences in hair appearance and quality favoring the soy-treated leg. Two-thirds of the study participants documented delaying hair regrowth, and smoothing and moisturizing of the skin (106–107). The non-denatured soy lotion was also found to reduce the facial hair area, length, and width for a male subject of Fitzpatrick skin type II, who shaved daily followed by applying a soy preparation daily for 4 weeks as illustrated in Figure 11.11.



**Figure 11.10** (a) A double-blind, placebo-controlled study examining leg hair regrowth comparing soy formula vs placebo. (b) Mean hair regrowth rate was stable on the placebo-treated leg but progressively decreased with application of the soy-based gel (active) after 2 weeks of the application of soy preparation.



**Figure 11.11** Three Caucasian male volunteers, Fitzpatrick skin type II, who shaved daily followed by application of a soy preparation daily for 4 weeks. (a) Shows the control and (b) the soymilk results for the shaved areas. (c) Average hair area, length, and width at the end of the study.





**Figure 11.12** Caucasian female subject (a) before and (b) after using non-denatured soy preparation. After 45 days of non-denatured soy product application, there was a statistically significant reduction in erythema and inflammatory papules ( $p < 0.001$ ).

#### *Improvement of Acneic Inflammatory Lesions*

The soy preparation was also proven to reduce acne-related blotchiness and erythema. A pilot clinical study was conducted to evaluate the potential of a non-denatured soy formulation to reduce erythema and acne lesions in acne sufferers and to look at the potential of this formulation to induce acne lesions in normal subjects. In the mild acne group, there was a statistically significant reduction in erythema and inflammatory papules after treatment with the soy preparation ( $p < 0.001$ ). There was a statistical trend in the reduction of the number of non-inflammatory comedones (Figure 11.12) (108). There was no incidence of acne induction by the treatment. Twenty-six subjects with mild acne and 29 subjects with no acne were entered into this open label study after Institutional Review Board (IRB) approval and informed consents. All subjects were female, 64% were Caucasian and 36% were black. Subjects applied the test product twice daily. Clinical safety evaluations were made on days 3, 7, 14, 21, 28, and 35. Dermatologist evaluations were made on day 0 (baseline) and day 45. In subjects with no acne, there was no statistically significant increase in comedones or papules and pustules. For those subjects with mild acne, a highly significant decrease from baseline in the number of inflammatory papules ( $p = 0.001$ ), a 41.9% decrease, was noted after 45 days of treatment with the soy preparation (79). This soy product was exceptionally well tolerated with no reports of stinging, burning, or itching at any time point evaluated. These results suggest that this non-denatured soy formulation may be considered as non-comedogenic and may be utilized as an important therapeutic option in acne sufferers.

#### *Enhancement in Skin Firming*

In addition to the enhancement in skin collagen production and elastin repair discussed in the section of "Preclinical Results of Non-Denatured Soy," clinical studies have further indicated that a soy composition containing a complete spectrum of soy components can improve skin firming and elasticity. In a 12-week double-blind randomized clinical study of skin aging, using a full-face design with twice daily applications, the non-denatured soy composition provided significant improvement in skin laxity versus baseline as early as week 4. Cutometer measurements further supported the dermatologist assessment in that 100% of the non-denatured soy-treated subjects showed improvement in skin firmness and distensibility.

#### *Sebum Production and Moisturization Balance in Combination Skin*

A 5-week, half-face, double blind placebo-controlled clinical study was performed in 23 female subjects of ages 20 to 35 years with combination skin (Fitzpatrick type I–II). Combination skin was defined as facial skin having at least one oily area and one dry area on each of the half face. The oily and dry areas were determined by sebumeter reading at  $>200$  mg/cm<sup>2</sup> and  $<66$  mg/cm<sup>2</sup>, respectively. Subjects applied soy preparation or placebo on the designated side of the face daily for 5 weeks. Measurements were taken at baseline and weeks 1, 3, and 5 on the forehead, cheek, and chin. Scaling, moisturization, oiliness, and smoothness were evaluated by instrumental measurements and digital photography. The results indicated this non-denatured soy preparation significantly reduced sebum in oily patches ( $p < 0.05$  for chin areas, as displayed in Figure 11.13) and enhanced moisturization for dry patches as



compared to placebo ( $p < 0.05$ ). Subject self-assessments demonstrated enhanced skin smoothness and oil reduction, which correlated with the instrumental results. Approximately 70% of the subjects noted improvement on the soy treated side while only 17% on placebo treated side noted improvement in overall tone and texture.

#### Effects on Ethnic Skin

Skin color is the most noticeable difference among different ethnic skins. Post-inflammatory hyperpigmentation (PIH) is of major concern to dark-skinned individuals. Non-denatured soy lotion combined with retinol and salicylic acid has clinically proven to reduce incidence and severity of PIH in as early as 1 week (108).

A 4-month double-blind, placebo controlled, randomized clinical study was conducted to evaluate the efficacy of a topical non-denatured soy formulation in improving the appearance of acne and post-acne PIH in 41 male and female Asian, African-American, and Hispanic subjects with Fitzpatrick skin type III–V and mild acne vulgaris (10–50 total facial acne lesions), 12 to 45 years of age. Subjects had physically distinct recent facial PIH acne marks. Subjects were asked to apply the test preparation twice daily (a.m. and p.m.) on the face.

The non-denatured soy composition led to a significant decrease in darkness, roughness, and erythema compared to baseline at week 4 and continued at weeks 8, 12, and 16, with reduction in darkness seen as early as week 1. Clinical expert grading of photos showed that the non-denatured soy lotion induced average improvements in PIH size of 41% at week 4, 68% at week 8, 82% at week 12, and 92% at week 16. This was statistically significantly more effective than the placebo composition at weeks 4, 8, 12, and 16. This composition was effective on 91% of subjects at week 4 and on 100% of subjects at weeks 8, 12, and 16.

Further statistical analysis of the data also showed that the non-denatured soy composition worked faster than the placebo in reducing the size and darkness of PIH marks.

Non-denatured soy also addressed the important issues of using natural ingredients for skin care, including being free of any trace pesticides/herbicides and any toxic heavy metals, e.g. lead and arsenic, being produced from non-GMO beans, and having very low microbial contents adequate for cosmetic

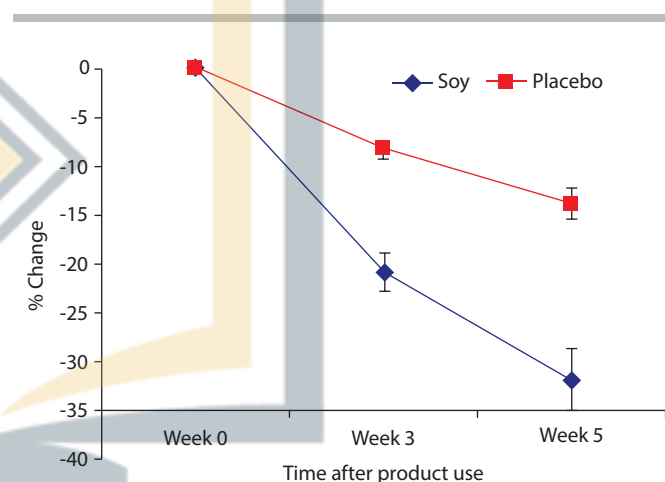
formulations. The skin care formulas prepared from non-denatured soy are stable throughout their shelf life, and possess the key bioactivities summarized in Table 11.6. These bioactivities were then proven in clinical studies to improve the skin condition, including reduction in hyperpigmentation, sun damage, unwanted hair, overly oily and dry skin, and acne.

## CONCLUDING REMARKS

Fundamental understanding of the mechanisms of action of soy actives led to the discovery of a new depigmenting option. Additional scientific studies demonstrated that non-denatured soy, which contains a complete spectrum of non-denatured soy components, provides broad skin care benefits. Thorough studies and safety reviews supported non-denatured soy as a long-term solution to skincare.

## ACKNOWLEDGEMENT

The authors thank Dr. Ying Sun for valuable scientific input.



**Figure 11.13** The non-denatured soy preparation significantly reduced sebum production in oily patches ( $p < 0.05$  for chin areas) versus placebo.

**Table 11.6** Summary of Recent Results in Understanding Soy's Bioactivity and Its Therapeutic Effects on Human Skin

In-vitro tests	Non-denatured soy's bioactivity/therapeutic effects on human skin	Reference(s)
Thiol retention activity antioxidant	Maintain the normal skin cell metabolism even when exposed to a harsh environment (e.g., smoke-induced loss of thiols)	73,83
Soybean trypsin inhibition activity	Protease-activated receptor-2 (PAR-2) in skin depigmentation (e.g., freckles, mottled hyperpigmentation, age spots) and delay hair regrowth	3–5, 37–38, 47, 90–92
Collagen synthesis	Enhance skin firming	86
Elastin	Enhance skin elasticity	86
Anti-inflammation	Reduce skin scaliness, erythema, and pain associated with sun exposure; reduce number of inflammatory papules ( $p < 0.001$ vs baseline) in mild acne subjects	47, 99–101

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# Kinetin

Stanley B. Levy

## INTRODUCTION

The success of retinoids and hydroxy acids as active ingredients in skin care products designed to improve the appearance of aging skin has stimulated the search for additional compounds. The use of both retinoids and hydroxy acids may be associated with skin irritation, further encouraging interest in alternatives. A possible addition to this armamentarium is kinetin (N6-furfuryladenine). Kinetin is a member of the plant growth hormone family cytokinins, known for growth-promoting and antiaging effects in plants. Zeatin is another cytokinin that has been incorporated into skin care, as has Pyratine-6 (furfurylaminoctetrahydropyranyladenine), a synthetic analogue. The incorporation of these materials into cosmetics has prompted a more detailed review.

## CHEMISTRY

Kinetin was first isolated from autoclaved herring sperm DNA in 1955 (1,2). It is a derivative of one of the nucleic acid purine bases, adenine. Kinetin has been reported to be present in various plants (3,4) and human cell extracts (5). It has been identified as a naturally occurring base modification of DNA (6). The chemical structure of kinetin suggests that it can be formed from adenine and furfuryl (Figure 12.1). The latter is a primary oxidation product of the deoxyribose moiety of DNA (7). It is not known if DNA repair enzymes remove this modified base from the DNA and make it available as free kinetin. Zeatin also contains adenine with the addition of an hydroxy-methylbutyl group (Figure 12.2). Pyratine-6 is structurally similar to kinetin except for the addition of a tetrahydropyranyl group.

## BIOLOGY

Kinetin was the first cytokinin identified (1,2) and the most studied. Cytokinins are plant growth substances that promote cell division and may play roles in cell differentiation. Most of the data for the biological properties of kinetin come from plant studies. Kinetin has been shown in plant systems to stimulate tRNA synthesis (8) and cell cycle progression (9). Calcium influx through the plasma membrane calcium channel in plant cells is stimulated by low levels of kinetin (10). More directly linked or related to antiaging, kinetin is known to prevent yellowing and senescence of leaves and slow down over ripening and degeneration of fruits (11).

Rattan and Clark (12) have reported the antiaging effect of kinetin on human skin cells and fruit flies. As little as 10 to 20 ppm of kinetin delay the onset of some biochemical and cellular changes associated with cellular aging in cell culture. Human skin fibroblast cell cultures of both young cells that had completed less than 20% of their potential in vitro life span and older cells that had completed 90% or more of their life

span were studied. Results were compared with cell cultures receiving no treatment (Table 12.1). Cytological manifestations of in vitro aging including cell enlargement, presence of multinucleated giant cells, accumulation of cellular debris and lipofuscin, and changes in actin filaments and microtubules were attenuated by the addition of kinetin. The number of cells per unit area in a confluent layer also markedly diminishes as a function of age. Kinetin treatment significantly diminished the age-associated reduction in cell yield (Figure 12.3). Kinetin did not affect the longevity of culture cells or their ability to multiply. Zeatin produced similar results in cell culture with less toxicity at higher concentrations (13).

A diet containing 20 to 50 ppm kinetin fed to fruit flies slowed down aging and development and prolonged average and maximum life span by 65% and 35%, respectively (14). The increase in life span was accompanied by a 55% to 60% increase in the antioxidant enzyme catalase (15). Catalase breaks down hydrogen peroxide associated with cell toxicity.

Kinetin has been demonstrated to have inhibitory activity on free radical formation of active platelets in vitro and thrombus formation in vivo (16). Kinetin may therefore be a potential therapeutic agent for arterial thrombosis. A cytokinin nucleoside, N6-furfuryladosine, has been shown to have antiproliferative and apoptogenic activity against various human cancer cell lines, suggesting potential anticancer activity (17). This activity has not been shown with kinetin.

## MECHANISM OF ACTION

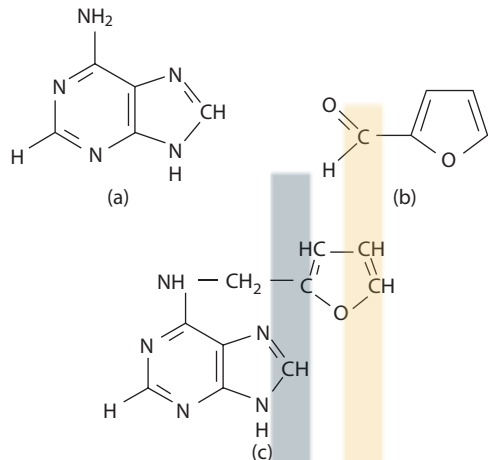
The exact mechanism by which kinetin acts to exert its effects is unknown. Kinetin may act directly as a signaling molecule, involved in signal transduction, stimulating defense pathways such as DNA repair (18). Kinetin modulates and promotes calcium-induced differentiation of normal human keratinocytes that becomes progressively delayed during aging (19,20).

Kinetin may also act indirectly as a natural antioxidant (21), preventing the formation of reactive oxygen species or as a direct free radical scavenger (22). Oxygen radicals could abstract hydrogen from the  $\alpha$ -carbon of the amine bond N6-furfuryladenine (23). Oxygen radicals undergo a faster dismutation reaction when kinetin is complexed with copper. A direct effect of kinetin on superoxide dismutase activity has been observed in plants (21). Kinetin has also been shown to protect against oxidative and glycoxidative protein damage generated in vitro by sugars and an iron/ascorbate system (22).

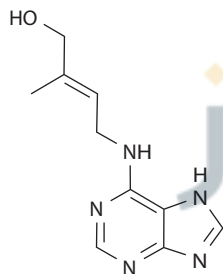
The biological significance of kinetin's interaction with DNA or its antioxidant properties are unknown. However, pluripotency may be a necessary prerequisite for effective antiaging activity (24). A multistep protocol utilizing in vitro and in vivo studies designed to compare the oxidative stress

capacity or various antioxidants demonstrated that kinetin performed favorably relative to other known antioxidants including tocopherol, ascorbic acid, and lipoic acid (25).

The activity of zeatin is attributable to its more stable trans form (26). Trans-zeatin inhibits UVB-induced matrix metalloproteinase-1 expression via MAP kinase signaling in human skin fibroblasts (27). It also has been shown to attenuate ultraviolet-induced down regulation of aquaporin-3 in cell-cultured keratinocytes, also attenuating delayed wound healing and decreased water permeability (28). Therefore, trans-zeatin might have protective effects on photoaging.



**Figure 12.1** Chemical structure of (a) adenine, (b) furan, and (c) kinetin.



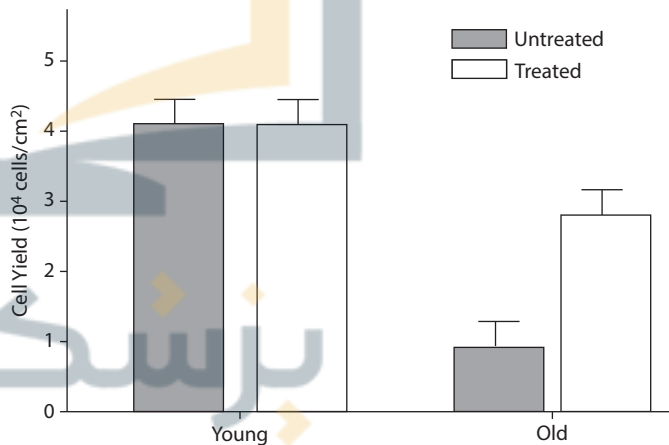
**Figure 12.2** Chemical structure of zeatin.

**CLINICAL STUDIES**

Percutaneous absorption studies of kinetin with human cadaver skin demonstrate significant skin penetration (McCullough, unpublished study). A dose-response was shown with 0.01% versus 0.05% kinetin with tissue levels for both serum and lotion formulations. There was no significant difference in transdermal absorption with the two test formulations. Topical treatment with low-concentration kinetin normalized hyperpigmentation and improved age-related skin structure changes in hairless dogs (29). Although cosmetic formulations containing a dispersion of liposomes with magnesium ascorbyl phosphate, alpha lipoic acid, and kinetin showed photoprotective effects (30), an 0.5% kinetin solution and 0.1% kinetin cream showed no photoprotective effects by themselves (31).

Thirty subjects with mild to moderate photodamaged facial skin were treated with topical kinetin 0.1% twice daily for 24 weeks (32). Significant improvements were seen in tactile roughness, mottled hyperpigmentation, and fine wrinkles at both 12 and 24 weeks. Overall photodamage was reported improved by both self-assessment and dermatologist grading. Transepidermal water loss decreased after 24 weeks, consistent with skin barrier function improvement. Other than some initial contact folliculitis, no significant skin irritation was seen.

Ninety-eight subjects with mild to moderate photodamaged facial skin each applied a kinetin-containing lotion and



**Figure 12.3** Cell yield in untreated and kinetin-treated young and old cells. (From Rattan SI, Clark BF, *Biochem Biophys Res Commun*; 201(2):665–72, 1994. With permission).

**Table 12.1** Kinetin's Effects on the Cytological Manifestations of In Vitro Aging

Characteristic	Untreated		Kinetin	
	Young	Old	Young	Old
Cell enlargement	None	Significant	None	Insignificant
Multinucleate cells	None	Present	None	None
Cellular debris	Minimal	Significant	Minimal	Minimal
Lipofusein	Low	High	Low	Low
Actin filaments	Diffuse	Highly polymerized	Diffuse	Less polymerized
Microturbules	Orderly	Disorganized	Orderly	Orderly

Source: Rattan SI, Clark BF, *Biochem Biophys Res Commun*; 201(2):665–72, 1994. With permission.

creams for 10 weeks (Revlon Research Center, unpublished studies). All subjects were assessed at baseline 4, 8, and 10 weeks for photodamage parameters. Statistically significant improvements were noted in all parameters, greatest with texture, skin clarity, discrete and mottled pigmentation, fine wrinkling, and global appearance. No significant irritation was noted.

Forty female subjects, ages 22 to 57, having mild to moderate facial skin photodamage, underwent a 12-week split face, double-blind, controlled and randomized study comparing a topically applied kinetin-containing lotion twice daily on one side and retinol containing lotion on the other (33). Evaluations at 4-week intervals demonstrated significant improvements for all attributes graded including discrete and mottled pigmentation, fine wrinkling, and overall photodamage. The kinetin lotion produced greater improvements in texture and clarity.

Another non-placebo controlled trial demonstrated a beneficial effect of topical kinetin 0.1% lotion in reducing erythema and overall clinical scores in 17 subjects with mild to moderate rosacea (34). A study of topical kinetin 0.03% with niacinamide 4% in 52 Asian subjects over a 12-week period suggested some synergistic cosmetic benefits to the combination (35).

Nine kinetin-containing products in 200 subjects each were subject to modified Draize repeat insult patch tests. No instances of sensitization were seen in the challenge phase. In addition, controlled use testing for up to 6 weeks demonstrated no significant irritation (Almay Research and Testing Corp., Edison, NJ, unpublished studies). Six kinetin-containing products were tested in 10 subjects each with skin phototypes I–III for UV sensitivity with a solar simulator (Ivy Research Laboratory Inc., Philadelphia, Pennsylvania, unpublished study). Panelists were treated with once-daily applications of 2 mg/cm<sup>2</sup> to the mid-back for 2 weeks at six sites. After the final applications, no difference in minimal erythema dose was noted between untreated control and treated sites. The above studies and clinical experience to date would suggest that kinetin has minimal or no potential to cause irritation, allergy, or photosensitization.

Furfuryl tetrahydropyranyladenine (Pyratine-6), a kinetin analogue, was utilized as a topical agent in a single 12-week open label photoaging study. Improvement in skin roughness, moisturization, hyperpigmentation, and fine wrinkling was noted (36). The same analogue showed some efficacy in a 48-week open label study in subjects with mild to moderate rosacea (37).

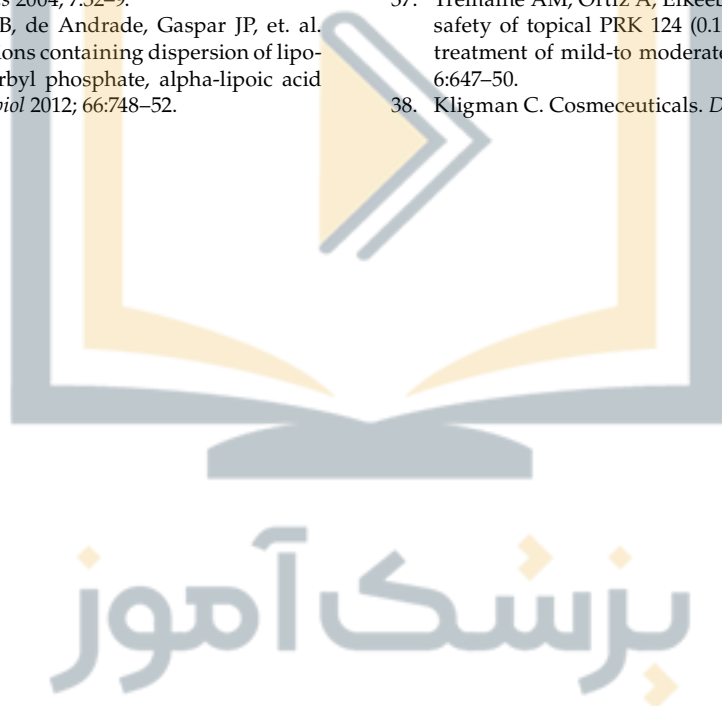
## CONCLUSION

Kinetin (N6-furfuryladenine), a plant growth regulator, has been demonstrated to delay a range of cellular changes associated with the aging of human skin cells in vitro. In addition, kinetin has antioxidant properties, formed as a response to free radical damage in human DNA. Before-and-after clinical studies have demonstrated improvements in photodamaged skin. As is very often the case with cosmetic ingredients, active to vehicle comparison studies are not available (38). Studies have clearly shown that the use of kinetin is not associated with significant irritation and is a potential alternative for individuals sensitive to retinoids and hydroxy acids. Analogues of kinetin may also prove useful in the future.

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# Urokinase and Plasmin in Dry Skin and Skin Aging

Yuji Katsuta

## INTRODUCTION

Urokinase (urokinase-type plasminogen activator, uPA) belongs to trypsin-like serine protease family and activates inactive plasminogen into its active form plasmin. Because plasmin degrades fibrin clots, urokinase and plasmin are called fibrinolytic enzymes. These fibrinolytic enzymes function not only in the blood systems but also in other organs, including the epidermis. They function in wound repair and in many disorders such as psoriasis and pemphigus. From the cosmetic point of view, urokinase and plasmin are involved in dry skin and skin aging. This chapter reviews the roles of urokinase and plasmin in dry skin and skin aging.

## UROKINASE AND PLASMIN IN DRY SKIN Dry Skin

Dry skin is visually characterized by dryness, scaling, and rough texture (1,2). This condition can be experienced by any healthy person, especially in a dry, cold season. There are many external and internal factors that change the skin surface morphology and cause dry skin. The external factors include exposure to extremes of climate (cold, wind, dryness) and chemicals (detergents, solvents), and ultraviolet radiation. The internal factors include various abnormalities in physiologic functions, illness, and mental stress. The effects of dry skin are not limited to the appearance. The barrier function of the stratum corneum is reduced. Proliferation of keratinocytes is accelerated and turnover of the epidermis is increased. The epidermis becomes hypertrophic. The differentiation of keratinocytes is also abnormal in dry skin, leading to the incomplete formation of the stratum corneum.

For these reasons, supplying moisture to the skin surface is not sufficient to prevent and improve dry skin. Applying creams topically for the purpose of occluding the skin surface is effective in improving the damaged barrier function, but this is not a fundamental treatment. To maintain the skin in a healthy state, it is essential to protect the epidermis from inside. Maintaining the normal proliferation of keratinocytes and keeping epidermal differentiation normal are important requirements for preventing dry skin.

## Approach to Finding Intra-Epidermal Secondary Factors That Cause Dry Skin

To elucidate the mechanism of dry skin occurrence and develop effective compounds for preventing dry skin, attempts have been made to identify intra-epidermal secondary factors that cause dry skin (1,3,4). These putative secondary factors are thought to respond to primary factors, such as a dry environment, and cause further changes in dry skin. Plasmin

is identified as one of the secondary factors by means of the following approach.

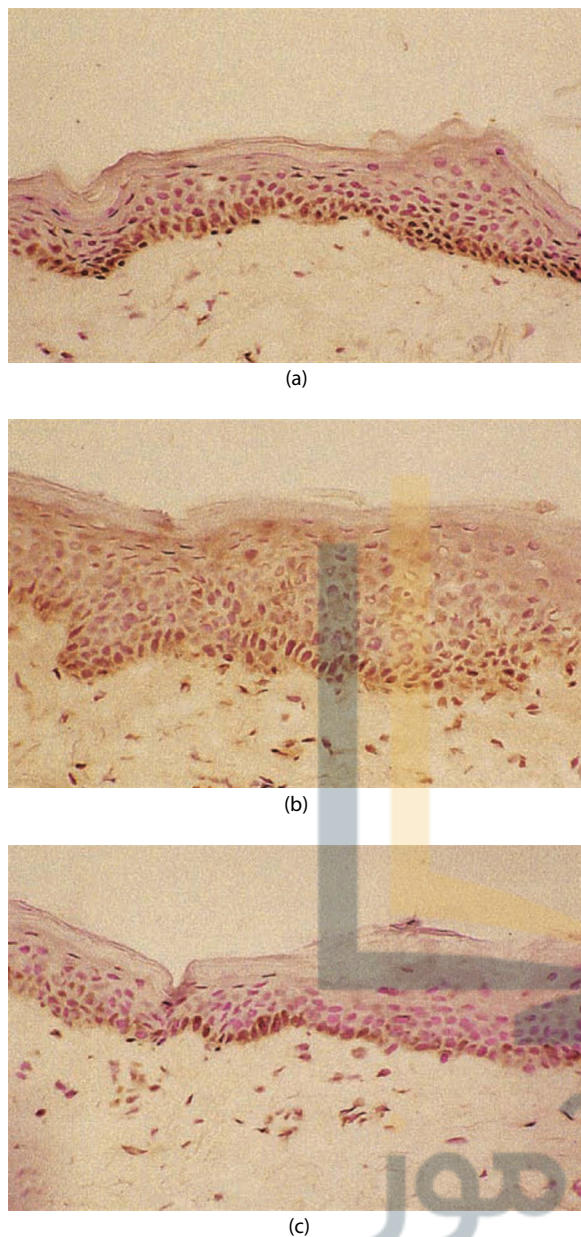
Detergent is a well-known cause of dry skin and is often used for an experimental dry skin model. Topical application of sodium lauryl sulfate (SLS) induces the features of dry skin, such as dryness, scaling, and rough texture. Physiologic values are also changed in the SLS-treated area. The water content of the stratum corneum is reduced and the transepidermal water loss (TEWL) values are increased. These physiologic features of the SLS-induced dry skin model resemble those of naturally occurring dry skin. This model was used in the screening for the secondary factors.

The barrier recovery test was also used, because the decrease of barrier function is one of the most important phenomena associated with dry skin (4). The barrier function of the stratum corneum was destroyed by tape-stripping or the application of a detergent or a solvent. Then the time course of TEWL was measured.

Many kinds of compounds were assayed in the experimentally dry skin model and the barrier recovery test. Nonsteroidal anti-inflammatory agents such as acetylsalicylic acid, indomethacin, mefenamic acid, ibuprofen, and sodium diclofenac were tested, but these compounds did not exhibit suppressive effects. Then protease inhibitors were tested and tranexamic acid [*trans*-4-(aminomethyl) cyclohexane carboxylic acid (*t*-AMCHA)] prevented epidermal hypertrophy and suppressed the appearance of dry skin. This compound is an inhibitor of plasmin. In contrast, ethylenediaminetetraacetic acid (EDTA; a metalloprotease inhibitor), pepstatin (an aspartate protease inhibitor) and chymostatin (a chymotrypsin-type serine protease inhibitor) had no beneficial effect.

Because *t*-AMCHA, a plasmin inhibitor, was effective in ameliorating dry skin, plasmin was the candidate for the secondary factor that causes dry skin. To confirm this, immunohistochemistry was performed. As shown in Figure 13.1, the localization of plasmin was localized is restricted to the basal layer in the intact skin of the inner forearm. On the other hand, plasmin was localized throughout the epidermis of experimentally induced dry skin on the inner forearm. This result shows that the amount of plasmin was increased with the induction of dry skin, suggesting that plasmin is one of the secondary factors of dry skin. Treatment with *t*-AMCHA was effective in preventing changes in the intra-epidermal distribution of plasmin associated with dry skin as well as in suppressing epidermal hypertrophy.

Efficacy of *t*-AMCHA was also shown in naturally occurring dry skin. A double-masked clinical test was carried out in the dry, cold winter season in Japan. The texture of the facial skin treated with *t*-AMCHA was significantly improved.



**Figure 13.1** Localization of plasmin in intact skin (a), in experimentally induced dry skin (b), and in experimentally-induced dry skin treated with *t*-AMCHA (c). (From Kitamura K et al., *J Soc Cosmet Chem Jpn*; 29:133–45, 1995.)

### Involvement of Plasmin in Skin Diseases

Plasmin is a trypsin-like serine protease that is distributed mainly in plasma. Although its main function is fibrinolysis in coagulated fibrin clots, plasmin also exists in adrenal, kidney, testis, heart, lung, uterus, spleen, thymus, and gut (5).

Plasmin exists in the epidermis as well. It is known to be expressed in diseases such as psoriasis (6). The epidermis of psoriatic skin is extremely hypertrophic, and the proliferation of keratinocyte is rapid. The expression of plasmin is scattered throughout the epidermis in lesional psoriatic skin. The increased plasmin activity may contribute to the disease manifestation.

Wounding epidermis also increases plasmin (7). At the wound edge, keratinocytes proliferate and migrate rapidly to cover the wounded area. Plasmin degrades the extracellular matrix at the wound edge to aid proliferation and migration of keratinocytes.

Epidermal hypertrophy is common in psoriasis, wounding, and dry skin. Plasmin may be involved in promoting the proliferation and migration of keratinocytes, resulting in epidermal hypertrophy.

### Urokinase in Dry Skin

Plasmin is biosynthesized as an inactive precursor called plasminogen. The cleavage of Arg560-Val561 of plasminogen activates this precursor molecule. Two major proteases are involved in plasminogen activation. One of them is urokinase-type plasminogen activator (uPA) and the other is tissue-type plasminogen activator (tPA). These plasminogen activators also belong to the trypsin-like serine protease family, as well as plasmin.

Aberrant plasmin activity in the epidermis may require increased levels of plasminogen activators. The lesional epidermis from patients with psoriasis contains elevated levels of plasminogen activators compared with nonlesional epidermis or epidermis from normal individuals (8,9). In psoriasis, tPA is thought to be the major plasminogen activator (10), whereas urokinase may be predominant in wounding.

The activity of plasminogen activator was detected in dry skin. Stratum corneum obtained from dry skin by tape stripping had the activity of lysing fibrin *in vitro*. Because this lysis was completely inhibited when antibody against urokinase was added, the major plasminogen activator in dry skin might be urokinase. *In situ* zymography was carried out to confirm the presence of plasminogen activator activity. The activity of plasminogen activator was detected in the epidermis of the SLS-treated skin, whereas little activity was seen in the control skin (4).

### Urokinase Activity in Stratum Corneum of Dry Skin

Urokinase is produced and secreted as an inactive single-chain precursor called pro-uPA. Cleavage of Lys158-Ile159 is essential for its activation. It was found that urokinase is activated in stratum corneum after barrier disruption (11). As a result of *in situ* zymography, plasminogen activator activity was detected in the stratum corneum 1 hour after barrier disruption (Figure 13.2). This indicated that urokinase is activated in the stratum corneum at an early stage of dry skin formation. The stratum corneum forms the surface layer of our bodies and is always exposed to the environment. This activation of urokinase in the stratum corneum might be the trigger process in dry skin formation.

The activation of urokinase in the stratum corneum was also tested *in vitro*. Human stratum corneum was homogenized in a glass homogenizer. The homogenate was washed with glycine buffer to remove endogenous urokinase. Pro-uPA was activated after the incubation with the insoluble components of the stratum corneum homogenate.

Pro-uPA activation must have taken place on the surface of solid stratum corneum, because only the insoluble components were used in the *in vitro* urokinase activation assay. To estimate the physical interaction of pro-uPA and the insoluble components of the stratum corneum homogenate, the mixture was filtrated to divide the soluble and insoluble components. Then the amounts of urokinase in both soluble and insoluble components were evaluated. Pro-uPA was found to bind to the insoluble components of stratum corneum homogenate in this assay. The physical interaction is likely to be important for the activation of urokinase.

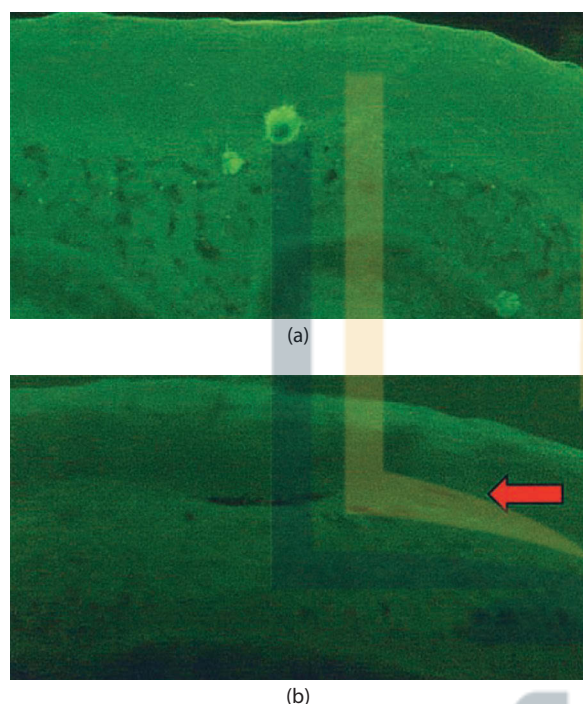
### ***t*-AMCHA Methylamide is Effective for Preventing Dry Skin**

The methylamide derivative of *t*-AMCHA (Figure 13.3) has less inhibitory activity against fibrinolysis than *t*-AMCHA (Figure 13.4a). However, *t*-AMCHA methylamide strongly inhibited the physical interaction between urokinase and the insoluble components of the stratum corneum homogenate in vitro (Figure 13.4b).

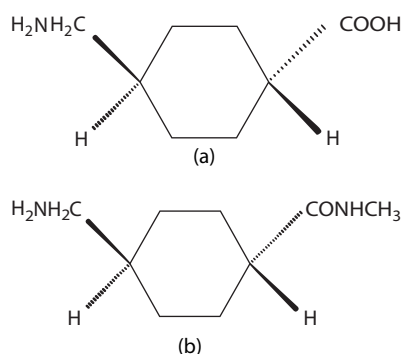
The effectiveness of *t*-AMCHA and *t*-AMCHA methylamide in preventing dry skin was compared in experimentally induced dry skin (Figure 13.4c). *t*-AMCHA methylamide suppressed dry skin more potently than *t*-AMCHA at the same

concentration. This result suggests that inhibiting the physical interaction between urokinase and the stratum corneum is more effective in preventing dry skin than inhibiting plasmin activity. Inhibition of urokinase activation in stratum corneum by *t*-AMCHA methylamide in vivo was confirmed by using in situ zymography (12).

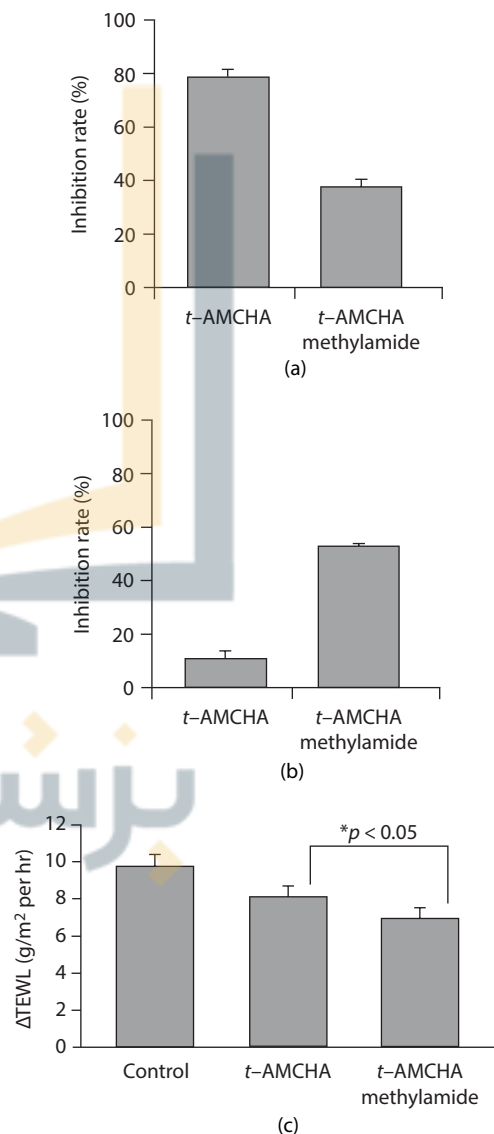
Efficacy of *t*-AMCHA methylamide in ameliorating naturally occurring dry skin was demonstrated in a double-masked clinical test. After 1 month's efficacy test in dry, cold winter in Japan, lotion containing *t*-AMCHA methylamide significantly improved dry skin. The skin surface texture of two cases before and after the treatment is shown in Figure 13.5.



**Figure 13.2** Detection of plasminogen activator activity in intact skin (a) and skin after barrier disruption (b). Loss of fluorescence indicates plasminogen activator activity. Plasminogen activator activity was detected in stratum corneum after barrier disruption (arrow).

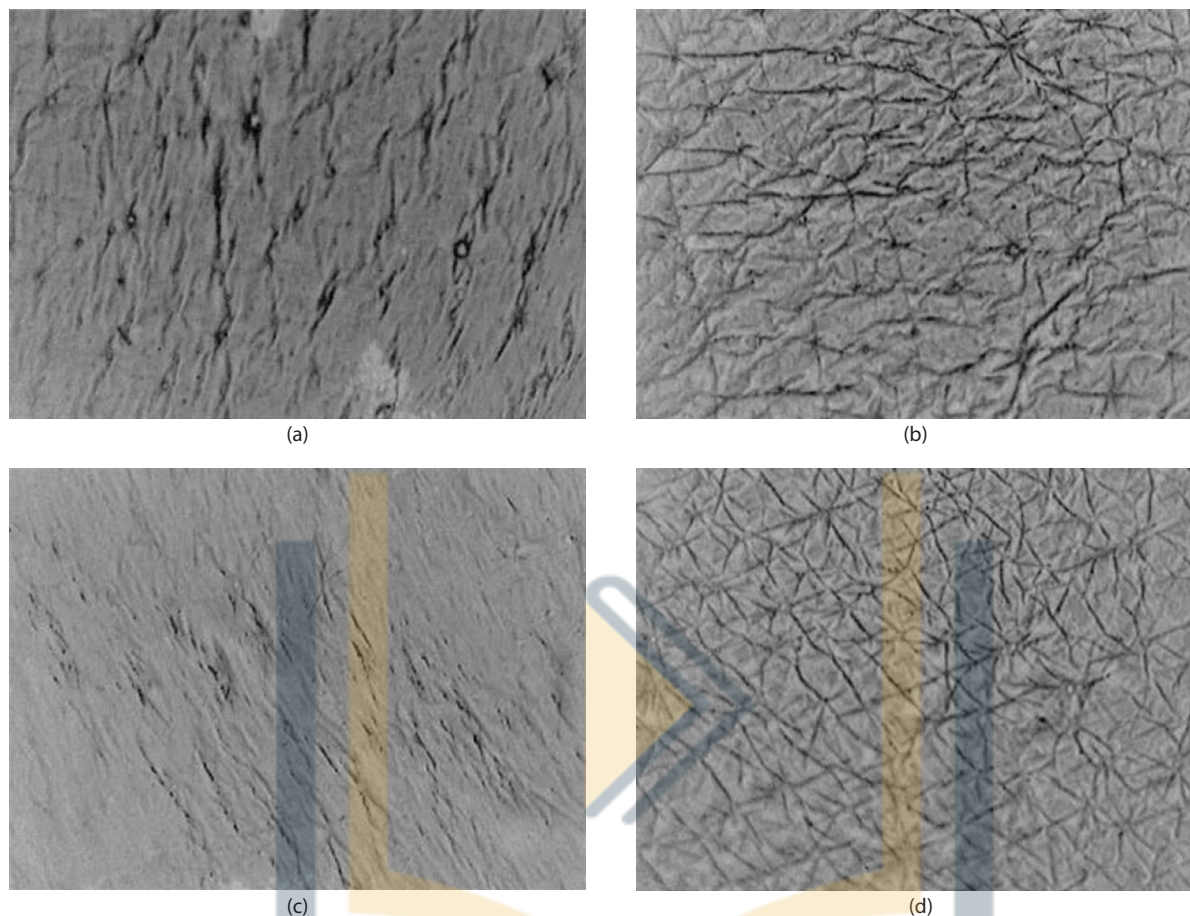


**Figure 13.3** Molecular structures of *t*-AMCHA (a) and *t*-AMCHA methylamide (b).



**Figure 13.4** (a) Inhibition of fibrinolysis by *t*-AMCHA and *t*-AMCHA methylamide. *t*-AMCHA methylamide (1 mol/L) had less inhibitory effect on fibrinolysis than *t*-AMCHA in vitro.  $\pm$ S.D. (b) Inhibition of physical interaction between urokinase and stratum corneum. *t*-AMCHA methylamide inhibited (1% solution) the attachment of urokinase to the insoluble components of stratum corneum homogenate more potently than *t*-AMCHA.  $\pm$ S.D. (c) Inhibition of experimentally dry skin by application of SLS. *t*-AMCHA methylamide (1% solution) suppressed the increase of TEWL than *t*-AMCHA  $\pm$ S.E.





**Figure 13.5** The improvement of skin surface texture after one month's treatment with *t*-AMCHA methyamide. Skin surface texture before treatment (a,c) and after treatment (b,d).

The efficacy of *t*-AMCHA methyamide indicates that inhibiting the physical interaction between urokinase and stratum corneum is a useful approach for preventing dry skin.

### UROKINASE AND PLASMIN IN SKIN AGING Basement Membrane in Photo-Aged Skin

The epidermal basement membrane (BM), located at the dermal-epidermal junction, plays several important roles in controlling skin functions. It links the epidermis and dermis tightly, and determines the polarity of keratinocytes (13). The proliferating keratinocytes remain attached to the BM and the daughter cells migrate into the upper layers and differentiate (14–16). The BM is mainly composed of type IV and VII collagens, laminins (laminin 332, 331 and 511), nidogen, and perlecan (17–19). Laminin 332,  $\alpha 2\beta 4$  integrin, type XVII collagen, and type VII collagen are essential to epidermal attachment, and mutations in the genes encoding these proteins lead to blistering at the dermal-epidermal junction (20–22).

Skin aging can be classified as intrinsic aging or photo-aging (23). Photo-aging is caused by chronic exposure of skin to ultraviolet radiation. Photo-aged skin is characterized by several clinical features such as wrinkling, laxity, roughness, sallowness, pigments, telangiectasis, and neoplasia (24,25). The histological changes of photo-aged skin include decrease in

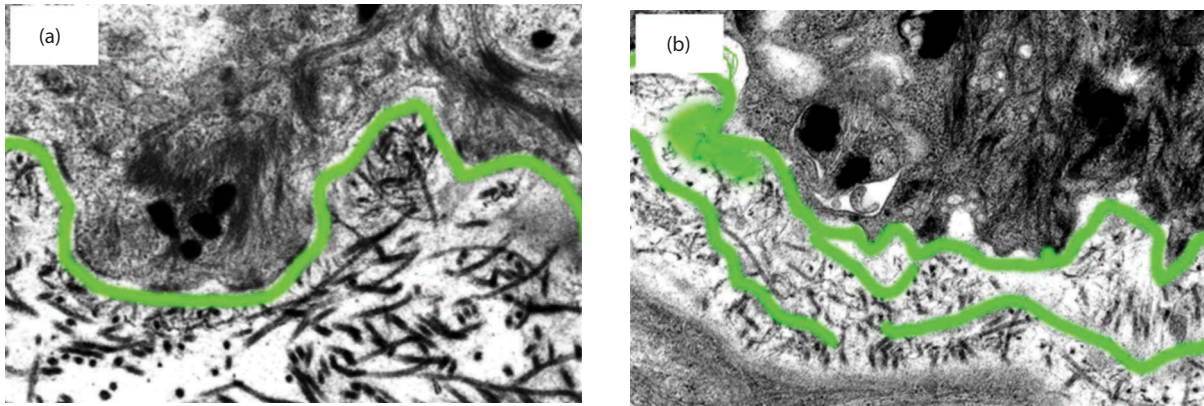
collagen, dermal elastosis, loss of polarity, and flattening of the dermal-epidermal junction (26,27). Another feature of photo-aged skin is disrupted BM structure. As shown in Figure 13.6, the BM is a single-layer sheet in the sun-protected skin, but it changes to multilayer in the sun-exposed skin (27–29).

### Involvement of Urokinase and Plasmin in the Disruption of BM

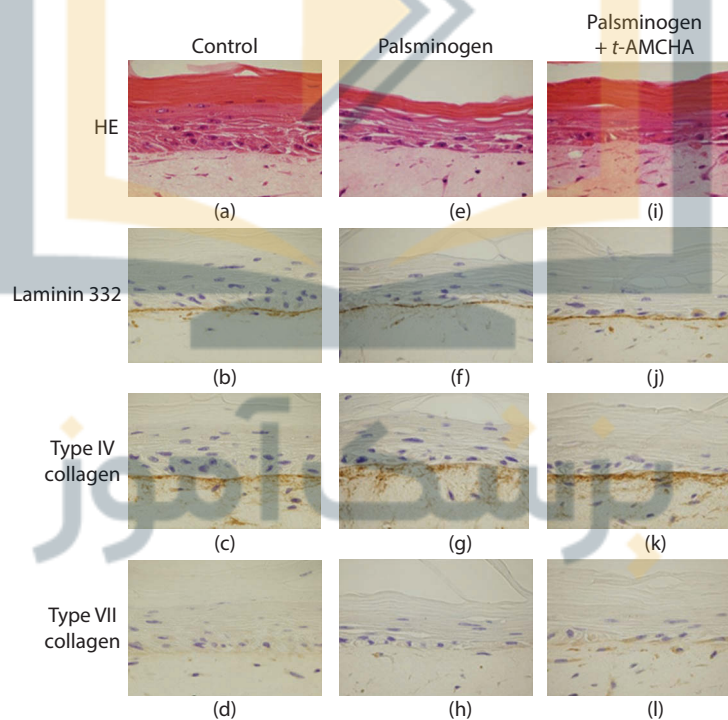
Ultraviolet exposure increases proteases such as matrix metalloproteinases (MMPs) and urokinase (30–33). These enzymes were reported to damage the BM. These enzymes were also confirmed in in vitro skin-equivalent models (34). Skin-equivalent models show only a faint BM because BM components were degraded by MMPs. In addition to MMPs, urokinase was detected in the condition medium by ELISA assay. The condition medium of the skin-equivalent model showed fibrinolytic activity in the presence of additional plasminogen. It indicated that the added plasminogen was activated by the urokinase in the skin-equivalent model.

BM in the skin-equivalent model is degraded in the presence of plasmin. The addition of plasminogen disrupted the deposition of the BM components such as laminin 332, type IV collagen, and type VII collagen. The depositions of these BM components are reduced and discontinuous at the





**Figure 13.6** Transmission electron microscopic images of the basement membrane (BM) of human skin. (a) Neither disruption nor duplication is observed in the sun-protected abdomen skin of a 34-year-old woman, but (b) disruption and reduplication of the BM are observed in the sun-exposed cheek skin of a 30-year-old woman (b).



**Figure 13.7** Disassembly of BM at the dermal-epidermal junction in the presence of plasmin, and the recovery of BM assembly by the addition of *t*-AMCHA. Skin equivalents (SEs) were cultured in the absence (a–d) or presence of plasminogen (e–l). The contro SE showed linear and sharply defined deposition of laminin 332, type IV collagen, and type VII collagen (b–d). In the presence of human plasminogen, impaired deposition of BM components is observed (f–h). The impairment of BM assembly is blocked in the presence of *t*-AMCHA (j–l). HE, hematoxylin and eosin staining (a,e,i).

dermal-epidermal junctions (Figure 13.7). Furthermore, the epidermis showed abnormal differentiation without filaggrin expression. The urokinase inhibitor *t*-AMCHA improved the deposition of these BM components, and the epidermis recovered to a normal differentiation state.

### Laminin 332 is Degraded by Plasmin

Because the deposition of laminin 332 was reduced by the addition of plasminogen, the degradation of laminin 332 by plasmin was analyzed by means of Western blotting. The processed form of laminin 332 is composed of two 160- and

150-kDa  $\alpha 3$  chains, a 140-kDa  $\beta 3$  chain and a 105-kDa  $\gamma 2$  chain. On the other hand, the plasmin-treated laminin 332 was composed of two 140- and 145- kDa  $\alpha 3$  chains, a 110-kDa  $\beta 3$  chain and a 105-kDa  $\gamma 2$  chain. These chains were presumably generated by the cleavage of a 5- or 10- kDa fragment from the carboxyterminal end.

## CONCLUSION

Urokinase and plasmin are activated in the process of dry skin. Inhibiting these enzymes is a useful approach for preventing dry skin. *t*-AMCHA and its methylamide derivative were effective in clinical tests.

The BM is located at the dermal-epidermal junction and is important for controlling skin functions. It is known to be degraded with photo-aging. In skin-equivalent models, urokinase and plasmin disrupted the BM structure and *t*-AMCHA improved the deposition of the BM components. It suggested that inhibiting these enzymes is also useful in anti-aging treatments of skin.

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## Ceramides and the Skin

David J. Moore, Clive R. Harding, and Anthony V. Rawlings

### INTRODUCTION

Prevention of desiccation of the body is a major function of the skin. This function is performed for the most part by skin's epidermis, with a particularly crucial contribution by the outermost layers, the stratum corneum (SC). At the skin's surface there is a delicate balance between the water content of the SC and the environment, and although the SC contains relatively little water, a critical level of moisturization is essential for the normal barrier function and health of the skin. To maintain the proper level of moisturization, the skin's epidermis has evolved a finely tuned differentiation program which generates and maintains an SC composed of cellular and macromolecular components that provide the required structure, humectancy, and barrier to water loss (1). The SC consists of three basic components: corneocytes (terminally differentiated keratinocytes), corneodesmosomes (proteinaceous rivets holding corneocytes together), and lipids. A widely employed, if oversimplified analogy of SC structural organization is a brick wall (2,3). This analogy provides an image of a heterogeneous structure of two major components: bricks and mortar (Figure 14.1). In this model, the corneocyte 'bricks' occupy most of the volume of the SC wall and are surrounded by a lipid "mortar." It is now recognized that the corneodesmosomes are a vital element of the mortar, ensuring structural integrity. Equally, the size and shape of the corneocytes influence skin barrier function as well as the total thickness of the SC. One must also not forget the role of filaggrin and its degraded components in SC functioning (1).

The lipid matrix constitutes approximately 20% of the SC volume (about 15% of the dry weight) and is the continuous phase of the skin barrier (4,5). The lamellar bilayer organization of this lipid matrix was first observed clearly using electron microscopy to examine ruthenium tetroxide (RuO<sub>4</sub>)-fixed samples (6) (typical examples are shown in Figure 14.2a and b). It has now been well established, using a variety of tape stripping and lipid extraction experiments, that the hydrophobic epidermal permeability barrier resides primarily in the lipid bilayers of the SC with the corneocytes and thickness of the SC dictating the tortuosity and thereby increased path length for the diffusion of water through the SC. Consistent with the "mortar" analogy, however, there is good evidence to indicate that the lipids also contribute to the intercellular cement, which helps to maintain the integrity of the tissue (7,8).

The SC lipid bilayers are unique among biological membranes in terms of composition, organization, and physical properties. The major lipid species of the SC are ceramides (about 50% by mass), fatty acids (10%–20% by mass), and cholesterol (25% by mass) (4,5,9). In addition, there are small amounts of cholesterol esters and cholesterol sulfate which, in particular, seems to play a critical role in normal barrier

function (10). There are no phospholipids in healthy SC (5). Many of the lipids of the SC are derived from the contents of the membrane-coating granules (MCG) (also called lamellar bodies or keratinosomes) formed in the keratinocytes of the stratum granulosum, the uppermost layer of the viable epidermis. At the interface between the stratum granulosum and SC the extruded phospholipids, sphingolipids, and plasma membrane constituents are enzymatically cleaved as they enter the SC to generate free fatty acids and ceramides (11). These components then fuse together to form the continuous lamellar bilayers characteristic of the SC (Figure 14.2a and b). It is estimated that the skin must synthesize approximately 100–150 mg of lipid per day to replace that lost in normal desquamation. The skin is therefore one of the most active sites of lipid synthesis in the body (9,12).

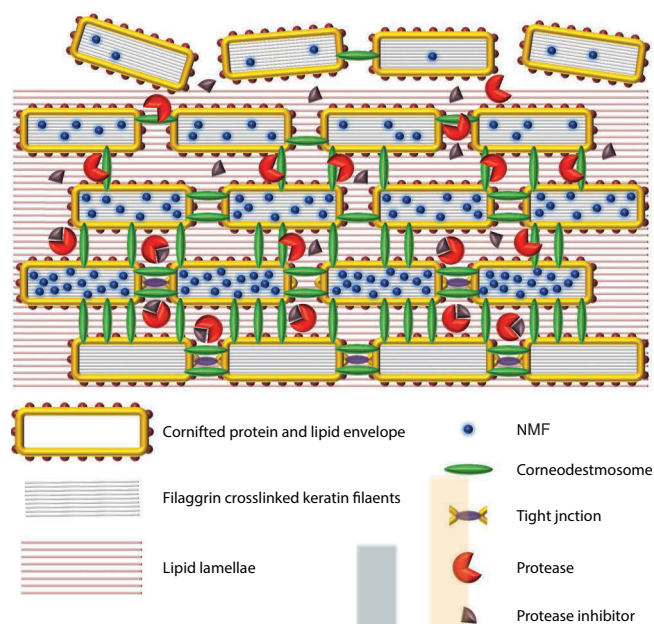
This chapter reviews developments in our understanding of the biological functions of ceramides, the major polar species from which the extracellular lipids of the SC are organized.

### THE STRUCTURE OF STRATUM CORNEUM CERAMIDES

As noted in the last edition of this chapter, our understanding of the heterogeneity of SC ceramides continues to increase in parallel with the development of new, highly sensitive analytical methodologies for their detection and measurement (13,14) and even visualization using specific antilipid antibodies (15). The previously described studies by Masukawa and colleagues employing normal-phase liquid chromatography (NPLC) connected to electrospray ionization-mass spectrometry (ESI-MS) which provided new quantitative insights into the diversity of ceramide classes and species in human stratum corneum (16,17) have been further advanced by several groups in The Netherlands, Belgium, and Germany (18–20).

At the time of writing this chapter (December 2014), 15 classes of free ceramides (Figure 14.3; non corneocyte-bound) have been identified including the newly identified and classified 1-O-acyceramides which contain long acyl chains in both the N- and O-positions (20,21). In addition, three classes of covalently bound ceramide (Figure 14.4) species have been identified (22–25). Collectively these ceramides are derived from three distinct lipid precursor pools synthesized in the underlying epidermis: epidermosides, glucosylceramides, and sphingomyelin. Epidermosides are glycosylated precursors of omega-hydroxyl-containing ceramides (26). Studies have shown that both glucosylceramides (MCG-content derived) and sphingomyelin (plasma membrane and MCG-membrane derived) can contribute to the formation of SC ceramides (27), although evidence suggests that glucosylceramides may represent the major source of





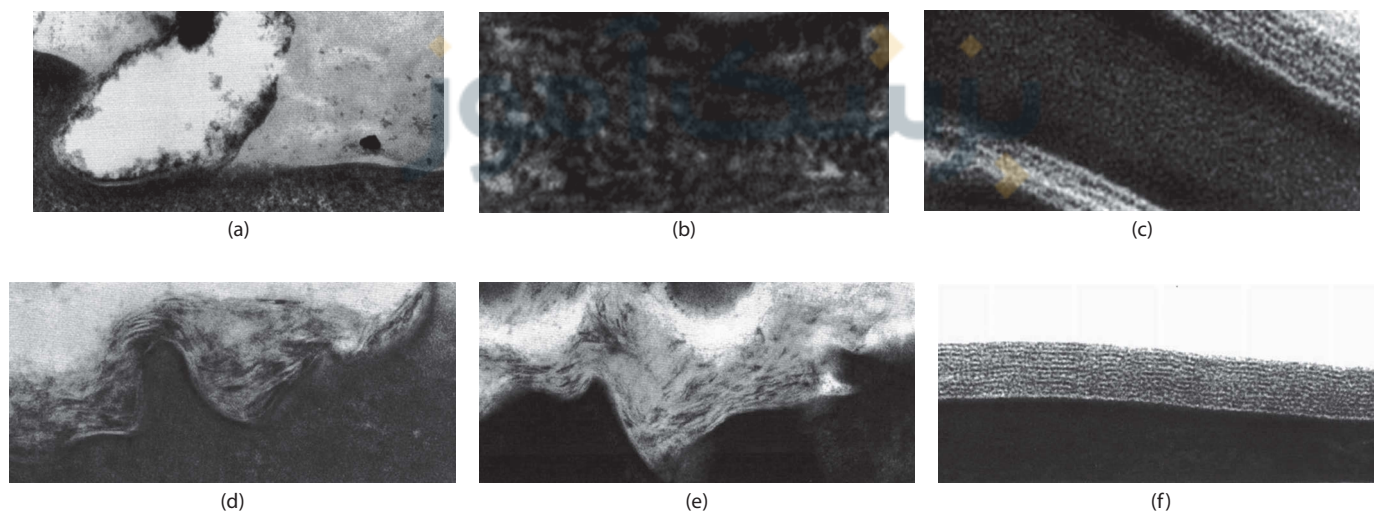
**Figure 14.1** Schematic representation of the stratum corneum “bricks and mortar” structure depicting the major structural components responsible for the water barrier function and overall tissue integrity. (From Rawlings AV, *Br J Dermatol*, 2014 Sep;171 Suppl 3:19-28.)

ceramide synthesis (28). The epidermosides are precursors to the covalently bound ceramides together with omega-hydroxy-containing ceramides (26). The general pathway

of ceramide synthesis is illustrated in Figure 14.5. At an initial glance the pathways of ceramide synthesis transportation and metabolism appears unduly complex and heavily dependent on metabolic resources and energy. However, the intrinsic insolubility of crystalline, high melting point ceramides, critical for barrier integrity, dictates initially their modification to facilitate transport and delivery to the stratum granulosum: the SC interface. This is achieved through their glycosylation (glucosylceramide synthetase), or conversion into sphingomyelin (sphingolipid synthetase) and transportation within MCGs. Once extruded into the intercorneocyte space the mature ceramides must subsequently be “regenerated” through a number of enzymatic processes. The integrity of the barrier function of the stratum corneum is therefore critically dependent on glucosylceramide and sphingomyelin synthesis (29). Ceramide synthesis in the epidermis, including the newly identified pathways for the 1-O-acyceramides, has been reviewed by Sandhoff and colleagues (21).

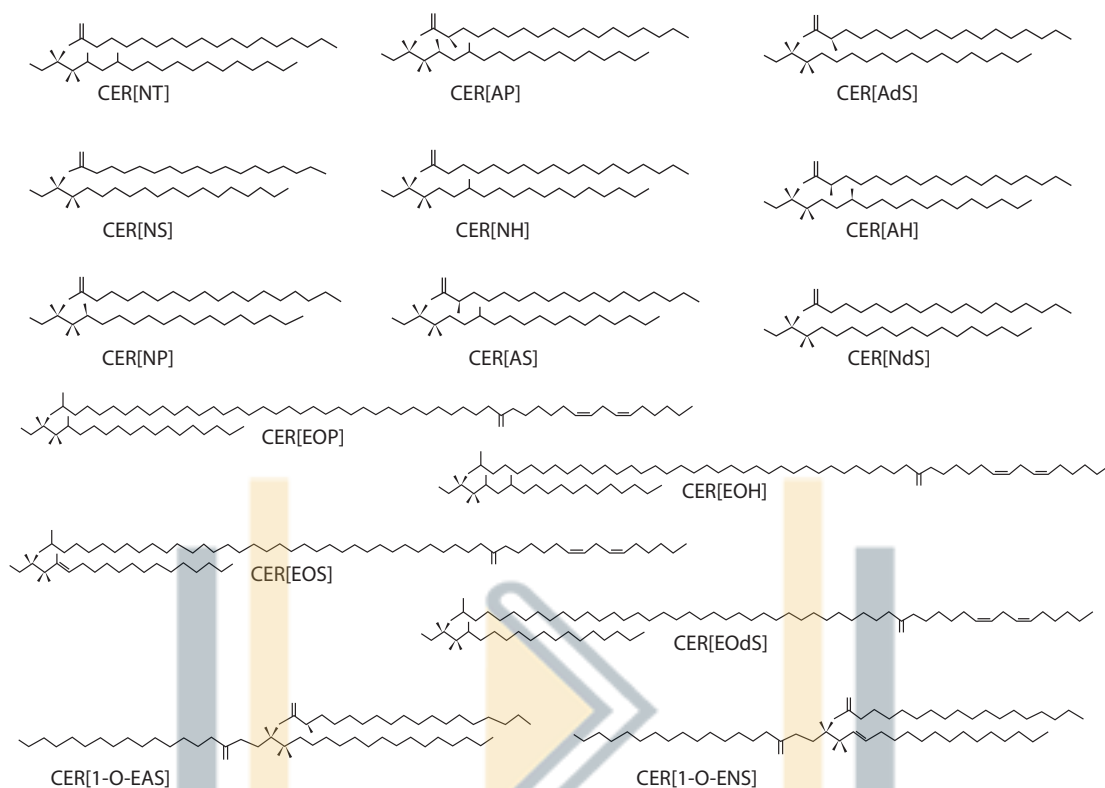
The chemical structures of the 15 free ceramides classes currently resolved are shown in Figure 14.3, where they are labeled according to the nomenclature system described below.

The old numerical ceramide nomenclature discussed in the earlier editions of this chapter has thankfully now largely been replaced by a nomenclature based upon chemical structure. Readers of older papers are cautioned to check the chemical structures of ceramides identified by either Arabic or Roman numerals as there is confusion and nomenclature inconsistency in regard to studies with ceramides isolated from porcine and human stratum corneum, and bovine brain-derived ceramides. The preferred ceramide nomenclature was first suggested some years ago (23,30) and is based on the molecular structures corresponding to the sphingoid base chains sphingosine



**Figure 14.2** Electron micrographs of lipid organization in tape strippings of normal (a–c) and xerotic (d–f) stratum corneum. Each case shows the first (a,d), second (b,e), and third (c,f) strippings. Normal lipid lamellae can be seen in the third strippings but loss of lipid organization is seen in the first for normal SC, whereas a totally disrupted structure is observed in xerotic skin ( $\times 200,000$ ; bar 0.05  $\mu\text{m}$ ). (From Rawlings AV et al., *J Soc Cosmet Chem*, 1994; 45:203–20.)





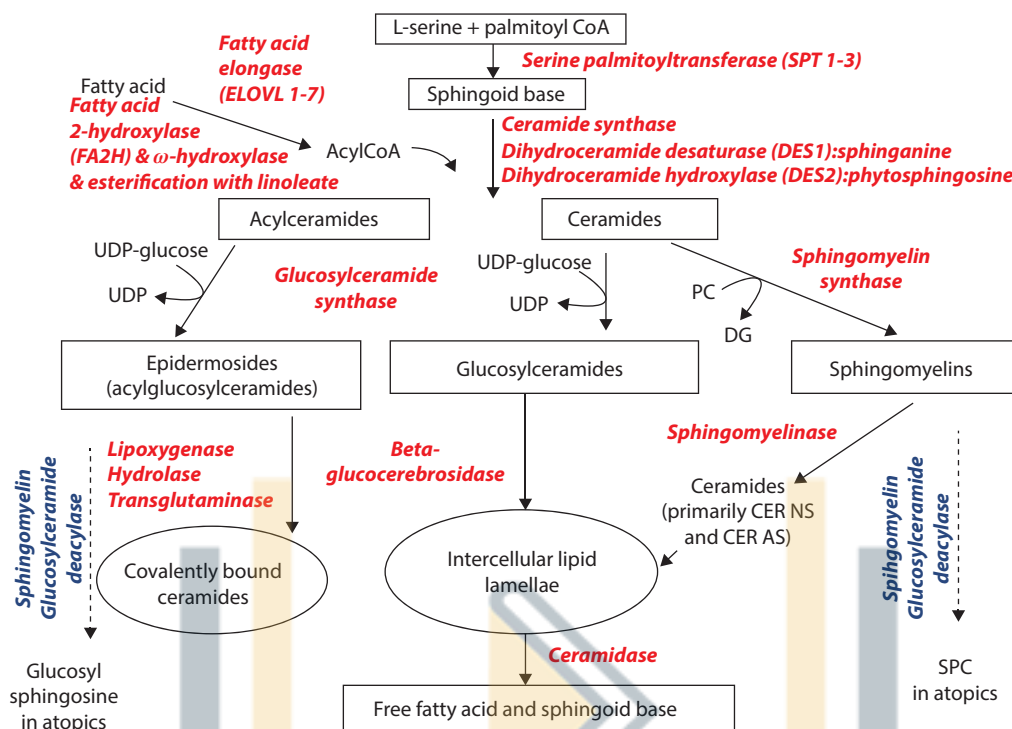
**Figure 14.3** Structures of the major free ceramide classes of human SC.



**Figure 14.4** Structures of the major covalently bound ceramides of human SC.

(S), 6-hydroxy sphingosine (H), dihydrosphingosine (DS), phytosphingosine (P), dihydroxy sphinganine (T), and the fatty acid chains alpha-hydroxy acid (A), nonhydroxy fatty acid (N) and omega-hydroxy fatty acid (O). The complete molecule is

designated by "ceramide-acid-base," i.e., the acid chain abbreviation precedes the base chain. In the case of ceramides esterified with an additional fatty acid, the letter E precedes the base and fatty acid chains. For ceramides covalently bonded to the



**Figure 14.5** Summary of the proposed pathways leading to synthesis of SC ceramides. Enzymes are shown in bold italics. PC, phosphatidylcholine; DG, diacylglycerol; UDP, uridine diphosphate, SPC, sphingosylphosphorylcholine. (From Wollenweber U et al., *SOFA*, 2004; 130.)

corneocyte protein envelope via the omega-hydroxy fatty acid the letter P precedes the base and fatty acid chain letters. For example, a 6-hydroxy sphingosine with an alpha-hydroxy fatty acid chain is denoted as CER[AH]. The free ceramide classes possible from the above combinations of acid and bases are shown in Figure 14.3 while the relative concentration of each class is listed in Table 14.1, based upon the work of t'Kindt et al (19).

Human SC ceramide base chains range from 18 to 22 carbons in length (12,22,31). For the non-hydroxy fatty acid ceramides the amide-linked fatty acid chains range from 16 to 32 carbons in length, with the major chain species being either 24 or 26 carbons (22,30). For the omega-hydroxy ceramides the fatty acid chains range from 30 to 34 carbons in length with linoleic acid (C18:2) esterified to the omega-hydroxy group (22,32). The pioneering analytical work of Masukawa and colleagues in evaluating SC ceramides was in general agreement (16,17) with the above and has been significantly added to by the work cited above (18–20). Furthermore, the identification of several odd-numbered fatty acid chains in SC ceramides has been confirmed in these studies (22,33). Table 14.2 lists the relative distribution of total ceramide carbon atoms as described by Bouwstra and colleagues (18). Using NPLC-ESI-MS methods, over 300 ceramide species had been to which approximately 100 new molecules are added with the identification of the 1-O-acylceramides (17,21). Further improvements in these techniques appear set to herald in the dawn of epidermal sphingolipid and ceramide lipidomics, defining precise changes in these species associated with body site, increasing age, gender, ethnic differences, and varying skin conditions. No doubt portending

**Table 14.1** Approximate Percentage of Major Free Ceramide Species to Total Ceramide Pool in The Stratum Corneum

Ceramide Nomenclature	Percent of Total Ceramide
Ceramide [NS]	7.4
Ceramide [NDS]	9.8
Ceramide [NP]	22.1
Ceramide [NH]	14.5
Ceramide [NT]	1.7
Ceramide [AS]	9.6
Ceramide [ADS]	1.6
Ceramide [AP]	8.8
Ceramide [AH]	10.8
Ceramide [EOS]	6.5
Ceramide [EOP]	1.1
Ceramide [EOH]	4.3
Ceramide [EODS]	0.4
Ceramide [1-O-ENS]	<2.0
Ceramide [1-O-EAS]	<2.0

the widespread application of such methods, a study from Kao's laboratories identified several significant differences in the profiles of 12 ceramide classes as a function of anatomical site and season. Changes in ceramide class and species (i.e., specific chain lengths) were correlated to skin properties such as barrier function and capacitance (34).

Analytical platforms now exist which allow for semi-quantitative analyses of all stratum corneum lipids. Thus, although not a focus of this chapter, it is worth noting that the development and application of increasingly sensitive lipidomic methods has increased our understanding of the free

**Table 14.2** Relative Distribution of Total Carbon Atoms (Both Chains) in the Ceramides of Human Stratum Corneum

Ceramide Chain Length (total carbon atoms)	Percent Abundance
34	2
40	3
41	4
42	9
43	6
44	13
45	6
46	14
47	5
48	10
49	3
50	5
51	2
52	2
66	2
68	2
70	2

fatty acids in the stratum corneum. The relative concentrations of free fatty acids are listed in Table 14.3, showing that saturated fatty acids make up some 95% of free fatty acids. Confirming earlier studies, C24 and C26 saturated chains are the major free fatty acid species (35). However, of note is the identification of several odd-numbered fatty acids as well as long-chain hydroxy free fatty acids such as C22:0-OH (18). The unusual physical properties of the SC lipid bilayers, compared to other biological membranes, are in large part due to these long, primarily unsaturated, hydrocarbon chains that characterize both the ceramides and the fatty acids. The critical importance of very long chain fatty acids (VLCFA) is emphasized by mutations in the enzymes (elongases) responsible for their synthesis. Mutations in the elongase, ELOVL4, are associated with the Stargardt-like macular degeneration. Deliberate deletion of ELOVL4 in murine models led to profound barrier defects characterized by a global decrease in VLCFA in the free fatty acid, ceramide, and glucosylceramide fractions (36,37). Strikingly, the SC is devoid of CER[EOS] and CER[EOH]. Similarly, synthetic short-chain ceramides (based on CER[NS]) with a 4 to 8 carbon acyl chain have been shown to dramatically increase SC permeability. The disruption to barrier was maximal with a C 6 acyl chain, whereas C2 and C12 ceramides did not increase permeability (38). In contrast, others have demonstrated that short-chain ceramides, including those with a C6 acyl chain, increase keratinocyte differentiation. (39,40).

Certain ceramides are covalently bonded to the outside aspect of the corneocyte protein envelope via the formation of ester linkages between hydroxyl groups on the ceramides and carbonyls of the beta-sheet proteins of the cornified cell envelope (41,42). This process appears to be catalyzed by transglutaminase 1 (43), a calcium-requiring enzyme previously thought to be involved solely with protein crosslinking *within* the cornified envelope. Lipoxigenases and an unknown hydrolase is also now known to be involved in this sequence of events prior to attachment to the corneocyte envelope via transglutaminase (44). Two novel species of

**Table 14.3** Relative Abundance of Free Fatty Acids in Human Stratum Corneum

FFA Carbon Chain Length	Saturated Chains (%)	Unsaturated Chains (%)
16	4.0	0.2
17	0.1	0.1
18	5.6	2.3
19	0.1	<0.1
20	0.7	0.1
21	0.1	<0.1
22	3.8	<0.1
23	2.8	<0.1
24	33.7	0.1
25	8.0	0.1
26	25.2	0.1
27	2.3	<0.1
28	7.1	<0.1
29	0.7	<0.1
30	1.0	0.2
>30	0.1	0.2

Table lists only species contributing >2%. It is noted that many minor species exist and have been quantified.

covalently bound ceramides have been identified—one consisting of a sphinganine base (C17-22), the other displaying a phytosphingosine base, and both linked to omega-hydroxy acid, designated CER[POS], and CER[POP], respectively (21). Downing suggests that all the omega-hydroxyceramides of corneocyte lipid envelopes are attached to proteins through their omega-hydroxyl groups (45). Clement et al. (46) found five classes of covalently bound ceramides in sparrows. Their structure awaits confirmation and their presence in humans needs to be identified.

It has been determined that there is enough lipid covalently attached to the corneocyte protein envelope to form a complete lipid monolayer over the surface of each cell (5,41). The very long chains of the envelope ceramides lipids will be conformationally ordered, thereby forming a water barrier around each corneocyte (41), and studies in mice suggest that the amount of covalently bound ceramide is highly correlated with the barrier function of the skin (47). A proposed critical function of this layer is to cover the corneocytes with a lipophilic coating and thereby act as a template or scaffold to direct the assembly of the extruded lamellar body lipids into lamellar bilayers (4,5). It has also been proposed that the corneocyte envelope may function as a semipermeable membrane permitting water transport but preventing the transport of natural moisturizing factor (NMF) out of corneocytes (1). Severe abnormalities to MCG formation, lipid organization, and barrier function result from the topical application of a specific inhibitor of omega-hydroxylation, emphasizing the important role this class of ceramides plays in barrier formation and integrity (48).

Although the majority of ceramides with the SC remain intact during SC maturation, ceramide-hydrolyzing enzymes have been identified in the SC, and these may be responsible for the formation of ceramide degradation products readily identified within the tissue (49). A lipid species called acyl acid, which appears to be the omega-esterified N-acyl fatty acid portion of CER[EOS], and free sphingoid bases were shown to be present in human epidermis (50–52). It is possible

that both of these lipid species could be derived from hydrolysis of CER[EOS] or acylglucosylceramides. Although the role of these degradation products is uncertain, sphingosine and other sphingoid bases may be involved in an SC–epidermis signaling function, as these have been reported to inhibit keratinocyte proliferation (53). It has been shown also that sphingosine is a potent anti-microbial and its presence in the SC may well form part of the skin's defense against invading microorganisms (54). Similarly, it has been reported that phytosphingosine has both antimicrobial and anti-inflammatory properties and has demonstrated potential to enhance existing anti-acne therapies. (55). The acyl acids may have additional lamellar lipid-structuring roles as has been shown for other esters (56–60).

As can be seen in Figure 14.5, the first step in the synthesis of ceramides is via condensation of serine and palmitoyl-CoA to form 3-ketodihydrosphingosine (not shown), which in turn is reduced by a reductase to dihydrosphingosine before being N-acylated by dihydro-CER synthase (CER synthase). Dihydro CER desaturase (DES1) produces ceramides whereas dihydro CER hydroxylase (DES2) produces phytoceramides. There are six CER synthases with specific fatty acyl-CoA preferences. CER S2 and S3 have the longest acyl chain length preferences. Once formed, these ceramides are converted to glucosylceramides and sphingomyelin and after secretion into the intercellular spaces are reconverted back to ceramides via beta-glucocerebrosidase and sphingomyelinase. CER[NS] and CER[AS] are primarily formed via the sphingomyelin pathway (61).

## LIPID ORGANIZATION IN THE STRATUM CORNEUM

The lamellar bilayer of most biological membranes consists of lipids in the liquid crystalline ( $L_\alpha$ ) state. In this state the lipid chains have considerable intramolecular conformational disorder. Aliphatic liquid crystal-forming lipids can undergo reversible transitions between the lamellar gel phase ( $L_\beta$ ) and the lamellar  $L_\alpha$  phase. In the  $L_\beta$  phase, hydrocarbon chains are in a fully extended all-trans conformation and the chains are packed in a two-dimensional hexagonal array which allows for some limited rotational freedom along the axes of the chains. Saturated long-chain lipids can also pack in lamellar bilayers in which the chains are packed in a two-dimensional orthorhombic array. In this phase the chains are conformationally ordered, packed in a very tight crystalline array, and have no rotational freedom (62).

Our understanding of biological membranes continues to advance as ever more sensitive analytical and biophysical techniques shed light on the molecular details of membrane lipid composition, organization, and dynamics. As discussed in the previous edition of this chapter there has been considerable modification of the original “fluid mosaic” model of cell membrane organization as lipid domains across cell membrane bilayers and within the plane of membrane leaflets have been observed in a variety of biological membranes (63–66). The complexity of the lipid composition of the SC, its unusual composition and unique physical properties, suggest the likelihood of a unique molecular organization. The very long carbon chain lengths of SC ceramides and free fatty acids (see Tables 14.2 and 14.3) along with the small polar headgroups of these lipids determine the unusual and highly specialized physical properties of the SC lipid lamellae.

A variety of biophysical techniques including nuclear magnetic resonance (NMR), wide-angle and small-angle x-ray diffraction, differential scanning calorimetry (DSC), Fourier transform infrared (FTIR) spectroscopy, and Raman spectroscopy have presented a detailed molecular level picture of lipid organization in the SC.

A variety of published reports have established that the hydrocarbon chains of SC lipids are highly ordered. McIntosh and co-workers, in x-ray studies of mixtures containing ceramides, fatty acids, and cholesterol, observed ordered gel phase lipids at 25 mole % cholesterol, which did not depend on the amount of water present or on the presence of protein (67). The repeat unit of 130 angstroms in these studies was postulated to arise from two bilayers. In early wide-angle x-ray studies of murine SC, White and colleagues reported the presence of some crystalline orthorhombic lipids at physiological temperature (68). Seminal studies by Bouwstra and colleagues utilizing x-ray diffraction and electron diffraction techniques demonstrated the presence of orthorhombic phase lipids in isolated human SC as well as ceramide/cholesterol/fatty acid models of SC (69–71). In addition, Bouwstra and colleagues have demonstrated the importance of CER[EOS] in producing the intermolecular organization necessary for healthy skin barrier function (72). Since the above-mentioned studies, many further studies have confirmed the presence of orthorhombic and hexagonally packed lipids in isolated human SC and in a range of ceramide-containing SC lipid models. Biophysical studies of complex mixtures of SC lipids, isolated SC, and even in vivo SC lipids is a vast topic well beyond the ceramide focus of the current chapter.

In one of the authors' laboratories (DJM), experimental infrared spectroscopy techniques were developed to measure lipid organization in lipid bilayers, cell membranes, and living cells (73–75) to explore the molecular behavior of diverse ceramide species, both alone and in lipid models of the SC (76–79). This work demonstrated that distinct ceramide species organize differently, reflecting distinct intermolecular interactions between their hydrocarbon chains and distinct head-group hydrogen bonding interactions. It is quite feasible that good overall cohesion in the SC, and therefore good barrier function, relies upon the diverse physical properties of these heterogeneous species.

CER[EOS] is worthy of particular mention. This is the predominant ceramide containing unsaturated fatty acids in the SC and it is remarkably enriched in linoleic acid, which comprises a minimum of 20%–30% of the omega-esterified fatty acid. The epidermis has an absolute requirement for linoleic acid in order to maintain a correctly functioning barrier and its absence leads directly to the dramatically perturbed barrier found in EFAD animals. The characteristic hyperproliferation seen in this condition may also reflect further perturbation in linoleic acid metabolism (80).

As the work of Bouwstra and co-workers and others has suggested, this particular ceramide species provides unique physical properties to the stratum corneum (72,81) that simply cannot be compensated for by the esterification of other unsaturated fatty acids within the CER[EOS] fraction. As we discuss later in this chapter, reduced levels of CER[EOS]-containing linoleic acid is a feature common to many skin disorders including acne, atopic dermatitis (AD), and winter xerosis.

In contrast, increases in CER[EOS] are a feature of the response of skin to repeated surfactant challenge (the so-called



“hardening phenomenon”), again emphasizing the key role this ceramide plays in skin protection (82).

The careful interpretation of many *in vitro* and *in vivo* investigations on lipid behaviour in the SC has led to several models being proposed to describe lipid organization in this structure. Some models are more theoretical/hypothetical, such as Forslind’s “domain mosaic” model (83) and Norlen’s related “single gel phase” model (84), whereas others are based on selected empirical data, such as Bouwstra’s sandwich model that relies primarily on x-ray diffraction data (69). Over the last decade no significant new models have been proposed for SC lipid organization, while the presence of orthorhombic lipid organization in both *ex vivo* and *in vivo* SC measurements has been repeatedly confirmed (85–89). Nevertheless, Iwai et al. (90) reported that the barrier is organized of fully extended ceramides with cholesterol molecules associated with the sphingoid moiety.

Although the prime function of lipids in the SC is that of providing the water barrier, lipid abnormalities associated with altered cornification have been reported in many common dermatological disorders (psoriasis and atopic dermatitis) (91). However, at present there is not a detailed understanding of how changes in the lipid composition, or specifically the ceramide composition, influence corneocyte cohesion and ultimately desquamation. There is indirect evidence for SC lipids being involved in cell cohesion from corneocyte re-aggregation studies *in vitro*. Numerous workers have re-aggregated previously dispersed corneocytes in the presence of SC lipids and found the physical properties of the reconstituted SC lipid-cell films to be similar to the intact tissue (92,93). In marked contrast, Chapman et al. (94) suggested intercellular lipids might actually have an anticohesive role, preventing close opposition of adjacent corneocytes. In those particular studies, when SC lipids were completely extracted, the intercorneocyte forces were dramatically increased, primarily due to the juxtaposition of the covalently bound lipids, and corneocytes became tightly cohesive. Taken together, these observations indicate that both the intercellular and covalently bound lipids may have a role in SC integrity.

Changes in physical properties of the SC ceramide lipids may also be important in promoting cell dyshesion toward the skin surface. It is known that the fine ultrastructure of the ceramide bilayers is disturbed in the superficial layers in normal skin (95). This loss of structure, critical for normal desquamation, may reflect hydrolysis by ceramidases (49). Alternatively, there is evidence that surfactant-like sebaceous fatty acids may lead to bilayer disruption (96) and the induction of an orthorhombic-to-hexagonal structure transition at the surface of the SC (97).

Ultimately, however, it is the corneodesmosome which is primarily responsible for inter-corneocyte cohesion (95–99), and it is this structure which must be effectively hydrolyzed to ensure desquamation. Ceramides, together with other lipid species, may play an important role in this process. Although the precise mechanism is far from understood, the phase behavior and organization of the intercellular lipids controls the water content in the SC and may influence the activity of the hydrolytic enzymes (100) present within the intercellular space which are responsible for corneodesmosomal degradation. In this respect it is of interest to note that the action of ceramidase in releasing free fatty acids may also contribute to the acidification of the stratum corneum that is critical for enzymatic regulation, normal desquamation, and barrier function (101).

## VARIATIONS IN SC CERAMIDE LEVELS

The total amount of ceramides in SC, as well as individual ceramide species, are influenced by disease and hormonal status, diet, age, race, external environment, and by circannual variation. Ultimately, it is the ratio of the different lipid classes as well as the levels of individual lipids in the SC that influence the physical properties of the extracellular lipid matrix and thereby barrier function, water content, and skin condition. These differences can be subtle; i.e., changes in the crucial ceramide:cholesterol ratio may explain the altered barrier characteristics in axillary skin (102). In this section we review specifically the changes in ceramide composition characteristic of various skin disorders.

### Psoriasis and Ichthyoses

Of the genetic diseases impacting skin condition, only lamellar ichthyosis and psoriasis have been investigated in any detail in relation to the levels of SC ceramides. In both of these conditions dramatic changes in SC lipid structure is observed, reflecting changes in lipid composition (103–105). These changes include increases in CER[NS] and CER[EOH], and decreases in CER[AS]. Together with the altered cholesterol and fatty acid levels, these alterations contribute toward some of the aberrations in SC function which are characteristic of these conditions, including corneocyte cohesion and faulty desquamation. Individuals suffering from lamellar ichthyosis have a defective gene for transglutaminase 1. As we have seen earlier, the inability to link ceramides to the cornified envelope has dramatic consequences for the skin, and the loss of this enzymatic function explains in part the dramatic skin phenotype seen in ichthyotic individuals. It has also been reported that the composition of the covalently bound lipids differs in psoriatic SC compared with healthy SC. In psoriatic skin, CER[OH] decreases while other components such as  $\omega$ -hydroxy acids and fatty acids, particularly the covalently-bound oleate and linoleate, are seen to increase (106). Focused studies on the discrete enzymes involved in the synthesis and degradation of ceramide have suggested that serine palmitoyl transferase (SPT), the rate-limiting enzyme in ceramide biosynthesis, is significantly decreased in lesional and nonlesional sites, whereas the levels of ceramidase were unchanged (106). The same authors also report on a negative correlation between the levels of this enzyme and the psoriasis area severity index score. The data on changes in ceramide levels in psoriasis were reviewed by Feingold (107) and indicate a shortening of ceramide chain length being dictated by the chain length of the amidated fatty acid. The proportion of CER[NH] with total higher carbon number between 40–43 was higher, whereas C47–50 was lower compared to healthy controls. Similar changes were observed for CER[AdS], CER[NP], and CER[AP]. Tawada et al. suggest that interferon gamma caused the effects, as *in vitro* it reduced the expression of ELOVL1, 4, 5, 6, 7, and CerS3, 4, and 6 (108). Ye et al. (109) have shown that the barrier recovery rates in uninvolved psoriatic skin are less compared with healthy skin.

Over the past 5 years the genetic basis of the congenital scaling syndrome harlequin ichthyosis has pointed to the importance of mutations in a member of the ABCA transporter family: ABCA12. Mutations in this gene lead to an abnormality in lamellar body formation, perturbed lamellar lipid organization in the SC, and loss of barrier function (110). These barrier perturbations are associated with profound decreases in the long-chain omega-hydroxyceramides

(CER[EOS]) and a corresponding increase in glucosylceramides (111). Understanding of molecular mechanisms indicates that epidermal ceramides may regulate ABCA12 gene expression through a PPAR delta-mediated signaling pathway (112). Although the SC lipid profiles in other ichthyotic diseases have not been fully determined, reduced levels of sphingosine have been found in a variety of subjects with various ichthyoses (113). This decrease in sphingosine may in part explain the cellular hyperproliferation observed in these conditions as sphingosine has been proposed to feedback to the epidermis and down-regulate keratinocyte turnover (53).

Refsum disease is a rare disorder of peroxisome metabolism due to a defect in the oxidation of phytanic acid, and Menon et al. (114) examined the barrier lipid structural organization in these patients. The SC intercorneocyte space were nonuniform, with some areas having appropriate lamellar structures and others completely lacking them. In many cases complete absence of corneocyte lipid envelopes was observed.

Sjögren-Larsson syndrome is caused by mutations in the fatty aldehyde dehydrogenase gene (ALDH3A2) which oxidizes medium- to long-chain fatty acids. Nakajima et al. (115) have reported that a patient had no difference in the quantity of SC-derived cholesterol but the fatty acids were increased two-fold while CER[EOS], CER[AP], and CER[AH] were decreased and membrane-bound ceramide as increased.

Dorfman-Charainin syndrome is a neutral lipid storage disorder in a gene that activates triglyceride lipases, yet acyl ceramides have been reported to be deficient in these subjects (116). Equally, reduced levels of corneocyte-bound omega-hydroxy ceramides and fatty acids were observed. It is proposed that the CGI-58 the protein that activates triglyceride hydrolysis also provides fatty acids for the omega-esterification of ceramides leading to acyl ceramides. Goto-Inoue et al. (117) also reported on the ceramide abnormalities in Dorfman-Charainin syndrome using imaging mass spectrometry, confirming the trace levels of acyl ceramides in these patients.

Chan et al. (118) highlighted the diagnostic implications of the skin ultrastructural changes in type 2 Gaucher disease, a disease lacking beta-glucosylcerebrosidase. Types 1 and 3 had normal lamellae due to low levels of the enzyme.

### Atopic Dermatitis and Netherton Syndrome

Atopic dermatitis (AD) is characterized by xerosis and reduced barrier function as measured by transepidermal water loss (TEWL) and corneosurfametry. This condition is also associated with a significant decrease in SC ceramide levels (119,120)—particularly CER[EOS]—containing linoleic acid (121)—and the presence of unusual, possibly diagnostic ceramide species (122). Research over the past 15 years has emphasized that many aspects of lipid metabolism are deranged in this condition; AD patients have significantly depleted covalently-bound  $\omega$ -hydroxyceramides (123) and reduced levels of prosaposin, an important regulator of sphingolipid metabolism (124).

Ishikawa et al. (125) observed increased levels of CER[AS] in AD patients and that the larger ceramide species (>50 carbons) was expressed at lower levels in CER[NS], CER[NdS], CER[NH], CER[AS], and CER[AH], whereas the smaller species (<40 carbons) were observed at higher levels in CER[NS], CER[NdS], and CER[AS]. CER[EOS], CER[EOP], and CER[EOH] were all at lower levels. Janssens et al. (126) also observed decreased levels of the EO species of ceramides including CER[EOdS] but increased levels of CER[NP] and of elevated C34 fatty acid chain length species in ceramide subclasses CER[AS], CER[AH], CER[NS], and CER[NH] (91,127).

Overall, the average ceramide chain length was significantly decreased by  $0.64 \pm 0.23$  total carbon atoms in atopic eczema patients, and no difference was observed between carriers and noncarriers of *FLG* mutations. When focusing on the lateral organization, atopic eczema patients show a less dense lipid packing compared with controls, which correlates strongly with a higher level of C34 ceramides. This finding shows that ceramide chain length is also an important determinant of the lateral lipid organization in SC. van Smeden et al. (128) also showed that a reduction in fatty acid chain length associates with a reduced ceramide chain length. An increase in unsaturated fatty acids is also observed in atopic eczema. Joo et al. (129) reported that representing lipids as a ratio to cholesterol rather than protein might be more sensitive for discrimination between AD patients and healthy controls. Nevertheless, Janssens et al. (130) observed a reduction in the lipid:protein ratio together with a thinner SC in both lesional and nonlesional skin. Angelova-Fischer et al. (131) observed that SC integrity was weaker in subjects with AD and filaggrin mutations but AD without filaggrin mutations was not different from healthy controls. Nevertheless, there was no difference in barrier recovery. Furthermore, the AD subjects with at least one filaggrin mutation had a lower ceramide-to-cholesterol ratio and reduced CER[EOH].

The total amount of ceramides is also reduced in Netherton syndrome while the amount of shorter chain ceramides (C30-40) is increased, much like the diseases reported previously (132). Of all the ceramide subclasses, CER[NP] was the most significantly reduced whereas CER[AS], CER[NS], and CER[AH] were the least affected. Equally, the levels of acyl-ceramides are dramatically decreased yet their glycosylated variants are not. Moreover, ceramides containing monounsaturated fatty acids were observed which lead to an altered lamellar lipid organisation with a high degree of disorder.

The lowered level of ceramides in AD has been linked to reduced activity of sphingomyelinase (133) and an altered (increased) expression of the enzyme sphingomyelin deacylase (134,135). This enzyme competes with sphingomyelinase for the ceramide precursor sphingomyelin. Although sphingomyelinase remains active in AD (136), significant levels of sphingomyelin are hydrolyzed by this alternative pathway to release free fatty acid and sphingosyl phosphoryl choline (SPC). The same enzyme can also degrade glucosylceramides to release glucosylsphingosine (GS) i.e. it also possess glucosylceramide deacylase activity (137).

The presence of SPC may partially explain the inflammation associated with this disorder as it is a potent modulator of epidermal function, stimulating proliferation and upregulating plasminogen activator (138). The vulnerability of the SC of AD patients to colonization by *Staphylococcus aureus* may reflect the reduced levels of sphingosine present in the tissue, in turn reflecting the decreased levels of ceramide (substrate) and the diminished activity of its metabolic enzyme acid ceramidase (139).

The shorter chain species identified in Netherton syndrome as related to reduced levels of ELVOVL1 and 6 and altered expression of glucosylcerebrosidase and sphingomyelinase was noted (132).

### Acne

Alterations in lipid species are evident in acne. Downing et al. (140) found reduced proportions of linoleate bound to CER[EOS] in acne patients and postulated that this reflected a localized decrease in the bioavailability of the essential fatty

acid due to a dilution effect of increased sebum production. Yamamoto et al. (141) have shown that the general decreases in both ceramides and free sphingosine in acne patients correlates to diminished water barrier function. Therefore, altered barrier functionality leading to epidermal hyperkeratinization and poor desquamation within the follicular epithelium may be responsible for comedone formation in susceptible individuals.

### Dandruff

Harding et al. have demonstrated (142) that in dandruff sufferers the intercellular lipid content of scalp SC, including ceramides, is dramatically reduced compared to healthy subjects. This depletion is associated with reduced barrier function, which may leave dandruff sufferers more prone to the adverse irritant effects of microbial metabolites, surfactants, or pollutants present on the scalp surface. These observations are consistent with studies that indicate the presence of a perturbed lipid ultrastructure in dandruff sufferers (143). Effective use of antidandruff treatments is accompanied by increased lipid levels, including ceramides (144).

### Senile Xerosis

It is widely experienced that as we age we suffer from more skin problems. Although these problems arise from a combination of many factors, an age-related reduction in the levels of SC ceramides may contribute to senile xerosis and other skin conditions. Age-related declines in SC ceramide have been reported in both Japanese (145) and Caucasian subjects (146–147). In the latter study, although the relative levels of the main ceramide subtypes did not change, overall SC lipid levels diminished with increasing age on face, leg, and hand skin. In addition, the same group reported an age-related decline in CER[EOS] linoleate levels, which may have a dramatic effect on SC barrier function (146). In another study with French Caucasians, a selective depletion of sterol esters and triglycerides, but not ceramides, was reported in aging leg SC (147). Researchers at L'Oreal have used high-performance liquid chromatography coupled with electrospray ionization mass spectrometry to elucidate ceramide change in dry skin. They report that phytosphingosine-containing ceramides are particularly depleted in dry skin compared with sphingosine-containing ceramide species (148). Moreover, shortening and lengthening of the acyl sphingoid bases sphingosine and 6-hydroxysphingosine have been reported in dry skin (148).

Ceramide subtypes have also been reported to change with age in Japanese women (145). Surprisingly, increases in CER[EOS] but decreases in CER[NP] and CER[AP] were found going from prepuberty to adulthood. These studies and others suggest that the impact of age on ceramide levels is likely to be influenced by ethnic background. For instance, in comparing SC lipid levels in several racial groups, Sugino and coworkers (149) reported that SC ceramide levels were lowest in African Americans compared with other racial types. Paradoxically, African-American skin is often viewed as being relatively resistant to damage, emphasizing the fact that the resilience of the skin barrier is determined by many factors including the size of the corneocytes and the thickness and integrity of the SC, although these factors were not discussed by these authors (150).

The most likely cause of an age-related decline in lipid levels is a reduced epidermal lipid biosynthesis capability,

as reported by Ghadially et al. (151). The increased activity of ceramidase reported by Akimoto may also contribute to declining ceramide levels (152). Studies in aged mice have shown reduced levels of epidermal acid sphingomyelinase and ceramide synthase consistent with a reduced age-related capacity to repair the barrier. In this study ceramidase did not show any age-related change (153). Further work is needed to extend and confirm these changes in man.

Although considerable progress has been made, our understanding of the influences of race, gender, and age on SC lipids remains incomplete. There is a large intra-individual difference in lipid levels without obvious physical manifestations of dryness (154). Similarly, in aged dry skin the demonstration of an altered lipid composition or differing molecular organization is not always apparent. Clearly, clinical dry skin is not monocausal and many other factors contribute to the phenotype (1). Nevertheless, and once again emphasizing the importance of the omega-hydroxy fatty acids, a deficiency of CER[EOS] and CER[EOH] is frequently correlated with an absence of the long periodicity phase as examined by x-ray diffraction in the dry skin disorder (81).

### Effects of Environmental Factors on The Expression of Winter Xerosis

It has become apparent that many factors influence the levels and types of SC lipids, and it is possible that their reduction leads directly to poor skin condition. Levels of lipids differ on different body sites, which may make some sites more or less prone to environmental damage (34,155). For instance, lower levels of SC lipids will be more susceptible to extraction (e.g. during hand washing) or perturbation of their structural organization which could lead to abnormalities in SC function and overall skin condition, leading to a visibly dry and flaky skin surface. Indeed, a picture is emerging that lipids influence the expression of this common problem. Lipids are easily extracted from the SC by solvents (156) and surfactants (157), leading to their depletion from the intercellular spaces of the SC, and resulting in skin scaling. In studies employing aggressive acute treatment regimes, solvent and surfactant extraction leads to changes in the relative amounts of the different lipid species in the outer layers of the skin, due to selective removal of lipids. However, during chronic treatments, particularly with surfactants, differences in SC lipid composition, but not total lipid levels, have been reported (158). Following chronic exposure to surfactants increases in CER[EOS], CER[NS], and cholesterol were observed, whereas the remaining ceramides, cholesterol esters, and long-chain fatty acids all decreased in concentration. Similar changes in SC ceramide profiles have been reported in other experimental models for scaly skin (e.g. tape-stripping), indicating that the changes in SC lipid composition are related to changes in epidermal lipid biosynthesis rather than lipid extraction from the SC (159). In skin suffering from soap-induced winter xerosis, the total levels of SC ceramides are decreased (160) and the levels of fatty acids are increased (160,161) leading to aberrations in the SC lamellar organization (Table 14.4 and Figure 14.2). Ishikawa et al. (162) demonstrated that all ceramide species negatively correlated with skin dryness, roughness, and scaliness.

Although the effects of climate on skin condition are well known, there have been very few studies examining circannual variation in SC lipids. An early study has shown a general decrease in epidermal cerebrosides in winter compared with summer (163). Following the analysis of SC lipids from the face, hand, and leg skin of female Caucasians in the winter,



**Table 14.4** Relationship of Skin Xerosis and Stratum Corneum Lipid Composition

Lipid Species	Skin Xerosis Grade			
	Grade 1	Grade 2	Grade 3	Grade 4
<b>Lipid Levels (ng lipid/<math>\mu</math>g protein)</b>				
Ceramides	64.9 $\pm$ 34.4	68.6 $\pm$ 30.4	39.2 $\pm$ 14.9*	37.5 $\pm$ 14.1*
Free acids	62.1 $\pm$ 34.6	67.4 $\pm$ 32.7	60.5 $\pm$ 37.0	54.9 $\pm$ 28.2
Cholesterol	3.9 $\pm$ 2.1	7.7 $\pm$ 4.2	4.4 $\pm$ 2.0	4.6 $\pm$ 2.3
<b>Relative Lipid Levels (% of total lipids)</b>				
Ceramide	47.1 $\pm$ 17.4	48.3 $\pm$ 8.6	40.2 $\pm$ 13.2	38.3 $\pm$ 11.2
Fatty acid	49.7 $\pm$ 18.6	46.2 $\pm$ 9.8	55.0 $\pm$ 12.0	56.0 $\pm$ 10.8
Cholesterol	2.0 $\pm$ 1.9	5.5 $\pm$ 2.6	4.8 $\pm$ 2.4	5.2 $\pm$ 3.2

Source: Rawlings AV et al., *J Soc Cosmet Chem*, 1994; 45:203–20.

Notes: Note reductions in ceramide levels with increasing skin xerosis. Values represent mean  $\pm$  standard deviation. Grade 1,  $n = 8$ ; grade 2,  $n = 8$ ; grade 3,  $n = 12$ ; grade 4,  $n = 12$ .

\*Significantly different from grade 1 ( $p, 0.05$ ).

summer, and spring months of the year, Rogers et al. reported decreases in all major lipid classes on all body sites during the winter months (146). Although the levels of ceramide subtypes were unchanged, the amount of linoleate esterified to CER[EOS] was reduced. These changes are likely to result in reduced barrier function. For instance, an increased susceptibility to treatment with SLS has been reported for the winter months of the year (164), and an inverse correlation has been shown between ceramide levels and TEWL following an SLS patch (165).

Xerosis is not confined to winter, and studies conducted almost 20 years ago reported that there were changes in ceramides associated with UV damage. Murine studies have indicated that there are dramatic differences in ceramide-relevant enzymatic activity during the early response to UV-B. Glucosylceramides accumulate in the SC due to attenuated activity of beta-glucocerebrosidase (166), and decreased levels of covalently bound lipids are measured potentially due to down-regulation of transglutaminase 1 (167). These and changes in other enzymes associated with lamellar body formation and organization will contribute to the reduced barrier function and scaly skin characteristically seen post UV irradiation.

During the past 15 years the pioneering studies by Denda and co-workers have begun to elucidate just how seasonal low humidity can influence epidermal processes and lead to disturbances in barrier function. The observation that low environmental humidity stimulates epidermal DNA synthesis and amplifies the hyperplasia associated with barrier damage (168) has helped to rationalize the characteristic seasonal exacerbation of inflammatory dermatoses. Perturbations to mast cell physiology have suggested the use of H1 and H2 histamine receptor antagonists to improve overall skin condition (169). The reader is referred to reviews by Denda (170) and Feingold and Denda (171) that explore several new strategies to improve barrier homeostasis.

Many studies only evaluate forearm or leg stratum corneum, and few have considered facial skin. Ishikawa et al. (34), however, examined the ceramide composition for a variety of body sites and observed that the skin on the lower legs, forearm, head, upper arm, buttock, and scalp have higher total ceramides compared with that of the palm, finger, lip, back of the hand, and cheek. The palm, finger, lip, and cheek showed a lower percentage of CER[NP], CER[EOS], CER[EOH], and CER[EOP] and a higher percentage of CER[NS] and CER[AS]. The skin on the scalp had the highest percentage of CER[NdS]

and CER[EOP], and the skin on the palm had the lowest percentage of CER[EOS], CER[EOH], and CER[EOP]. Equally, the average carbon numbers of CER[NS] were shorter on the lip, palm, cheek, and fingers compared with other body sites. C34-CER NS was higher on the scalp, forehead, cheek, and lip compared with the other body sites. Seasonal variations in these body comparisons were observed, and all studies need to consider this.

### Ethnicity

Muizzuddin et al. (172) compared the levels of SC ceramides in African-American, Caucasian, and East Asian skin. African-American skin had the lowest ceramide-to-protein ratio, with the East Asians being identical to the Caucasians. However, Jungersted et al. (173) found increased ceramide:cholesterol ratios for Asian subjects compared with Africans and Caucasians, with the Africans having the lowest values. Much more work is needed in this area to understand the importance of these differences to the known differences in skin barrier function.

## BIOSYNTHESIS OF SC CERAMIDES AND BARRIER REPAIR

### Endogenous Regulation of Ceramide Synthesis and Barrier Function

Elias and co-workers developed several models of barrier repair in order to decipher the biochemical control mechanisms of barrier homeostasis (reviewed in 174). When the SC barrier is damaged, a series of homeostatic processes are immediately accelerated, and in the absence of further damage, the barrier recovers to its original level. These processes include lipid biosynthesis, lipid processing, and the acceleration of the exocytosis of lamellar bodies. Although altered water flux is a key factor in initiating barrier repair following SC perturbation (175), the precise signal is not understood. Moreover, it should be noted that many of these studies were conducted on murine models and may not necessarily reflect the situation in human skin (176).

Studies using inhibitors to the key rate-limiting enzymes have indicated that all major species of SC lipids are synthesized during barrier repair and all are required for full barrier homeostasis. In contrast to the synthesis of cholesterol and fatty acids, which increase almost immediately after barrier disruption, synthesis of glucosylceramides, the precursors of the SC ceramides, is delayed until approximately 7 hours later (177). It is possible that the synthesis of the other lipids occurs



more quickly, as the rate-limiting enzymes (hydroxymethylglutaryl CoA reductase and fatty acid synthetase) involved in their synthesis are subjected to acute metabolic control mechanisms, such as phosphorylation. SPT (see earlier section) is not subjected to such control and requires the transcription and translation of further enzyme (178). Furthermore, studies on transcriptional control of lipid synthesis in mammalian cells have shown that the expression of genes involved in cholesterol and fatty acid synthesis and uptake is regulated by the sterol regulatory binding proteins (SREBP 1 and 2). In contrast, ceramide synthetic machinery does not appear to be regulated by this system (179).

Studies on animal models (48) have emphasized that hydrolysis of the glycosylated ceramide precursors by beta-glucocerebrosidase is a critical step in correct barrier formation and repair, and once again activity of this enzyme is regulated by barrier permeability (180). Although factors controlling the synthesis and activity of this enzyme are poorly understood, there is evidence emerging that the saposins (sphingolipid activator proteins) are intimately involved. This class of proteins stimulates enzymatic hydrolysis of sphingolipids including glucosylceramide and is essential for epidermal barrier permeability barrier formation and maintenance (181). Prosaposin is reported to be depleted in certain skin disorders (132,182). Many studies have indicated that ion flux plays a critical role in barrier homeostasis, and there is a drastic alteration of calcium gradient following barrier insult (183). It is possible that regulation of calcium ion dynamics after the barrier damage might control the skin barrier homeostasis (184). Mixtures of magnesium and calcium salts have been shown to accelerate skin barrier recovery and improve surfactant-induced or tape-stripping-induced dry skin (185). Although such studies indicate the importance of these ions for epidermal homeostasis, more work is needed with cosmetic formulations. Nevertheless, based on these observations some work has demonstrated that manipulation of ligand-gated ion channels can influence barrier recovery. Gamma-aminobutyric (GABA) type A receptor agonists musimol and isoguvacine accelerate barrier recovery following barrier disruption (186). Conversely, ATP (purinergic) receptor (P2X) agonists delay barrier recovery whereas P2Y antagonists accelerate it (187). These molecules also reduced the epidermal hyperproliferative response induced by acetone treatment under low environmental humidity.

Importantly, a variety of cytokines are released/secreted by the epidermis during barrier repair and they equally may contribute as key lipid biosynthetic switches in barrier development and barrier homeostasis (188–190). Readers are referred to the reviews of Denda together with Feingold and Denda for additional information (170,171).

### Influence of Topically Applied of Ceramides on SC Barrier Function

Imokawa and co-workers were the first to investigate the effects of topical application of human SC ceramides to solvent and surfactant-induced scaly skin (156,157). When the extracted lipids, and in particular the ceramide fraction, were applied back to the damaged skin, reductions in scaling and improvements in skin moisturization, as measured by skin conductance, were observed. This amelioration of skin condition was superior to placebo and corresponding formulations containing sebaceous lipids. In these studies the ceramides were either solubilized in squalene or emulsified in water in

oil cream containing monomethylheptadecylglyceryl ether. Interestingly, these effects were not observed without the glyceryl ether. The glyceryl ether may be aiding penetration of the influencing SC lipid phase behavior. Agents such as glycerol and glyceridacid are known to influence the physical properties of the ceramide-containing SC lipids.

Beradesca et al. (191) and Lintner et al. (192) have demonstrated that exogenously supplied CER[EOS] and CER[NS] respectively reduced the detrimental effects of SLS on disturbing skin barrier function. Chamlin et al. (193) reported that a ceramide dominant barrier repair cream helped to alleviate AD over a 6-week period. However, the title was incorrect in this paper, as a pseudoceramide was actually used (N-(2-hydroxyethyl)-2-pentadecanoylhexadecanamide (personal communication Y. Uchida). Studies by Imokawa and co-workers have reported on the skin condition benefits derived from topical application of formulations containing 5%–8% synthetic ceramides (so-called pseudoceramides) that mimic the physical properties of SC ceramides (194). Many laboratories continue to examine novel synthetic ceramide structures for both improved keratinocyte differentiation and impact upon barrier repair (195,196).

Invariably, optimal improvements in dry and damaged SC are observed when complete lipid mixtures are topically applied. De Paepe observed that a mixture of ceramides (CER[NP] and CER[AS], together with cholesterol, linoleic acid, and phytosphingosine provided significant improvements in barrier function and hydration over use of ceramides alone (197). Elias and co-workers have also focused on the use of exogenously supplied lipids to repair water barrier function (198,199). Although equimolar mixtures of ceramides, cholesterol, and fatty acids allow the barrier to repair at normal rates, an optimized mixture (cholesterol, ceramide, palmitate, and linoleate: 4.3:2.3:1:1.08) was noted to accelerate barrier repair following disruption of the murine water barrier by acetone. Although this mixture was seen to accelerate barrier repair following a range of barrier insults (tape-stripping, treatment with N-laurosarcosine or dodecylbenzene sulphonic acid), the mixture was not effective after barrier damage with SLS or ammoniumlaurylsulphosuccinate (200). These studies suggest that customized mixtures of the critical lipid species may be required to repair barrier damage resulting from differing insults. Further studies are required to relate the significance of these observations to human skin.

So called “ceramide-based” emulsions such as EpiCeram and TriCeram are based upon the physiological 3:1:1 molar ratio although they contain pseudoceramides. Other pseudoceramides have also been used topically (201–203). Many of the studies conducted with these formulations suffer from not having an appropriate vehicle control but at the same mass level in a product they should be superior to a non bilayer forming ingredients (204,205). However, if a non-bilayer-forming ingredient can be formulated into a product at much higher concentration because it is less expensive (e.g. petrolatum), then this will give a superior clinical benefit (206). These types of products may, however, be inferior in terms of product aesthetics.

Huang et al. (207), however, reported that CER[EOS] and CER[NS] act synergistically on skin hydration and transepidermal water loss of SLS-irritated skin relative to a control non-ceramide-containing vehicle. This is the best comparison potentially demonstrating the effects of ceramides but the vehicle simply had less “emollient” in the formulation. To be confident of these effects, further studies need to be conducted

with the same mass level of emollients and barrier lipids in the products.

It is also becoming apparent that in order to optimize barrier integrity through the topical application of ceramides that the correct stereochemistry of these lipids is considered. X-ray diffraction studies have reported that chiral CER[NP] forms the characteristic short (SPP) and long periodicity phases (LPP), while a racemic CER[NP] mix prevented formation of the LPP, resulting in a disrupted lipid matrix (208). Moreover, one of the big problems in interpreting the clinical data on use of ceramides is the lack of vehicle-controlled studies. Nevertheless, amphiphiles that mimic their behavior have been shown to be clinically very effective compared to non-bilayer-forming species (204,205).

### Enhanced Ceramide Synthesis through Delivery of Ceramide Precursors

Given the critical importance of ceramides to the barrier function, many researchers have sought routes to increase the synthesis of ceramides within the skin using lipid precursors. The earliest work relates to the correction of ceramide levels and barrier function in essential fatty acid deficiency by the application of linoleic and linolenic acid (209). Similarly, it has also been demonstrated that the low ratio of CER[EOS] linoleate to CER[EOS] oleate which is characteristically seen in skin in the winter months, and which may predispose such skin to winter xerosis, can be improved by up to 85%, through topical application of formulations containing linoleic acid in the form of natural oils (210). Brod et al. have also demonstrated similar effects on dry skin (211). Thus, increasing the proportion of CER[EOS] linoleate may improve SC function in such conditions.

Lactic acid, especially L-lactic acid, can also function as a general precursor to ceramides and this may explain the improvements in SC resilience observed following treatment with this alpha-hydroxy acid. The L-chiral isomer improved SC barrier function, as measured by both reduction in TEWL values following a challenge with SLS, and by improved resistance to the appearance of dry skin in moisturization efficacy studies (212). These improvements were related to the overall increase in SC ceramide levels and especially ceramide 1-linoleate levels following use of the prototype formulation. Furthermore, in vitro studies with keratinocytes established that lactic acid was utilized for lipid biosynthesis and it is possible that this also takes place in vivo, leading to increased ceramide levels and a more effective barrier.

Other precursors such as serine, the primary substrate for SPT, are utilized by keratinocytes in the presence of thiols (lipoic acid and n-acetylcysteine) to stimulate ceramide biosynthesis (213). These thiols presumably activate SPT by thiol disulphide exchange mechanisms, and might be expected to provide benefit to the skin of subjects with a perturbed barrier.

Another option for enhancing ceramide biosyntheses is to use substrates, which can feed into the ceramide biosynthesis pathway beyond the rate-limiting enzyme. Carlomusto et al. have shown that the modified sphingoid base tetracetylphyto-sphingosine (TAPS) is a substrate for ceramide biosynthesis in vitro (214). In vivo topical application of TAPs lead to an increase in ceramide biosynthesis in vivo as measured by lipid analysis of tape-stripped SC and a corresponding increase in resistance to surfactant damage (215). In further studies, a synergistic improvement in SC ceramide levels and a corresponding increase in barrier function was achieved when TAPS was

combined with omega-hydroxy acids and linoleic acid. This triple lipid combination preferentially increased CER[EOS] level above other ceramides, supporting its proposed mode of action as a CER[EOS] precursor (215).

Activation of key enzymes is a promising route to increase lipid biosynthesis. Studies by Tanno and coworkers (216) have shown that nicotinamide can increase synthesis of ceramide and free fatty acid levels in the SC and decrease TEWL in dry skin. The mechanism of this action involves increased synthesis of acetyl CoA (as a general precursor for both lipid species) and of mRNA for SPT with an associated increases in enzyme activity.

Peroxisome proliferator-activated receptors (PPAR) are nuclear receptors that belong to the steroid/thyroid/retinoid receptor superfamily and are specifically involved in lipid homeostasis. Evidence suggests that activation of these receptors by specific ligands can also increase the mRNA expression of several key enzymes involved in ceramide (SPT and beta-glucocerebrosidase) and cholesterol (HMG CoA reductase) synthesis. (217). It is of interest to note that expression of PPAR alpha is down-regulated in involved regions of psoriatic skin (218). Studies by Hara and coworkers have suggested that certain glycosphingolipids may also improve dry skin condition through activation of beta-glucocerebrosidase (219). There is also an indication that, in vitro at least, vitamin C can influence the hydroxylation and synthesis of specific ceramides, notably CER[AP] and CER[AH], to improve the overall organization and water-retaining properties of these model systems (220). Whether vitamin C can influence the spectrum of ceramides synthesized in vivo remains to be established.

Novel routes to enhancing ceramide levels have appeared in the literature; increased levels of ceramides are reported in the skin of elderly subjects following topical application of a preparation of *Streptococcus thermophilus* containing sphingomyelinase (221), and the potential of dietary manipulation leading to increased barrier function has been shown in mice following dietary supplementation of glucosylceramides obtained from rice bran and germ (222). Dietary supplementation with wheat germ extracts are also claimed to improve barrier function in humans. Peptides from potato hydrolysate have also been shown to increase ceramide synthesis (223), as well as eucalyptus extract, with macrocarpal A being the key component, which also increases the expression of the ceramide-generating epidermal enzymes (224). Moreover, Kato and Takahashi (225) reported that sodium alpha-tocopheryl-6-o-phosphate increases ceramide biosynthesis. In addition, Hashizume et al. (226) found that N-acetyl-L-hydroxyproline increased ceramide synthesis and expression of SPT.

### Influence of Ceramides on Keratinocyte Differentiation

In this review we have been concerned primarily with the role of ceramide in barrier function. Extracellular or exogenously added ceramides may play an important pro-differentiation role, indirectly leading to improved SC resilience. For instance, exogenously supplied short chain ceramides, and certain pseudoceramides, are known to induce keratinocyte differentiation in vitro (39,195). Short-chain ceramides and pseudoceramides also potentiate the effects of vitamin D, which is essential for keratinocyte differentiation (214).

Moreover, sphingolipids act as potent second messengers in diverse cellular signaling pathways (227–230). Ceramides are intimately involved in cellular decisions on

proliferation (231), differentiation (39,40,214,232), and apoptosis (233), and ultimately the ubiquitous roles fulfilled by ceramides impact skin condition at many different levels. Much of the initiation of mammalian apoptosis and epidermal differentiation takes place at the mitochondrial level where the physicochemical properties and integrity of the membrane plays a critical role. Mitochondria are essentially sensors of sphingolipids, and ceramides can influence membrane permeability, effecting activation of the critical proteases (caspases) that can initiate apoptosis or differentiation (234). The mitochondrial membrane appears to represent a common destination where mediators of stress converge and where critical decisions about cellular adaptation or apoptosis are taken. It is becoming clear that ceramides directly or indirectly profoundly affect mitochondrial functions but it must be remembered that this is also necessary for epidermal differentiation. (235,236). The most compelling evidence for a lack of effect of these compounds on apoptosis comes from *in vivo* studies. In humans Jatoti et al. find no effects of C2 and C6 ceramides on apoptosis (237). In light of these studies and *in vitro* studies, using realistic dose levels of short-chain ceramides can clearly induce epidermal differentiation by up-regulating ABCA12 expression via the PPAR delta-mediated signaling pathway, which are both important for epidermal differentiation and lipogenesis (112). These studies are consistent with the earlier investigations reported by Pillai et al. (39) and Paragh et al. (40).

There is a lot of confusion in the literature on whether ceramide or pseudoceramides and especially their short chain variants cause keratinocyte apoptosis or induce keratinocyte differentiation. This has been fueled by the studies of Uchida et al. (195) who showed that cell permeant C-2 ceramides and natural ceramides decreased mitochondrial membrane potential but pseudoceramides PC104 and BIO301 did not. The authors then emphasized the safety of the latter but not the former. This was despite earlier studies demonstrating the keratinocyte prodifferentiation benefits of short chain ceramides and pseudoceramides (39,40,214,232). Kim et al. (238) also demonstrated the apoptotic effects of tetraacetyl phytosphingosine in HaCat cells which was enhanced in the presence of UVB light. Takeda et al. (239), however, found that only C6 and not C2-ceramides induced apoptosis in HaCat cells. Conversely, Kwon et al. (196) established that the pseudoceramides K6PC-4, 5, and 9 induced differentiation of primary keratinocytes. Philips et al. (240) however, found that C-2 ceramides increased caspase-3 expression in primary keratinocytes. Nevertheless, Jiang et al. (112) found that short-chain ceramides increased ABCA12 expression via stimulation of PPAR delta expression, indicating the role of short chain ceramides in epidermal differentiation that was long established (39,40,214,232). Murakami et al. (241) later showed that phytoceramide and sphingolipid bases derived from brewer's yeast activated PPAR, which was followed by a study of the effects of sphingoid bases in keratinocytes (242) and the effects of phytosphingosine-1-phosphate inducing EGF-dependent dermal fibroblast proliferation together with phytosphingosine ameliorating skin inflammation (243). Farwick and Rawlings reviewed the effect of sphingoid bases as PPAR agonists (244). Thus on balance, despite the earlier study of Uchida et al. (195) demonstrating the pro-apoptotic effects of ceramides, this class of lipids would appear to induce keratinocyte differentiation. In support of this, Jiang et al. (245) demonstrated that C2 and C6 ceramides (but not C8 glucosyl ceramides) increased caspase-14 expression. Nevertheless, the most compelling evidence for a lack of effect of these compounds on apoptosis comes from

*in vivo* studies described above (237). Additionally, a study by Grether-Beck et al. (246) confirmed the lack of effect of phytosphingosine, N-hexanoyl phytosphingosine, and N-stearoyl phytosphingosine on UVB-induced apoptosis. Increased caspase-3 activity was observed in basal and differentiating keratinocytes with UVB, but co-stimulation with the ceramides did not further increase caspase-3 activity and nucleosomal fragmentation, corroborating the safety of these ceramides. Moreover, Janusova et al. (247) found that medium- to long-chain ceramides (C8-24) had no detrimental effect on barrier function, whereas short-chain ceramides adversely affected the physical properties of the barrier. This is similar to the studies of Novotny et al. (38). Thus the short-chain ceramides may enhance their own delivery.

In support of this, several dietary studies have also confirmed the effects of dietary sphingolipids on keratinocyte differentiation. Hasegawa et al. (248) showed that glucosylceramide enhances corneocyte envelope formation via transglutaminase expression and involucrin production in hairless mice irradiated by UVB. Ideta et al. (249) showed that orally administered glucosylceramide improves epidermal tight junction and corneocyte envelope expression in SDS-treated mice. Duan et al. (250) showed that glucosyl ceramide and sphingomyelin increased the expression of ceramide synthases 3 and 4 in the epidermis of an AD-like skin model, and Kawada et al. (251) demonstrated that dietary glucosyl ceramide enhanced the expression of claudin-1 in UVB-irradiated mice.

## CONCLUSIONS

Research into the structure and function of skin ceramides has increased dramatically over the past 20 years. These complex lipids have been shown to influence many aspects of cutaneous biology, including the barrier, mechanical, and desquamatory properties of the SC. In this respect our understanding of the relationship between SC lipids and cosmetic and pathological skin conditions has been essential in our continued desire to improve these abnormal skin conditions, through the use of either defined barrier lipid species or their biosynthetic precursors. Nevertheless, as our techniques of investigation become more sophisticated it is becoming increasingly evident that our understanding of these molecules remains in its infancy. The observations that the SC contains over 300 distinct ceramide species, derived from 13 classes, suggests an exquisitely subtle relationship between the types of ceramides and their correct physical and chemical characteristics and the essential barrier function of the skin. There is much to learn about the critical role that ceramides play in epidermal signaling and differentiation, the factors that control their biosynthesis, and not least, the subtleties of their organization that maintain the essential barrier function. Moreover, the safety of ceramides, whether short or long and in the presence of UV or not, has now been completely established through the exhaustive studies conducted over the last two decades both *in vitro* and *in vivo*.

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## 4-Hexyl-1,3-Phenylenediol, an NF- $\kappa$ B Inhibitor, Improving Clinical Signs of Aging

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### INTRODUCTION

Aging can be defined as a physiological process that is characterized by an alteration of the physical and intellectual abilities of the human body, the fight against aging being one of the major challenges of the twenty-first century.

Skin aging could be considered as a reflection of the overall aging of the human body. Indeed, the appearance of wrinkles is one of the first visible signs of aging. Among several features, skin aging can be characterized by the appearance of wrinkles but also an emergence of brown spots, loss of skin tissue, or reduced wound healing capacity. Cosmetic industries through their “anti-aging” product lines attempt to mitigate the appearance of these signs of aging. Therefore, the development of these products requires prior knowledge of skin aging mechanisms and many research teams try to identify markers of aging and evaluate the cellular and molecular alterations responsible for these skin changes. Briefly, skin aging is associated with epidermal atrophy, decreased proliferative capacity of skin cells, and loss and/or alterations of dermal extracellular matrix (ECM) protein expression.

At the cellular level, the aging process, also called cellular senescence, involves a cell cycle arrest accompanied by a certain loss of function and an alteration of the gene expression pattern. Over the past 20 years, NF $\kappa$ B (nuclear factor kappa B) activation has been recognized as a hallmark of the aging process as it regulates the expression of numerous genes, some of which are involved in senescence entry and maintenance.

### NF $\kappa$ B SIGNALING

First described in 1986 by Sen and Baltimore (1), NF $\kappa$ B is a well-known transcription factor involved in the regulation of numerous target genes related to innate and adaptative immunity, inflammation, apoptosis, or cellular growth (2,3). NF $\kappa$ B was also shown to be involved in several diseases including arthritis, asthma, and cancers (4).

The NF $\kappa$ B family members such as p50, p52, p65, c-Rel, and RelB are found as homodimers or heterodimers in the cytoplasm of unstimulated cells, the most common form being a heterodimer of p65 and p50. These proteins share highly conserved sequences of amino acids known as the Rel homology domain, containing a DNA-binding site, a dimerization domain, and a nuclear localization domain.

Both homodimers and heterodimers are sequestered in the cytoplasm in an inactive form due to their association with a member of the I $\kappa$ B family of inhibitory proteins masking their nuclear localization domain and preventing their translocation to the nucleus (5). Activation of the dimers requires the activity of I $\kappa$ B kinases (IKK) which phosphorylate two

serine residues (Ser32 and Ser36) on I $\kappa$ B proteins leading to their ubiquitination and degradation in the proteasome. The degradation of I $\kappa$ B proteins unmasks the nuclear localization sequence of NF $\kappa$ B which can translocate to the nucleus and finally bind to specific sequences on the promoter region of various target genes (6).

### NF $\kappa$ B AND SKIN HOMEOSTASIS

NF $\kappa$ B pathway was first studied for its role in the development and function of immune cells (7). More recently, its implication in non-immune cell growth and function regulation has been largely investigated, especially for skin homeostasis. More specifically, several studies have shown that NF $\kappa$ B signaling had a particularly important role for the maintenance of immune homeostasis in epithelial tissues (8).

The skin is comprised of two major compartments, the epidermis and the dermis. The latter is mainly composed of fibroblasts surrounded by ECM proteins such as collagen and elastin. The epidermis is a stratified epithelium containing various layers of keratinocytes. The basal layer is mitotically active and contributes to cell renewal, whereas in the upper layers, proliferation stops and cells migrate and undergo terminal differentiation.

Khavari's team has evidenced a critical role of NF $\kappa$ B in epidermis homeostasis. They observed a cytoplasmic localization of NF $\kappa$ B proteins within cells of the basal layer and a nuclear localization in non-proliferating cells of the upper layers, suggesting that NF $\kappa$ B activation plays a role in the switch from the proliferative basal cell phenotype to the non-proliferative one of suprabasal layers (9). Furthermore, blockade of NF $\kappa$ B function resulted in epithelial hyperplasia demonstrating a potential role for NF $\kappa$ B activation in negative growth regulation, in contrast to other tissues. In vitro overexpression of active NF $\kappa$ B subunits in normal epithelial cells inhibits cell cycle progression and favors cell cycle inhibitor expression such as p21 (10). Likewise, IKK deficient mice display abnormal skin development with thicker epidermis associated with hyper-proliferation and defects in keratinocyte terminal differentiation (11).

The epidermis is perpetually subjected to harmful UV radiation. In this context, NF $\kappa$ B binding activity was shown to be rapidly induced in nuclear extracts from skin submitted to UV radiation, suggesting a role for the transcription factor in keratinocyte protection and survival (12).

At the dermis level, NF $\kappa$ B was shown to inhibit the expression of collagen 1 alpha 1 (COL1A1) gene (13). This inhibition is mediated through the recruitment of p65 subunit to the COL1A1 gene promoter by physical interaction with COL1A1

transcription activators c-Krox, Sp1, and Sp3 (14). NFκB is thus a potent negative regulator of dermal ECM homeostasis.

Furthermore, overexpressing an NFκB super-repressor in adult mice resulted in defective morphogenesis of hair follicle and other appendices (15), confirming important functions of NFκB in skin biology. Altogether, these data clearly indicate that NFκB proteins are strongly involved in skin homeostasis.

## NFκB ACTIVATION UNDER (SKIN) AGING

There is now growing evidence for an increased NFκB activity during aging. This alteration was first observed on rodents by Salimen's group, which demonstrated an age-related increase in NFκB binding activity in mouse cardiac muscle and rat brain (16,17). However, neither IκB inhibitor protein levels nor IKK protein levels and phosphorylation activity were affected by aging (18), suggesting a potential retention of the transcription factor into the nucleus. The role of NFκB signaling during aging was further investigated by motif module mapping technique. These experiments on various tissues from mice and humans revealed that the NFκB motif was the motif most strongly associated with aging (19). Another study suggested that NFκB might be activated upon senescence (20). More recently, a genome-wide expression profiling identified various up-regulated and down-regulated genes during cellular senescence as downstream targets of NFκB. Reversion of senescence by abrogation of p53/p21 and p16/pRb pathways reversed up-regulation of these NFκB target genes as well as silencing of NFκB subunits did manage to overcome growth arrest. This suggests that NFκB signaling has a causal role in promoting senescence (21).

Of interest, various studies demonstrated an association between NFκB signaling and skin aging. Indeed, senescent keratinocytes were shown to display higher NFκB DNA binding activity than young ones and inhibiting its activity resulted in decreased senescence-associated β-galactosidase (SA-βGal) staining in old cells. Also, overexpression of NFκB subunits in normal young keratinocytes induced premature senescence of these cells (22). Likewise, Bigot et al. observed elevated amounts and binding activities of p65 and p50 NFκB subunits in dermal fibroblasts isolated from aged skins (23). This alteration is associated with a significant loss of COL1A1 expression in older cells compared to younger ones. NFκB was previously shown to inhibit the transcription COL1A1 gene (13,14); thus, as expected, forced expression of p65 and p50 NFκB subunits in young cells resulted in significantly lower expression of COL1A1. Also, overexpression of p65 did induce senescence in fibroblasts from young and old donors. Altogether, this set of data strongly suggest that NFκB signaling might be involved in the skin aging process by favoring skin cell senescence and preventing collagen synthesis, both features leading to the appearance of the clinical signs of aging. Supporting this hypothesis, the work of Adler et al. revealed that tissue-specific NFκB blockade in mice did reverse key features of epidermal aging such as loss of proliferative potential of keratinocyte from the basal layer, p16 expression, and SA-βGal-positive staining (19).

NFκB pathway can also be activated in response to oxidative stress triggered by ionizing radiation or ultraviolet exposure with a subsequent enhanced DNA binding activity and NFκB protein expression (24, 25). Skin being chronically exposed to sun radiation along life, enhanced NFκB activity might favor cellular senescence and participate to skin aging, in particular photo-aging.

UV radiation and photo-aging are often associated with an accumulation of DNA damage through the oxidative metabolism of reactive oxygen species (ROS) (26). Our preliminary experiments suggest that NFκB might favor DNA damage accumulation after UV exposure (27). Indeed, topical pretreatment of skin equivalents with an NFκB inhibitor (4-hexyl-1,3-phenylenediol) significantly reduced UV-induced DNA damage as determined by T-T dimer formation in skin cells. However, NFκB was also shown to be involved in double strand break removal and repair by stimulating homologous recombination, potentially through BRCA2 which expression can be induced by NFκB (28,29). Conversely, our experiments, where human primary keratinocytes were treated with NFκB inhibitors (Bay-11-7082 and 4-hexyl-1,3-phenylenediol) after UV exposure, indicate that NFκB activity could delay DNA repair capacities of keratinocytes (27). Cells immediately treated with NFκB inhibitors (0.1μg/mL and 1μg/mL) displayed significantly lower DNA damage after 1 or 2 hours of treatment as measured by Comet assay compared to untreated cells, strongly suggesting improved DNA repair capacity with NFκB inhibition. Sauvaigo and her team recently demonstrated that both base excision repair and nucleotide excision repair were significantly reduced in human skin fibroblasts under aging (30,31). As previously mentioned, NFκB binding activity is significantly increased in dermal fibroblasts from older donors compared with younger ones (23). Taken together, these data clearly suggest that elevation of NFκB transcriptional activity might contribute to the decrease in DNA repair capacity of skin cells under aging.

## A NEW TARGET FOR SKIN AGING?

Since NFκB is now clearly associated with skin aging, it appears as a promising target. As mentioned, the work of Adler et al. clearly supports the idea that NFκB blockade could reverse the skin aging phenotype (19). Indeed, tissue specific blockade of NFκB in the epidermis by using a mutant of p50 unable to bind DNA under control of the keratin 14 promoter did reduce gene expression of 225 genes in older skins to gene expression levels observed in younger ones. It also reversed key features of epidermal aging such as loss of proliferative potential of keratinocyte from the basal layer, p16 expression, and SA-βGal-positive staining (19).

NFκB inhibitors are also frequently investigated for their protective effects against skin aging. For example, Tanaka and his colleagues have widely explored this topic and demonstrated that some plant extracts such as parthenolide (from *Tanacetum parthenium*), magnolol (from *Magnolia ovovata*) or cynaropicrin (from *Cynara scolymus* L.) efficiently protected skin cells from photo-aging (32,33,34). Likewise, our results strongly indicate that the NFκB inhibitor 4-hexyl-1,3-phenylenediol, also known as HEXINOL™, can protect skin cells from UV-induced DNA damages and increase DNA repair capacity, thus preventing the photo-aging process (27).

## 4-Hexyl-1,3-Phenylenediol: A New Technology against Skin Aging?

4-hexyl-1,3-phenylenediol is commonly used in food processing (35) and has a long history of use in topical disinfectant and throat lozenges for its antioxidative and antimicrobial properties (36,37,38). It contains a 4-substituted phenylenediol motif, and compounds with such chemical moiety are known as potent inhibitors of tyrosinase activity in vitro (39,40), an enzyme involved in melanin synthesis. Our recent study



described that 4-hexyl-1,3-phenylenediol significantly reduced melanogenesis in primary human melanocytes, murine melanoma cells, and pigmented human epidermal equivalents through inhibition of tyrosinase enzyme activity and protein expression (41). A double-blinded, randomized controlled clinical study confirmed that topical application of a formula containing 4-hexyl-1,3-phenylenediol significantly reduced skin hyperpigmentation (41). These findings clearly establish 4-hexyl-1,3-phenylenediol as an effective agent for the treatment of undesirable human skin hyperpigmentation such as the appearance of brown spots, one of the key features of skin aging process.

4-hexyl-1,3-phenylenediol is also known as a potent inhibitor of NF $\kappa$ B (42). Given the prominent role of the transcription factor in aging and in particular during skin aging, we investigated whether NF $\kappa$ B inhibition through 4-hexyl-1,3-phenylenediol could improve photo-damaged skin and clinical signs of aging. Our recent results (43) first confirmed an increased NF $\kappa$ B transcription activity in cells from older skins compared with younger cells, activity that was dose-dependently inhibited with 4-hexyl-1,3-phenylenediol. Furthermore, treatment with 4-hexyl-1,3-phenylenediol restored collagen and elastin synthesis that had been inhibited by NF $\kappa$ B signaling enhancement. At the clinical level, an 8-week, double blinded, placebo-controlled, randomized controlled study demonstrated significant improvements regarding crow's feet fine lines, cheek wrinkles, and forehead wrinkles in females treated with 4-hexyl-1,3-phenylenediol compared with those treated with placebo. Skin radiance, mottled pigmentation, and age spots were also significantly improved (43). These results are in accordance with the anti-tyrosinase activity of 4-hexyl-1,3-phenylenediol, as previously described (41).

This set of data strongly indicate that 4-hexyl-1,3-phenylenediol, by reducing skin hyperpigmentation and inhibiting NF $\kappa$ B-mediated skin damage such as reduced ECM expression or decreased DNA repair capacity, is a potent tool for anti-aging strategies.

## Clinical Study

To further investigate the effects of HEXINOL technology on skin aging, we performed a clinical study on 42 female volunteers from 45 to 70 years old. A product containing the 4-hexyl-1,3-phenylenediol (1%) or a placebo was applied by the subjects on half face and neck for 12 weeks, twice a day. Enrolled volunteers presented wrinkles to the forehead, in crow's feet area, underneath eyes, in cheeks and nasolabial folds, and to the corner of the lips. They also displayed pigmented spots to the face (at least one of 3 mm in diameter on each half-face), tonicity problems, and dull complexion.

Skin aging features were clinically assessed by a dermatologist using a visual analog scale. Brown spots were also characterized using a colorimeter, and viscoelasticity of the skin was measured. Assessments were performed before the first application (T0), then after 4 weeks (T4), 8 weeks (T8), and 12 weeks (T12) of product application.

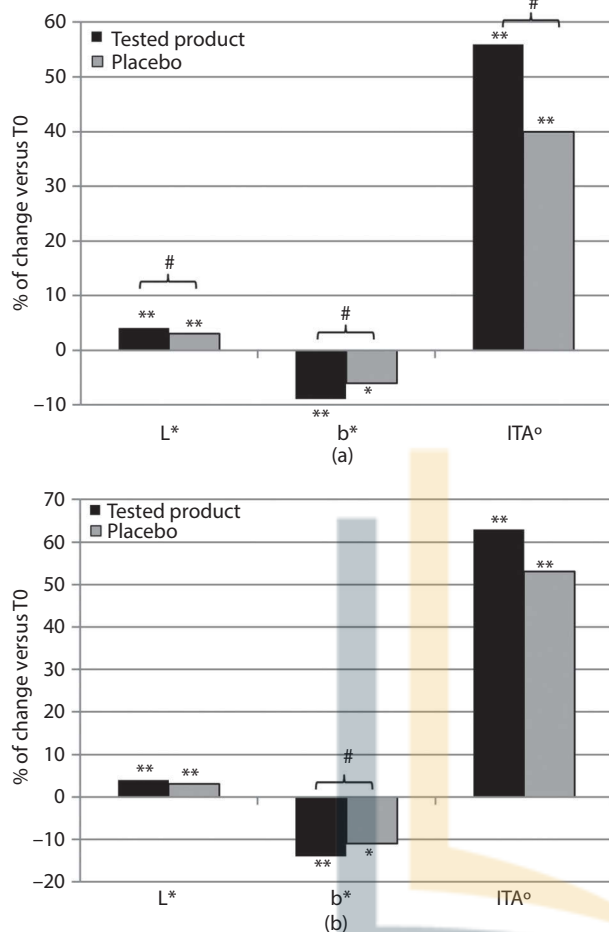
Results of the clinical grading are presented in Table 15.1. For nearly each parameter, there was a significant improvement after treatment with both the product and the placebo at T8 and T12. Moreover, for almost all improved parameters, the improvement was significantly higher in the tested product when compared with placebo. At T4, no improvements were observed with both tested products. Regarding skin viscoelasticity, as measured by a cutometer, both placebo and product significantly improved skin elasticity. However, no significant differences were observed between the two products. This set of data clearly indicate that HEXINOL technology contributes to the reduction of wrinkles and fine lines, favors skin firmness and elasticity as well as skin softness and smoothness, and improves skin radiance and complexion.

Colorimetric assay results on brown spots are consistent with the clinical grading performed by the dermatologist (Figure 15.1). After 8 weeks and 12 weeks of treatment, individual typological angle (ITA°) values, which define the skin pigmentation degree of a subject and the L\* values, were significantly increased, indicating a decrease in the spots' darkness with both product and placebo. Of note, at T8 the tested product had a significantly

**Table 15.1** Percentage of Improvement for Each Parameter with The Tested Product at T8 and T12

	T8		T12	
	% Improvement	Significance vs Placebo	% Improvement	Significance vs Placebo
Forehead wrinkles	5,00	*	8,00	*
Crow's feet wrinkles	17,00	*	25,00	*
Under the eye wrinkles	19,00	*	29,00	*
Cheek wrinkles	10,00	*	18,00	*
Marionette wrinkles	5,00	NS	7,00	NS
Nasolabial fold	4,00	*	7,00	*
Crow's feet fine lines	9,00	*	13,00	*
Under the eye fine lines	13,00	*	21,00	*
Color intensity of pigmented spots	11,00	*	24,00	*
Size of the pigmented spots	3,60	NS	6,00	NS
Number of pigmented spots	8,00	*	16,00	*
Overall photodamage	9,00	*	16,00	*
Skin brightness	18,00	NS	26,00	NS
Complexion homogeneity	26,00	*	33,00	*
Skin firmness	26,00	*	35,00	*
Skin elasticity	21,00	*	28,00	*
Softness	21,00	*	30,00	*
Smoothness	32,00	*	46,00	*

Note: Results are presented as percentage of improvement as compared to T0. \* $p < 0.05$  versus placebo, NS: Non significant.



**Figure 15.1** Percentage of change of L\*, b\*, and ITA° values at (a) T8 and (b) T12 versus T0. Color is expressed in a three-dimensional coordinate system, in terms of three units: L\* (black-white), a\* (green-red) and b\* (blue-yellow). The skin color is an admixture of the L\* a\* b\*-values. Individual Typological Angle (ITA°) defines the skin pigmentation degree of a subject,  $ITA^\circ = (\arctan(L^* - 50/b^*)) \times 180/\pi$ . The higher the ITA° and L\*, the lighter the skin is, and the lower the b\* parameter, the less yellow/brown the skin is. \*\* $p < 0.001$  vs T0; \* $p < 0.05$  vs T0; #  $p < 0.05$  between products.

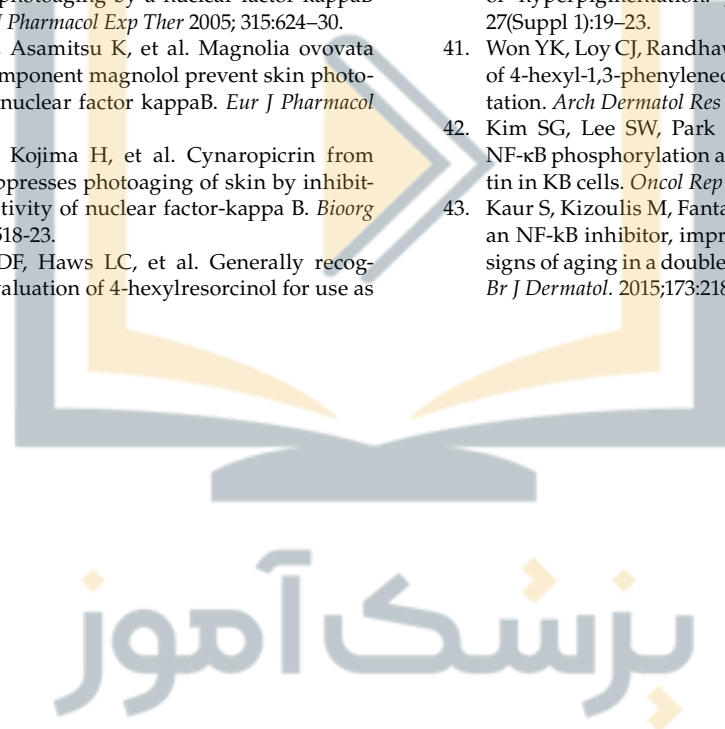
higher activity as compared with placebo. Moreover, b\* values were significantly reduced by both treatments, indicating a decrease in spot coloration. The tested product also had a significantly higher activity on b\* values as compared with placebo. Altogether, these data strongly demonstrate that HEXINOL technology is effective in reducing age-induced brown spots.

Finally, the clinical study performed on a product that contains the active ingredient 4-hexyl-1,3-phenylenediol clearly confirms the anti-aging properties of 4-hexyl-1,3-phenylenediol and validates the NFκB pathway as a promising target to fight skin aging.

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# Perfumes

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## DEFINITIONS

A fragrance ingredient is any basic substance used for its odorous, odor-enhancing, or blending properties in the composition of perfumes. A perfume is a blend of fragrance ingredients diluted in ethanol and experienced over a certain time frame. It is also named the fragrance formula and may consist of 10 to several hundred ingredients. A fragrance ingredient may be either a chemical or a natural product (1).

Natural fragrance ingredients are produced from natural products such as plant parts—for example, petals, leaves, bark, roots, or fruits from certain plant families (1). Animal secretions such as musk from the male musk deer have been used for their odoriferous properties, but are now replaced by blends of chemicals for the reason of animal welfare protection. The fragrance ingredients are extracted from the plants by processes such as distillation, extraction, or expression.

The resulting natural products are often complex mixtures consisting of numerous ingredients. In some cases, the characteristic odor is due to a particular ingredient, and the isolation of these odor determinants is the original basis of production of chemical ingredients. Fragrance chemicals may be isolated from the natural products or synthesized from basic organic chemicals. The synthesized chemicals may be nature-identical; that is, imitations of naturally occurring substances or entirely new chemicals never identified in nature. Most fragrance ingredients are natural or nature-identical (1). About 2500 fragrance ingredients are in current use for compounding perfumes.

## APPLICATIONS OF PERFUMES

Perfumes are added to many types of consumer products such as cosmetics, detergents, air fresheners, sanitary napkins, and toys. Perfumes are also used in aromatherapy and herbal remedies as well as industrial settings. Perfumes are products in themselves sold as parfum, eau de toilette, and eau de cologne. A perfume is developed for one particular type of product, and the composition may have to be changed to retain the same odor if it is incorporated into a different type of product.

Perfumes may be used to mask unpleasant odors from the basic ingredients, for example, in skin care products. If a perfume is added for this purpose only, it is termed a masking perfume and is usually of a simple composition, consisting of few ingredients. Certain fragrance ingredients such as farnesol and geraniol have antibacterial properties and may be used as preservatives.

Plant extracts are regarded by some as functional ingredients, claimed to act as, for example, anti-irritants. The same ingredients may in other cases be used just to provide a pleasant odor.

Perfumes are part of daily life in modern society as it was in ancient times. The most intense skin exposure comes from cosmetic products, especially stay-on cosmetics, which is also the cause of most contact allergic reactions.

## ADVERSE REACTIONS TO FRAGRANCE INGREDIENTS

A few fragrance ingredients are banned by industry because of their neurotoxicity or carcinogenic properties ([www.ifraorg.org](http://www.ifraorg.org)), but the majority of fragrance ingredients have not been evaluated at all for systemic effects or poorly so. Effects on the respiratory organs from inhalation of perfumes have been described, mediated by sensory or irritant mechanisms (2). Skin reactions as contact urticaria, photoallergy, and phototoxicity are well recognized but infrequent skin side effects. Psoralens in naturally occurring fragrance ingredients were previously the cause of phototoxic reactions giving rise to acute erythema and followed by long-standing hyperpigmentation. The content of the light sensitivity-inducing substances is now regulated and the problem has diminished. In the 1970s, the fragrance ingredient musk ambrette was the cause of an epidemic of photoallergy, particularly in men following the use of after-shave. The usage of musk ambrette was reduced and in 1995 the substance was prohibited from use in cosmetics in Europe (<http://ec.europa.eu/enterprise/cosmetics/cosing>).

Immediate contact reaction to fragrance ingredients is often of the nonimmunological type (3). This means that most individuals will react with local erythema and edema if exposed to the substance in a sufficient concentration. The fragrance ingredient cinnamal is capable of producing nonimmunological contact urticarial reactions, as are Balsam of Peru and cinnamic acid (3). Allergic contact dermatitis is the most frequently reported adverse reaction in relation to fragrance ingredients (4).

## ALLERGIC CONTACT DERMATITIS

Fragrance ingredients are low molecular weight substances, which easily penetrate intact skin. About 100 of the 2500 ingredients used for compounding perfumes have been described as capable of inducing contact allergy in humans (4,5). Fragrance ingredients are the most common cause of allergic contact dermatitis because of cosmetics, being responsible for 25% to 45% of the allergic reactions depending on the population under study, closely followed by the preservatives (5). Allergic contact dermatitis due to fragrance ingredients may involve the face, hands, the axilla, or be generalized, depending on the causative products (4).

Contact allergy to fragrance ingredients is diagnosed in 10% to 15% of eczema patients in Europe and North America (4). Estimates show that in terms of population frequencies, it



can be translated into 1.7% to 4.2% of the general population with either a high or low degree of sensitivity to fragrance ingredients. In Germany, this corresponds to at least 1.4 to 3.4 million people (6).

In Denmark, the frequency of contact allergy to the fragrance ingredients in a sample of women aged 18 to 41 years recruited from the general population rose from 0.7% in 1990 to 3.9% in 1998 and decreased again to 2.3% in 2006 (7). A statistical significant relationship between fragrance allergy, cosmetic dermatitis within the past 12 months, and seeing a doctor for this condition was established in the population (7).

While contact allergy in children to cosmetic ingredients previously was rare, allergy to fragrance ingredients is seen in about 2% of children 12 to 16 years of age with almost equal frequency in boys and girls (8). Furthermore, fragrance ingredients are among the most common causes of allergic contact dermatitis in the pediatric population, when patch tested (9). Investigations in the general population are in the pipeline concerning the newer marker of fragrance allergy, fragrance mix II (see later in the chapter), which may add to the prevalence of fragrance allergy.

## CAUSATIVE INGREDIENTS AND DIAGNOSIS

Since the beginning of the 1980s, a mixture of eight fragrance ingredients, named fragrance mix I, has been used for screening purposes in the routine investigation of allergic contact dermatitis (Table 16.1). The mixture has been valuable in diagnosing fragrance contact allergy, and only few modifications have been made to its original composition over the years. In 1984 the initial patch test concentration of 16% was reduced to 8% because of many irritant reactions, which seemed to be caused mainly by the presence of cinnamal. As many fragrance ingredients are used in composing perfumes, investigations have been conducted to identify significant fragrance ingredients not present in the fragrance mix I (10,11,13,14). From this knowledge base a new test has been designed, fragrance mix II, which contains six fragrance chemicals (15–17) (Table 16.1), compiled to a test concentration of 14% in pet. This has also been included in the European baseline series together with its main ingredient hydroxyisohexyl 3-cyclohexenecarboxaldehyde (Lyrall) (18). In a Belgian investigation, fragrance mix I gave positive reactions in 9.0%; fragrance mix II and hydroxyisohexyl 3-cyclohexenecarboxaldehyde in 2.1% of patch tested eczema patients (19). Contact allergy to hydroxyisohexyl 3-cyclohexenecarboxaldehyde is much less frequent in North America than in Europe (20), where a positive reaction to the substance in 0.4% of consecutively patch tested patients was seen. The reason could be that hydroxyisohexyl 3-cyclohexene carboxaldehyde is not used to the same extent in underarm preparations in North America as in Europe. Deodorants/antiperspirants are product types carrying a high risk of sensitization to fragrance ingredients (21–23).

The ingredients of fragrance mix I and II are present in most consumer products in combinations and not infrequently in concentrations capable of eliciting allergic contact dermatitis (24). This explains the value of the two fragrance mixes as diagnostic tools and links the patch test reactions to exposure and clinical disease. A natural *Myroxylon pereirae* is also part of the European baseline series and is used in some countries in topical medicaments and also in the form of extracts and distillates in fragrances (25). Work has been done to optimize the identification of contact allergy caused by natural extracts (10,11,26)

(Table 16.2). Some natural extracts have a simple composition, consisting of only a few chemicals; an example is clove oil, that contains up to 80% geraniol, a well-known allergen. Other extracts have a very complex composition, and even though they are well recognized as causes of allergic reactions, the allergens in the extracts are not known. An example is *Evernia prunastri* (oakmoss absolute). As *E. prunastri* is a major cause of allergic reactions, investigations have been undertaken to pinpoint the major allergens, chloroatranol and atranol; still other less important allergens exist in the mixture (27). This opens up the possibility of producing extracts of *E. prunastri* with no or low levels of these two strong allergens (28).

Some natural products from the terpene family, such as limonene and linalool, will form allergens when oxidized, but while they in their unoxidized state have only weak or no allergenic properties. The allergens formed are mainly hydroperoxides with strong allergenic potential (30). These oxidized terpenes are major causes of fragrance contact allergy and should be included in the test series for fragrance contact allergy (31). Antioxidants can prevent oxidation for some time, but when the antioxidant is consumed, oxidation starts immediately.

## COSMETIC PRODUCTS AND ALLERGIC CONTACT DERMATITIS TO FRAGRANCE INGREDIENTS

Investigations of fragrance-allergic individuals have shown that initiation of disease typically is caused by a fragranced deodorant, a cologne/perfume, or both (21). High levels of well-known allergens have been shown particularly in fine fragrances. A parfum may contain up to 30% of fragrance ingredients, while 1% is typical for deodorants. This means that the individual ingredients are present in much higher concentrations in parfums than in other products. The same allergens have been found also in significant concentrations in naturally based perfumes, deodorants, and toy perfumes (4).

The risk of sensitization will depend primarily on exposure concentration. The exposures as well as the sensitivity of the individual are decisive factors in whether eczema develops in a sensitized individual. A key determinant in exposure is the concentration of allergen per unit of skin surface. However, the formulation of a product also influences the risk of elicitation of allergy; for example, from experiments it seems that deodorant sprays are more likely to provoke reactions, given the same conditions and contents of allergens, as deodorant sticks. Other factors that affect the risk of elicitation in humans are the number of allergens in a product, frequency of exposure, region of application, the skin condition (4). The intensity of skin contact is important, which means that stay-on products generally give a higher risk of sensitization and elicitation than wash-off products given the same concentrations of allergens. However, frequent use of a product such as a liquid soap may compensate and cause the same risk as a stay-on cosmetic product. Another frequent cause of fragrance contact allergy is topical medicaments in some countries (32).

## THE FRAGRANCE ALLERGIC PATIENT

There is considerable inter-individual variation in the sensitivity of fragrance-allergic individuals. Some cannot tolerate any fragrance products at all; others may be able to use some type of products such as shampoos without problems because of the

**Table 16.1** Fragrance Ingredients Included in the List of Substances to be Labeled as Ingredients If Present in Cosmetics in Europe and Their Inclusion in Diagnostic Test Preparations

Name (INCI)	CAS no.	In FM I	In FM II
Amyl cinnamal	122-40-7		
Benzyl alcohol	100-51-6		
Cinnamyl alcohol	104-54-1	X	
Citral	5392-40-5		X
Eugenol	97-53-0	X	
Hydroxycitronellal	107-75-5	X	
Isoeugenol	97-54-1	X	
Amylcinnamyl alcohol	101-85-9	X	
Benzyl salicylate	118-58-1		
Cinnamal	104-55-2	X	
Coumarin	91-64-5		X
Geraniol	106-24-1	X	
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrall)	31906-04-4		X
Anisyl alcohol	105-13-5		
Benzyl cinnamate	103-41-3		
Farnesol	4602-84-0		X
Butylphenyl methylpropional	80-54-6		
Linalool	78-70-6		
Benzyl benzoate	120-51-4		
Citronellol	106-22-9		X
Hexyl cinnamal	101-86-0		X
D-Limonene	5989-27-5		
Methyl 2-octynoate	111-12-6		
Alpha isomethyl ionone	127-51-5		
Evernia prunastri (oakmoss extract)	90028-68-5	X	
Evernia furfuracea (treemoss extract)	90028-67-4		

**Abbreviations:** INCI, International Nomenclature of Cosmetic Ingredients; FM, mixture of fragrance ingredients used for diagnosing fragrance allergy.

**Note:** The presence of the substance must be indicated in the list of ingredients when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products according to the Cosmetic Directive in EU (<http://ec.europa.eu/enterprise/cosmetics/cosing>). Information regarding the presence of other fragrance ingredients in cosmetics may be made available by fragrance industry on a case-by-case basis (38).

**Table 16.2** Natural Extracts Frequently Reported as Causing Positive Patch Tests in Eczema Patients

Cedarwood oil
Clove bud oil
Dwarf pine needle oil
Eucalyptus oil
Evernia furfuracea (treemoss abs)
Evernia prunastri (oakmoss abs)
Geranium oil bourbon
Jasmine abs
Lavender oil
Lemongrass oil
Lemon oil
Myroxylon pereirae (Balsam of Peru)
Narcissus abs
Neroli oil
Orange oil
Patchouli oil
Peppermint oil
Sandalwood oil
Spearmint oil
Ylang-ylang oil I
Ylang-ylang oil II

**Sources:** Simonsen AB et al., *Contact Dermatitis*; 65:254–65, 2011; Larsen W et al., *Am J Contact Dermatitis*; 9:202–6, 1998; Frosch PJ et al., *Contact Dermatitis*; 47:279–87, 2002; Uter W et al., *Contact Dermatitis*; 63:277–83, 2010.

**Note:** A more extensive list can be found in ref. 4.

short contact time and the dilution factor, and may even tolerate some stay-on products. The products most difficult to tolerate on the skin are perfumed deodorants, perfumes/colognes, and skin care products. In a questionnaire study of the strategies of

fragrance-allergic eczema patients in their choice of cosmetic products, 45.3% answered that they had found some scented products that they could tolerate, 31.6% had not tried to find any scented products, and 22% had tried but could not find any (30). The methods most often used were trying different products and reading the ingredient label. The quality of life in patients, especially younger women with fragrance allergy, has been shown to be severely affected (33).

Indicative of the clinical problems the fragrance-allergic individual may have is their level of sensitivity at patch testing with standard test concentrations. It has been shown that patients giving a +++ or ++ grading at patch testing with the fragrance mix or one of its ingredients are very sensitive and likely to react to normal use of cosmetics containing the allergen they cannot tolerate.

A total 82.1% of a group of eczema patients who had been diagnosed with fragrance contact allergy within the past 2 years reported some degree of eczema presently. Most had hand eczema (55.5%), followed by facial eczema (33.3%), and 42.7% had eczema at multiple locations (30).

Fragrance-allergic patients are advised to avoid the specific fragrance ingredient(s) they cannot tolerate according to the patch test results. However, if they are allergic to multiple fragrance ingredients, fragrance ingredients not obligatorily declared on the label, or if they have no interest in using fragranced products, they are advised to use fragrance-free products only. They should be made aware that some products marketed as fragrance-free may contain fragrance ingredients in spite of this marketing. Various flower or plant extracts or chemicals are often used as preservatives (34). Such products may cause adverse reactions in the perfume-allergic patient. It

is often possible to tell if a product contains fragrance ingredients by the (pleasant) scent.

## INTERVENTIONS AND REGULATIONS

The International Fragrance Association (IFRA) was founded in 1973 by fragrance manufacturers and has since issued recommendations for the safe use of fragrance ingredients. Guidelines for more than 100 ingredients are published ([www.ifraorg.org](http://www.ifraorg.org)). Most recommendations are due to skin effects, especially allergic contact dermatitis. In spite of these guidelines, fragrance ingredients still are one of the most frequent causes of allergic contact dermatitis. The fragrance industry has developed new models for estimation of exposure levels which are not expected to sensitize (35). The efficacy of these models is unknown, and already-sensitized consumers will not be protected.

In Europe, ingredient labeling of cosmetics was introduced in 1997, while it has been in force in the United States for decades. In both cases, fragrance compositions were referred to as "parfum," and disclosure of the individual fragrance ingredients was not given. By the amendment of the Cosmetic Directive in Europe (2003), labeling of certain fragrance ingredients recognized for their sensitizing potential has been introduced (Table 16.1) to improve diagnostics and help the sensitized consumer to avoid relevant allergens. The list has been proposed to be expanded to more than 100 substances in a 2012 (4) revision of the original opinion from 1997. Whether this will be implemented in practice is not known. A number of other fragrance ingredients have been restricted or have been banned from use in cosmetic products mainly because of their sensitizing properties (<http://ec.europa.eu/enterprise/cosmetics/cosing>). The fragrance ingredient hydroxyisohexyl 3-cyclohexene carboxaldehyde as well as the main allergens in *E. prunastri* (oak moss absolute) are under consideration for ban in cosmetic products in Europe due to their sensitizing properties.

A decline in contact allergy to FMI and MP has been noted (7,19,36), which may to some extent be an effect of these actions, change in fashion, and other initiatives; however, as new causative ingredients have been identified (FMII/HICC), the collective sum of fragrance allergy seems unchanged or even increasing, as recently shown in Germany (37).

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# Alternative and natural treatments in dermatology

Daniel Oxman and Cheryl Levin

Supplementation to traditional drugs has gained popularity in recent years, particularly within the field of dermatology where diseases are often chronic and treatments may at times be frustrating (1,2). The intent of this review is to survey some of the more recently reported alternative and complementary dermatologic treatments, with an emphasis on those that follow evidence-based dermatology guidelines (3,4). Specifically, this review focuses on those difficult to treat skin conditions in which significant developments have been made, including skin pigmentation disorders, onychomycosis, psoriasis, and atopic dermatitis.

## HYPERPIGMENTATION

Melanin is produced by melanocytes in the basal layer of the skin and is primarily responsible for skin pigmentation. Humans all have approximately the same number of melanocytes, implying that variation in skin color is attributable to a variety of other factors, such as the number and size of melanosomes within each melanocyte, the ratio of different types of melanin produced,\* degree of melanocyte activity, and environmental factors. From a biochemical standpoint, tyrosinase catalyzes three reactions in melanin formation: hydroxylation of tyrosine to DOPA, oxidation of DOPA to DOPAquinone, and oxidation of DH1 to indolequinone. Consequently, this enzyme is a popular target for inhibition in treating subjects with hyperpigmentation (5).

Melasma is quite common, yet the precise pathogenesis is not fully understood. It is thought that sun exposure is the most common trigger. Mechanistically, the thought is that sunlight stimulates nitric oxide production, which increases melanocyte tyrosinase activity, thereby increasing melanin production, resulting in patches of hyperpigmentation in sun-exposed skin. The second most common trigger is believed to be estrogen, with a predilection often observed in pregnancy, oral contraceptive use, or hormone replacement therapy. Pregnancy-associated melasma often clears within several months postpartum, whereas the other forms may take years (6). Topical hydroquinone is the gold standard treatment. A novel formulation, described by Kligman and Willis in 1975, utilizes hydroquinone in combination with a topical corticosteroid and topical retinoid to provide additional depigmentation (7). Many alternative therapies have been investigated, including kojic acid, azelaic acid, and glycolic acid (8).

Ascorbic acid is a known tyrosinase inhibitor. It also is known to prevent the absorption of UV light. Finally, it acts as an antioxidant, preventing free radical generation and thereby preventing increased melanin production (9). Espinal-Perez et al. conducted a double-blind randomized trial that assessed the efficacy of 5% ascorbic acid versus 4% hydroquinone in

treating 16 women with melasma. Participants included in the study had bilateral and symmetric facial melasma, and were instructed to apply hydroquinone to one side of the face and ascorbic acid to the other side daily for 16 weeks. Endpoints of the study were subjective assessment by the patient<sup>†</sup> and objective colorimetric assessment. Of the participants, 62.5% rated their response to ascorbic acid as “very good” or “good,” whereas, 93.75% rated their responses to hydroquinone as “very good” or “good.” Objective colorimetry found no difference in success rates between the two compounds. However, this may be due to several factors including the small sample size or the short 16-week endpoint. Notably, positive results from hydroquinone were visible within the first month of treatment, while positive results from ascorbic acid were not noticeable until month three. Greater than two-thirds of patients experienced skin irritation from the hydroquinone, and only one patient experienced irritation from the ascorbic acid. The authors propose that a higher percentage ascorbic acid solution will likely produce more dramatic results. Ascorbic acid is known to be an unstable molecule. It is possible that as ascorbic acid is better stabilized in cream or solution formulation, it will have a greater efficacy. Hakozaki et al. conducted several in vitro and in vivo studies assessing the impact of niacinamide<sup>‡</sup> on melanin and hyperpigmentation. Regarding the in vivo studies, investigators determined that niacinamide does not impact the activity of mushroom tyrosinase,<sup>§</sup> nor does it impact melanocyte production of melanin. Interestingly, the final in vivo study demonstrated that niacinamide inhibits transfer of melanosome from melanocytes to keratinocytes, when melanocytes, keratinocytes, and niacinamide are cultured together. Two keratinocyte cell lines were used and melanin transfer was inhibited by 35% in one cell line and 68% in the other. Two in vivo clinical studies were conducted assessing the efficacy of niacinamide at reducing hyperpigmentation. Study 1 involved 18 Japanese women with areas of facial hyperpigmentation—slight–moderate solar lentigines, melasma, or freckles—who applied 5% niacinamide moisturizer to one side of the face and the vehicle to the other side, for 8 weeks. Study 2 included 120 Japanese women with “moderate to deep facial tan,” randomized to one of three groups. Group 1 members applied the vehicle to one side of the face and sunscreen to the other. Group 2 members applied sunscreen to one side of the face and 2% niacinamide + sunscreen to the other side. Group 3 members applied the vehicle to one side of the face and 2% niacinamide + sunscreen to the other. Outcome measures included objective imaging with computer analysis of percent change in pigmentation,

<sup>†</sup> Subjectively rated as “very good,” “good,” “moderate,” and “mild” in terms of improvement.

<sup>‡</sup> Activated vitamin B3.

<sup>§</sup> Enzyme responsible for the rate limiting step in melanin formation.

\* Eumelanin and pheomelanin.

visual assessment by blinded judges, and self-assessment. In study 1, all three outcome measures demonstrated a greater reduction in pigmentation with niacinamide as compared to the vehicle, with the greatest difference observed with objective image analysis. In study 2, the lightening ability of niacinamide + sunscreen compared to sunscreen alone on “tan” skin was less conclusive. Self-assessments did not demonstrate a significant difference between the two arms of the study. However, imaging with objective computer analysis *did* demonstrate a statistically significant difference between sunscreen + niacinamide and sunscreen alone in lightening capacity at 4 weeks; however, at 8 weeks, the difference between these two categories were no longer significant. Both the niacinamide + sunscreen and sunscreen alone showed significant lightening capacity compared to the vehicle alone at 4 and 8 weeks (10).

Hakozaki et al. performed a subsequent *in vivo* clinical trial that explored the efficacy of ascorbyl glucoside (AG)\* plus niacinamide, in conjunction with sonophoresis, in treating hyperpigmentation. The subjects were 60 Japanese women with bilateral facial hyperpigmentation—solar lentigines, melasma, or freckles. A 5 MHz ultrasound device set at an intensity of 0.7 W/cm<sup>2</sup> and the gel containing 2 AG and 3.5% niacinamide were used. The subjects were divided into two groups of 30. Group 1 applied 2.6 mL of the gel to one side of their face and used the ultrasound for 10 minutes nightly, and applied nothing to the other side of their face. Group 2 used the gel and ultrasound on one side as described above, and gel only on the other side. The duration of the study was 4 weeks. Results of the study were measured in a variety of manners, though most telling was the high resolution digital imaging with computer quantification of pigmentation reduction. In group 1, the side of the face treated with ultrasound and gel showed a 9.4% greater reduction in pigmentation compared to the no-treatment side. In group 2, the ultrasound plus gel demonstrated a 4.8% greater reduction in pigmentation when compared to gel alone. This implies that not only are niacinamide and AG effective at reducing hyperpigmentation, but that ultrasound is likely responsible for approximately half of the efficacy.<sup>†</sup> No adverse effects were reported by any subjects during the study (11). Interestingly, there is a cream, Fair & Lovely, that was launched in Kenya, and is now available worldwide, that contains niacinamide and ascorbyl phosphate, among other ingredients (8).

Assorted plant extracts are potent inhibitors of melanin formation and many have been investigated as potential depigmenting agents. The bioflavonoids as a class, and specifically the flavanones, have been identified as potent tyrosinase inhibitors (12). Flavanones, including hesperidin, eriodictyol, and naringenin, have a chemical structure similar to hydroquinone. In a study by Zhang et al., hesperidin, found in the peel and membranes of citrus fruits, was applied to melanoma B16 cells and human primary melanocytes. A dose-dependent inhibition of tyrosinase activity versus control was observed (13,14). Flavonoids from licorice roots, such as glabrene, glabridin, and isoliquiritigenin, have also been found to inhibit tyrosinase (15). In one study, glabridin applied to cultured B16 murine melanoma cells at concentrations from 0.1 to 1.0 µg/mL inhibited tyrosinase activity without affecting DNA synthesis (16,14).

The licorice extract liquiritin, another flavonoid, was also found to be effective in decreasing skin pigmentation. In a clinical trial, 2% liquiritin cream at a dose of 1 g day<sup>-1</sup> was applied to 20 women with melasma for 4 weeks. Eighty percent of the treated cases had a reported excellent response (17). Of note, liquiritin does not inhibit tyrosinase activity. Depigmentation is thought to be secondary to melanin dispersibility and epidermal stain-removing property. Pycnogenol, a flavonoid-containing bark extract, has also decreased pigmentation in melasma patients. In a study of 30 women with melasma, a 25-mg pycnogenol tablet taken three times daily led to a statistically significant decrease in melasma pigmentation, as measured by the average pigment intensity and average melasma area (18).

A recent study by Yamakoshi et al. studied the effects of grape seed extract in treating melasma-associated hyperpigmentation. Twelve Japanese women with melasma took proanthocyanidin-rich grape seed extract three times daily for between 6 and 12 months while not using any other treatments for their melasma. There was a significant decrease in their melasma as measured by reflectance spectrophotometry and clinical parameters as compared to baseline (19).

Arbutin, a hydroquinone derivative found in the bearberry plant, has been found to inhibit tyrosinase and DHICA (5,6-dihydroxyindole-2-carboxylic acid) polymerase activities (20), possibly through competitive inhibition (14). A clinical trial of topical deoxyarbutin (dA,4-[tetrahydrofuran-2-yl-oxy]-phenol) applied to solar lentigines for 12 weeks led to significant lightening in light-skinned individuals and minimal lightening in dark-skinned individuals (21). Alpha-arbutin (4-hydroxyphenyl alpha-glucopyranoside) exhibits greater chemical stability and stronger inhibition of tyrosinase when compared to arbutin (22).

Multiple *in vitro* studies were conducted to investigate the inhibitory capacity of aloesin, an aloe extract, on tyrosinase. First, experimenters looked at aloesin's inhibitory capacity on fungal, murine, and human tyrosinase, and compared to it to two well-known tyrosinase inhibitors, arbutin and kojic acid. Aloesin was consistently shown to be a less potent inhibitor than kojic acid and a more potent inhibitor than arbutin. Additionally, aloesin was shown to inhibit melanin production in a dose-dependent fashion in murine melanoma cells, stimulated with alpha-MSH. Specifically, aloesin was shown to inhibit both the tyrosine hydroxylase and DOPA oxidase reactions. However, aloesin dissolved in ethanol when applied to cadaveric skin was found to have poor penetration. Subsequently, the authors conducted a preliminary trial<sup>‡</sup> that demonstrated a localized decrease in melanin production when a hydrophilic patch impregnated with 1% aloesin was applied to human volunteers' skin (5).

Khan et al. conducted *in vivo* and *in vitro* studies exploring the efficacy of extracts from two plants known to have medicinal properties. *Cassia fistula*, also known as the golden shower tree, is a yellow-flowering tree, previously described in Indian literature in the treatment of a variety of skin conditions, including boils, leprosy, ringworm, and burning skin sensation. *Hippophae rhamnoides*, common sea-buckthorn, is a berry-producing shrub found throughout Europe whose medicinal properties were discovered over 1000 years ago. Extracts from both of these plants—found to be rich in the antioxidant catechin, part of the flavonoid family, were tested.<sup>§</sup> This was a single-blinded,

\* Vitamin C derivative.

<sup>†</sup> Group 1: 9.4% difference between gel+US and no treatment. Group 2: 4.8% difference between gel+US and gel alone. Efficacy of gel is 9.4%–4.8%=4.6%.

<sup>‡</sup> Data not available in this study.

<sup>§</sup> Formulation 1 (F1) = *H. rhamnoides* extract, F2 = *C. fistula* extract, F3 = placebo.

12 week study that involved 50 Asian subjects with melasma, divided into two groups of 25 subjects each. One group applied *H. rhamnoides* to one cheek (referred to as F1) and *C. fistula* (F2) to the opposite cheek, while the other group applied F2 to one cheek and placebo to the other; both groups applied the formulation twice daily. Skin pigmentation was measured objectively by the Mexameter®, a spectrophotometer sensitive to the presence of melanin. Results at 12 weeks were significant for a mean melanin reduction of 16.35% in those using F1, the *H. rhamnoides* formulation, and a reduction of 13.0% in those using F2, the *C. fistula* formulation. The side of the face receiving F3, the placebo, actually showed mild increases in melanin. Side effects of F1 and F2 were limited to mild to moderate itching and skin irritation experienced by approximately 10% of patients (23).

Just as the golden shower tree and common sea-buckthorn are known to be rich in tyrosinase-inhibitory catechins, *Acacia nilotica*, a tree long reputed for its medicinal properties, is known to possess similar phenolic catechin compounds. Ali et al. conducted a 12-week, blinded, controlled study assessing the efficacy of *Acacia* bark extract in reducing skin pigmentation and erythema content in 11 subjects. Subjects applied the vehicle to one side of the face and the experimental cream to the other twice daily. Skin melanin and erythema content were measured using the Mexameter spectrophotometer every other week. Throughout the study, the *Acacia* extract cream produced continuous reduction in erythema and melanin content, while the control cream did not. At the conclusion of the study, there was a statistically significant reduction in skin melanin and erythema content when the active cream was used and insignificant reductions when the control cream was used (24).

Keratinocyte phagocytosis of melanosomes, regulated by protease-activated receptor 2 (PAR-2), has been confirmed to be an important biochemical pathway in pigmentation regulation. Serine protease inhibitors found in soybeans—soybean trypsin inhibitor (STI) and Bowman-Birk protease inhibitor (BBI)—modulate this pathway, potentially resulting in depigmentation. Wallo et al. conducted a 12-week, parallel, randomized, double-blind, and vehicle-controlled study involving 63 subjects with evidence of photoaging, and compared the effects of a facial moisturizer with sunscreen, STI, and BBI to one with only sunscreen when applied twice daily. The study endpoint was improvement in skin tone\* and texture† as evaluated by the patient, dermatologist, and digital photography with colorimetry. While both groups showed clinical improvement, the experimental group showed a statistically significant greater improvement than the control group in skin tone and texture as per dermatologist evaluation. However, objective colorimetry found no significant difference (25). See Table 17.1 for alternative treatments.

## ONYCHOMYCOSIS

Onychomycosis is a condition that results in disfigurement of the nails, and can often be embarrassing (26). In addition to social and emotional ramifications, there are medical concerns, including secondary bacterial infection, pain, and spread of infection to others (27). Onychomycosis is responsible for one-third of all fungal skin infections and approximately one-half of all nail disease. *Trichophyton rubrum* is the most common causative organism, primarily infecting the nail bed and underside

of the nail plate (26). While oral systemic treatments have been the traditional choice and are among the most effective in treating this condition, they are associated with a higher rate of adverse effects and drug–drug interactions than other treatment modalities (28). This section addresses the alternative therapies that can be used in managing onychomycosis.

## Ciclopirox

Ciclopirox is a “substituted pyridone” topical agent with broad antifungal activity. Its exact mechanism of action is unknown; however, it is thought to exert its antifungal effects by interfering with fungal transmembrane transport as well as DNA and RNA synthesis (29).

Two U.S., multicenter, randomized, double-blind, vehicle controlled studies, conducted with similar protocols, were performed to assess the efficacy of Ciclopirox 8% lacquer in the treatment of toenail onychomycosis. Four hundred sixty subjects with 20%–65% involvement of the target nail were included in the study and randomized to apply the Ciclopirox or vehicle to all nails daily for 48 weeks. The mycological cure, defined as negative culture and KOH stain, was 34% for the Ciclopirox group and 10% for the vehicle group. Treatment cure rate, defined as 100% clearance of clinical signs of fungal infection as well as negative culture and KOH, was 7% for the Ciclopirox group compared to less than 1% in the vehicle group (30).

Friedlander et al. conducted a prospective, randomized, double-blinded, vehicle-controlled study assessing the efficacy of Ciclopirox 8% nail lacquer in treating toenail onychomycosis without matrix involvement, in a pediatric population. Of the 40 subjects enrolled in the study, 30 were assigned to the experimental arm and 10 were assigned to the vehicle arm of the study. Subjects applied their assigned lacquer to all toenails daily for 32 weeks, and 7/9 of the subjects in the vehicle arm were crossed over to the experimental arm at week 12, for lack of noticeable improvement. Results at 32 weeks were as follows: 34% of the Ciclopirox group achieved complete cure,† 71.4% of the Ciclopirox group achieved effective treatment (defined as ≥50% clearance and negative culture),§ none of the crossover subjects achieved complete cure, and the two subjects remaining in the vehicle group achieved complete cure. Of those who achieved complete cure at 32 weeks, 92% remained disease free at 1 year follow-up. Authors of the study suggest that the thinner and potentially faster growth rate of nails in the pediatric population may be part of the explanation for the higher efficacy in this treatment population than in adults. Additionally, given the two children achieving complete cure in the vehicle arm, watchful waiting may be a feasible option in the pediatric population. Interestingly, *T. rubrum* was found to be more resistant to treatment than other infectious fungi (31).

## Tea Tree Oil

Several studies have been performed to assess the efficacy of tea tree oil in the treatment of onychomycosis. Tea tree oil is derived from the Australian tree *Melaleuca alternifolia*, and the active ingredient is Terpinen-4-ol (32).

A double-blind, multicenter, randomized controlled trial involving 117 subjects with distal subungual onychomycosis compared the efficacy of 1% clotrimazole solution to 100% tea

\* Improvement in skin tone defined as reduction in mottled hyperpigmentation, lentigines, and blotchiness with increase in brightness.

† Improvement in skin texture defined as reduction in surface roughness or improvement in fine lines and wrinkling.

‡ Complete cure is defined as IGA (Investigator's Global Assessment) score of 0 and negative culture. IGA ranges are: 0 (complete clearance); 1 (75%–99% clearance); 2 (50%–74% clearance); 3 (<50% clearance); 4 (no change); and 5 (worsening).

§ Effective treatment is defined as IGA score ≤2 and negative culture.



**Table 17.1** Alternative Treatments for Hyperpigmentation

Alternative Medication	Study Type	Experimental Result	Source
Acacia bark extract	In vivo, blinded, controlled	Decrease melanin and erythema	(Ali, Akhtar et al. 2012)
Aloesin	In vitro	Decrease tyrosinase activity	(Jones et al. 2002)
Arbutin	In vitro	Decrease tyrosinase and DHICA	(Chakraborty, Funasaka et al. 1998)
Ascorbic acid	In vivo - double-blind, randomized	Decrease melasma pigmentation	(Espinal-Perez et al. 2004)
Ascorbyl glucoside with sonophoresis	In vivo	Decreases hyperpigmentation	(Hakozaki et al. 2006)
Deoxyarbutin	In vivo	Decreases solar lentigines	(Boissy, Visscher et al. 2005)
Glabridin	In vitro	Inhibit tyrosinase	(Fu, Li et al. 2005)
<i>C. fistula</i> and <i>H. rhamnoides</i>	In vitro and in vivo (single blinded)	Tyrosinase inhibition (in vitro) Reduction in melanin (in vivo)	(Khan et al. 2013)
Grape seed extract	In vivo—open design study	Decrease melasma pigmentation	(Yamakoshi, Sano et al. 2004)
Hesperidin	In vitro	Inhibit tyrosinase	(Zhang, Zhu et al. 2008)
Liquirtin	In vivo—clinical trial	Decrease melasma pigmentation	(Amer and Metwalli 2000)
Niacinamide	In vitro and in vivo	Inhibition of melanosome transfer from melanocytes to keratinocytes (in vitro) Pigmentation reduction (in vivo)	(Hakozaki et al. 2002)
Pycogenol	In vivo	Decrease melasma pigmentation	(Ni, Mu et al. 2002)
Soybean—soybean trypsin inhibitor and Bowman-Birk protease inhibitor	In vivo—parallel, randomized, double-blind, vehicle controlled	Improvement in skin tone and texture	(Wallo et al. 2007)

tree oil in treating this condition. Patients were instructed to apply the assigned solution to the affected nails twice daily for 6 months, with follow up at 1-, 3-, and 6-month intervals for clipping and debridement. Outcome measures were fungal culture, clinical appearance, and subjects' perception of appearance and symptoms at 9 months after initiation. Of the 108 participants to complete the trial, one-half to two-thirds demonstrated clinical improvement or reported subjective improvement of their fungal nail infection, without statistically significant differences between the two groups. Analysis of fungal culture results was limited due to 35% dropout at 6 months, when the culture was to be performed (32).

Another randomized, double-blinded, placebo-controlled study involving 60 patients compared the efficacy of 5% tea tree oil with 2% butenafine, a phenol-substituted benzylamine derivative, which has been shown in prior studies to have fungicidal effects vs. 5% tea tree oil alone in the treatment of toenail fungal infections. Mycological cure was defined as negative fungal culture and potassium hydroxide stain, clinical cure was defined as 100% remission or > 90% improvement, and overall cure was defined as both mycological and clinical cure. Patients applied their assigned cream three times daily to the affected toenail for 8 weeks, with debridement of the nails occurring as deemed necessary by the examiners. They were evaluated on a weekly basis for the first 16 weeks, then monthly until week 36. Eighty percent (32/40) of the experimental group achieved overall cure, while none of the control group (0/20) achieved overall cure. Mild inflammation was the only reported side effect.

The investigators suggested that 8 weeks may not have been a long enough duration for tea tree oil to achieve its full effect, explaining its lack of efficacy in this study (33).

### Vicks® VapoRub

Vicks VapoRub has been reported as a folk remedy for onychomycosis. Ramsewak et al. conducted an in vitro study investigating the inhibitory activity of both the active and inactive ingredients found in VapoRub on fungal pathogens which cause onychomycosis. The study demonstrated that camphor, menthol, thymol, and *Eucalyptus citriodora* oil (composed of monoterpenes) are all effective inhibitors when used singly as well as when used in combination (34). Disruption of the fungal cell membranes and inhibition of ergosterol synthesis by thymol and monoterpenes has been suggested as the mechanism of action in laboratory studies (35,36).

A clinical case series examined the effect of daily topical application of VapoRub in 18 human subjects with clinically confirmed and culture-positive fungal nail infections. Outcome measures at the study endpoint of 48 weeks were defined as "mycological cure," based on KOH and nail sample cultures, and "clinical cure," which could be either "complete," "partial," or "no change," dependent on the appearance of the nail. Five subjects experienced mycological cure, ten demonstrated partial clinical cure, and three showed no significant change. Interestingly, the six subjects who were culture-positive for *T. rubrum*, the most common causative pathogen of onychomycosis, had only "partial cure" or



“no change,” whereas the five individuals who demonstrated “complete cure” were culture-positive for either *Candida parapsilosis* or *Trichophyton mentagrophytes* (37).

### Tavaborole

Tavaborole is a boron-containing compound developed by Anacor Pharmaceuticals (Palo Alto, CA) that has been shown to inhibit a fungal enzyme needed for fungal protein synthesis. Two phase III trials—approximately 600 subjects per trial—assessing the efficacy of topical application of tavaborole in treating onychomycosis were recently completed and demonstrated promising results (38). Primary publication data could not be found with a Pubmed search for “Tavaborole,” however, a recent review article quoted complete cure\* rate of 6.5% in the treatment arm versus 0.5% in the vehicle arm (39).

### Efinaconazole

Efinaconazole is the first triazole antifungal developed for the treatment of onychomycosis. Two identical multicenter, randomized, double-blind, vehicle-controlled studies involving a total of 1655 subjects were conducted to assess the efficacy of topical efinaconazole 10% solution in treating onychomycosis. Subjects were randomized to efinaconazole or vehicle and applied the assigned solution daily for 48 weeks and were evaluated at 52 weeks, with endpoints defined as complete cure† or treatment success‡. In studies 1 and 2, 18% and 15% of subjects in the treatment arm achieved complete cure, respectively. Complete cure rate was only 3% and 6% in the vehicle arm in studies 1 and 2, respectively. For those treated with efinaconazole, treatment success was achieved in 45% and 40% in studies 1 and 2, respectively, whereas only 17% (study 1) and 15% (study 2) reached treatment success in the vehicle arm. Given the high treatment success rate achieved with efinaconazole at 48 weeks and that toenails may take up to 78 weeks for completely new growth, the authors suggest that longer treatment duration may increase complete cure rates. Additionally, the authors commented that given the minimal side effect profile of efinaconazole, it could be used as a maintenance medication or adjunct to prevent recurrence of onychomycosis in those previously treated with systemic therapy (40).

### Photodynamic Therapy

Photodynamic therapy (PDT) refers to the use of light-activated compounds that react with a certain wavelength of light to produce oxygen free radicals which interact with cells, resulting in cell damage and/or death (41). While the U.S. Food and Drug Administration (FDA) has not approved PDT for the treatment of onychomycosis (42), there is evidence to suggest that PDT likely has a role and can be used in treating this condition.

An in vitro study by Smijs et al. demonstrated the fungicidal activity of certain photosensitizing porphyrin molecules when exposed to wavelengths of light in the red spectrum. Notably, PDT was demonstrated to not only eradicate the fungal hyphae, but to also inactivate the *T. rubrum* spores, which have been implicated in the recurrence of tinea unguium infections after treatment with traditional oral agents. Use of red light has the additional benefit of increased tissue penetration when compared to white light, making it an attractive agent for treating fungal nail, which requires adequate tissue penetration (41).

\* See footnote 14.

† Complete cure defined as 0% clinical involvement of the nail, as well as negative culture and KOH stain.

‡ Treatment success defined as <10% clinical involvement of the nail.

A subsequent in vitro study demonstrated that *T. rubrum*, when exposed to 5-aminolevulinic acid (5-ALA), generates the photosensitizing molecule protoporphyrin IX, which can be irradiated with red wavelengths of light—636nm and 708nm—resulting in a nearly 50% inhibition of fungal growth compared to control (43).

Utilizing the technology discussed above, there have been several in vivo case reports of successfully treated onychomycosis using PDT. One case was that of a 78-year-old woman with onychomycosis of the bilateral great toenails. Treatment included removal of the affected nails after softening with 40% urea ointment, application of 5-ALA (Metvix 160 mg/g) cream, and irradiation with 37 J/cm<sup>2</sup> light-emitting diodes in the red spectrum—630nm wavelength. This regimen was repeated every 15 days for 45 days, totaling three treatment sessions. While KOH stain and cultures were positive immediately after the last session, follow-up at months three through month 24 demonstrated complete mycologic and clinical resolution of the infection (44).

Additionally, two case reports of successfully treated onychomycosis involving urea, 5-ALA, and irradiation with red light have been described by Watanabe et al. However, in these cases, 20% urea was used to soften the nail without subsequent removal and an excimer-dye laser with a fluence of 100 J/cm<sup>2</sup> at 630nm wavelength was used. While the laser caused “some pain” for the patients during treatments, it was temporary in nature, and the laser was chosen as the light source due to its greater tissue-penetrating capabilities than other light sources. One patient required seven treatments and the other required six treatments with no recurrence of infection recorded at 6-month and 3-month follow-ups, respectively (45).

Sotiriou et al. conducted a single-center open trial involving 30 Caucasian subjects with diagnosed toenail onychomycosis. Therapeutic regimen included treatment with 20% topical urea cream with subsequent removal of the affected nail, 3-hour treatment with 20% 5-ALA, and irradiation with a noncoherent, 40 J/cm<sup>2</sup> dose of red light ranging in wavelength from 570–670nm. Three treatments were performed at 2-week intervals for 6 weeks. During irradiation, approximately one-third of patients endorsed pain or burning requiring a brief interruption, and almost all patients experienced other minor side effects such as erythema, edema, and blistering around the site. Cure at 12 and 18 month follow-up was defined as “100% absence of clinical signs” or “subungual hyperkeratosis” affecting less than 10% of the nail plate with “negative mycological laboratory results.” This study demonstrated a cure rate of 43.3% (13/30) at 12 months and 36.6% (11/30) at 18 months, suggesting that PDT is somewhat efficacious, though room for improvement exists (46).

### Laser Therapy

In addition to PDT, many studies have begun exploring the therapeutic effect of lasers on onychomycosis. The following in vitro study by Vural et al. investigated the effects of a variety of laser systems, wavelengths, and fluences on *T. rubrum* growth inhibition. *T. rubrum* colonies were grown on culture plates and irradiated by a variety of lasers, with fungal colony size and growth rate measured on days 1, 3, and 6 post-irradiation. While a majority of the lasers were ineffective at inhibiting growth, the Q-switched Nd:YAG laser used at independent wavelengths of 532nm and 1064nm, with varying fluences, both retarded fungal growth. The authors suggest that the growth retardation observed with 532nm irradiation is

likely due to xanthomelanin, a *T. rubrum* pigment that absorbs in that spectrum, leading to “photothermolysis,” as opposed to “nonspecific thermal damage.” Similarly, irradiation with the 1064nm wavelength is postulated to cause its “photothermolytic” damage due to absorption by melanin, a pigment found in the cell walls of *T. rubrum*. The Q-switched Nd:YAG laser’s “short pulse width” adds to its inhibitory effects on *T. rubrum* growth by inducing thermal and mechanical damage via rapid heating and cooling, as well as “acoustic shock wave” production, respectively. Fluences chosen in this study were similar in energy to fluences used in other dermatologic procedures, such as port-wine stain and unwanted hair or tattoo removal (47).

A subsequent clinical study demonstrated strong efficacy of the VSP long pulse Nd:YAG 1064nm laser therapy in treating four different types of onychomycosis—total dystrophic form, distal, proximal, and endonyx—in 72 patients with 194 affected nails caused by *T. rubrum*, *T. mentagrophytes*, *Candida*, or *Aspergillus niger*. After microscopic and culture confirmation of positive fungal infection, subjects underwent four sessions—three irradiations during each session—at 1-week intervals, in which affected nails were irradiated at fluences of 35–40 J/cm<sup>2</sup>, pulse duration 35ms, and rate 1Hz. Variation in fluence was due to variations in nail thickness, with thicker nails requiring higher fluence. Three participants had particularly thick nails, which were pretreated with a urea preparation to improve laser penetration. Nail plate temperatures were raised to 45±5°C, with cold air cooling used during treatments, with the majority of patients rating their pain as “mild,” none rating their pain as “severe” or “intolerable.” At 3-month follow-up, 69/72 subjects were cleared of fungal infection and the three affected subjects repeated the therapeutic protocol. All 72 subjects were free of infection at 6- and 12-month follow-ups. The mechanism of action underlying this therapy is in line with the previously discussed theory that 1064nm wavelengths are absorbed by fungal cell wall melanin, leading to “local hyperthermia,” and consequent fungal destruction. This study extrapolates on the “local hyperthermia” and destruction, suggesting that thermal injury likely results in a combination of fungal DNA damage, production of reactive oxygen species, and protein denaturation, all triggering fungal cell apoptosis, leading to mycological cure (48).

Kalokasidas et al. conducted a clinical study involving 131 subjects, investigating the inhibitory effects when both 1032nm and 532nm wavelengths are used together. Prior to irradiation, the severity of onychomycosis was graded and subjects’ affected nails were mechanically debrided with a nail file. Using the Q-switched Nd:YAG 1064nm/532nm laser, affected nails were first irradiated with a 1064nm wavelength laser, received a 2-minute break, and then irradiated with a 532nm wavelength laser. Participants attended two treatment sessions—on days 0 and 30—and the above regimen was conducted during each session. The results of this study reflect that of the prior study, with 125/131 subjects demonstrating mycological cure (negative microscopy and culture) at 3-month follow-up. Notably, of the types of onychomycosis, “distal subungual” demonstrated the best response to treatment and of the infecting organisms, *T. rubrum* showed the most prompt response. Given the promising results, if laser therapy does not supplant systemic therapy, it could potentially be used in conjunction with it, reducing treatment duration. This study’s clinical outcomes have yet to be fully assessed at 12-month follow-up, and as the authors point out, negative cultures do not always equate with mycological cure, as there is high false negative rate (27).

While many studies have shown laser therapy to be efficacious in the treatment of onychomycosis, a recent in vitro and in vivo study by Carney et al. brings the efficacy of Nd:YAG 1064nm laser therapy into question. A few common nail dermatophytes, including *T. rubrum*, were chosen for the in vitro portions of the study. In phase one of the in vitro portion, suspensions of the fungi were heated to varying temperatures—45°C, 50°C, and 55°C—for varying durations, then plated on agar and monitored for growth. *T. rubrum* growth was retarded when exposed to 50°C for 5 minutes and a fungicidal effect was observed when exposed for 15 minutes. In phase two, *T. rubrum* suspensions were plated on agar, then irradiated with the laser at varying combinations of fluence, pulse width, and Hz, and temperature was recorded. In phase 3, *T. rubrum* was grown on an agar plate until red pigmentation was visible; 3-mm punch biopsies of mycelium were taken and transplanted to another plate, which was subsequently irradiated with the same variations as used in phase 2. In both phases 2 and 3, no growth inhibition of *T. rubrum* was noted after irradiation and temperatures only reached 40°C in phase 2, suggesting that prior clinical trials proposing fungicidal effects due to thermal destruction is unlikely. Phase 4 was the 24-week in vivo portion of the study in which five laser treatments were performed on eight individuals with 14 affected nails over the course of the initial 7 weeks. Nail assessments, microscopic and culture evaluation were performed intermittently throughout the study. Notably, eight of the 14 affected nails showed only mild clinical improvement, though this did not correlate with microscopic or culture findings, and notably, two subjects changed Onychomycosis Severity Index (OSI)\* categories, both of which increased in disease severity. While recognizing differences in study protocols, particularly the wide variation in laser settings, the results of this study have compelled the authors to conclude that laser therapy is thus far not a reliable treatment modality for onychomycosis and thermal damage is unlikely the mechanism underlying clinical improvement when laser therapy is performed (49).

See also Table 17.2.

## ATOPIC DERMATITIS

Atopic dermatitis is a common disease affecting up to 20% of the pediatric population and about 1%–3% of adults in the industrialized world. The disease is characterized by pruritus, rash on the extensor surfaces and face of young children, lichenification of the flexural surfaces of older children, recurrent skin infections, and personal or family history of atopic disease. The pathogenesis of the disease is complex and multifactorial, involving interplay of genetic susceptibility resulting in decreased skin barrier and immune system dysregulation and hyper-reactivity (50). Many traditional therapies exist; however, side effects may limit their use (51). This has prompted a search for additional, alternative treatment modalities for this condition.

Pruritus is a common symptom of atopic dermatitis and is thought to be due to increased nitric oxide and cytokines. Vitamin B12 is a nitric oxide scavenger, thereby reducing pruritic symptoms through reduction of nitric oxide levels. A double-blinded, randomized, placebo-controlled,

\* OSI: Area of involvement is rated from 1–5. Proximity of disease to the nail matrix is rated from 1–5. The two values are multiplied. Ten points are added if a longitudinal patch or streak is present or if subungual hyperkeratosis greater than 2 mm is present. Categories: Mild = 1–5, Moderate = 6–15, Severe = 16–35

**Table 17.2** Alternative Treatments for Onychomycosis

Alternative Medication	Study Type	Experimental Result	Source
Ciclopirox	Multicenter, randomized, double-blind, vehicle-controlled in vivo	34% mycological cure rate; 7% clinical cure rate	(Gupta and Joseph 2000)
Ciclopirox	Randomized, double-blinded, vehicle-controlled, crossover, in pediatric population in vivo	34% achieved complete cure; 72% with greater than 50% clearance	(Friedlander et al., 2013)
Efinaconazole	Multicenter, randomized, double-blind, vehicle-controlled in vivo	15%–18% complete cure rate and 40%–45% with <10% clinical involvement	(Elewski et al., 2013c)
Laser therapy: Q-switched Nd: YAG	In vitro	Retardation of fungal growth	(Vural et al., 2008)
Laser therapy: VSP long pulse Nd: YAG	In vivo	100% cure rate	(Kozarev and Vizintin, 2010)
Laser therapy: Q-switched Nd: YAG	In vivo	9% with excellent clinical improvement and 95% mycological cure rate	(Kalokasidis et al., 2013)
Laser therapy: long pulsed Nd:YAG	In vivo and in vitro	In vitro demonstrated no fungal growth inhibition In vivo showed 57% with clinical improvement and 43% without clinical improvement or worsening	(Carney et al., 2013)
Photodynamic therapy	In vitro	Eradication of fungus	(Smijs et al., 2004)
Photodynamic therapy	In vitro	50% Inhibition of fungal growth	(Kamp et al., 2005)
Photodynamic therapy	Single case report in vivo	Clinical and mycological cure	(Piraccini et al., 2008)
Photodynamic therapy	2 case reports in vivo	Clinical improvement and mycological cure	(Watanabe et al., 2008)
Photodynamic therapy	Single-center open trial in vivo	36.6% Cure rate	(Sotiriou et al., 2010)
Tavaborole	Phase 3 clinical trials in vivo	6.5% Complete cure rate	(Anacor Pharmaceuticals)
Tea tree oil (100%)	Multicenter, double-blind, randomized in vivo	Improvement in onychomycosis	(Addino et al., 1994)
Tea tree oil (5%)	Randomized, double-blinded, placebo-controlled in vivo	No improvement	(Syed et al., 1999)
Vick's Vapo Rub	In vitro	Effective inhibition of fungal growth	(Ramesewak et al., 2003)
Vick's Vapo Rub	Clinical case series in vivo	28% mycological cure rate and 55.5% partial clinical cure rate	(Derby et al., 2011)

intra-individual study conducted in 2009 examined the effects of topical vitamin B12 on atopic dermatitis in the pediatric population. Participants' ages ranged from 6 months to 14 years old. Parents were instructed to apply the vitamin B12 cream to one side of their child's body and the vehicle to the other twice daily for 4 weeks, the study duration. Efficacy of the creams was evaluated using the modified SCORAD scale.\* At the study's completion, there was a statistically significant difference between SCORADs. When B12 was used, the average baseline SCORAD value of 13.19 decreased by 4.52 points, whereas the controls' baseline SCORAD value of 12.57 only decreased by 1.61 points. Side effects were minimal; 22 subjects started the 4-week study and 21 subjects completed the study,

\* SCORAD (SCORing for Atopic Dermatitis) scale. This is the gold standard for evaluation of atopic dermatitis disease severity. The modified SCORAD scale contains six objective items (erythema, edema, excoriation, oozing, lichenification, and dryness) and three subjective items (pruritus, sleep loss, and overall parental rating), each rated from 0–3.

with one subject experiencing side effects to both the experimental and control creams. A similar study had previously been conducted in adults. This was the first to be conducted in the pediatric population (51).

Oats, a secondary crop derived from a weed of the primary cereal domesticates wheat and barley (52), are commonly used to treat skin conditions such as atopic dermatitis. In fact, oatmeal has been deemed an effective skin protectant by the FDA (53). In an assessor-blind study of 35 burn wound healing patients, 5% colloidal oatmeal in a liquid paraffin suspension applied twice daily was compared to liquid paraffin alone. Patients applying colloidal oatmeal reported significantly less itch and required significantly less antihistamine than those applying vehicle alone (54).

Essential fatty acids (EFAs) are fatty acids that need to be obtained through the diet, because the body cannot synthesize them. As noted above, immune system dysregulation is thought to play a role in atopy, and it is known that EFAs—notably n-3 and n-6 fatty acids—have a role both in



inducing inflammation and modulating it. Previous studies have suggested that having an optimal ratio of n-3 fatty acids to n-6 fatty acids\* plays an important role in properly programming the fetal and infant immune system.

Blackcurrant is a commonly cultivated berry in Europe, and blackcurrant seed oil (BCSO) possesses the optimal ratio of these EFAs. Therefore, Linnamaa et al. conducted a double-blind, placebo-controlled trial assessing the efficacy of BCSO dietary supplementation in preventing the development of atopic dermatitis in infants. Pregnant women were randomly assigned to receive 3 g† of either BCSO or olive oil, the control. The pregnant women received their first dose at 8 weeks' and 16 weeks' gestation, then daily through the end of the exclusive breastfeeding phase. After breastfeeding was complete, infants received the oils orally, in the form of drops, until 2 years of age. Infants were evaluated for atopic dermatitis at 3, 12, and 24 months of age, and the SCORAD scale—mentioned above—was used to assess severity. Interestingly, 81.7% of the infants in this study had either one or two parents with a personal history of atopy, thereby increasing the infants' predisposition to atopy. At both 3 months and 24 months of age, there was no statistically significant difference between the two treatment groups with regard to the atopic dermatitis rates. However, at 12 months of age there was a statistically significant difference between the two study groups, 33% and 47.3% with atopic dermatitis in the BCSO and olive oil groups, respectively. Additionally, according to the SCORAD, those with atopic dermatitis in the BCSO group had statistically significantly less severe disease than those in the placebo group. The lack of statistically significant differences at ages 3 and 24 months may be explained by the general low prevalence and adoption of a less healthy diet at those ages, respectively. Previous studies have shown no side effects to blackcurrant supplements, making this a viable option for preventing and reducing the severity of atopic dermatitis early in life (55).

Chronic inflammation associated with atopic dermatitis is in part thought to be associated *Staphylococcus aureus* colonization, and the *S. aureus* burden has been shown to correlate with eczema severity.‡ Gentian violet (GV) is a known antimicrobial agent, and Brockow et al. performed a study assessing the efficacy of topical GV, diflucortolone-21-valerate (DC),§ and liquor carbonis detergens (LCD)¶ in treating *S. aureus*-colonized atopic dermatitis. Twenty-one adults with *S. aureus*-colonized atopic dermatitis were assigned to one of three groups—GV, DC, or LCD—and were instructed to apply their assigned topical agents—active and vehicle—to both eczematous and normal skin twice daily for 4 days. The severity of the eczematous lesions was evaluated prior to and 2 days after completing the treatment regimen using the modified SCORAD scale, and *S. aureus* burden was quantified daily. In patients treated with GV, both unaffected and eczematous skin showed statistically significant decreases in *S. aureus* density and there was a statistically significant difference between those areas treated with GV and those treated with control. Two days after treatment, *S. aureus* density returned to pretreatment baseline. Eczematous patches showed statistically significant reductions in SCORAD values during treatment with GV, as well as control. Subjects treated with DC and LCD did not show significant reductions

in *S. aureus* colonization in either affected or unaffected skin during the treatment phase, though interestingly, *S. aureus* density decreased 2 days following treatment. SCORAD values improved for the eczematous lesions treated with DC and LCD, as well as their associated controls; however, the improvement was more dramatic when the active agents were used. The in vitro portion of the study demonstrated the bactericidal effect of GV on *S. aureus* and DC's and LCD's lack of antibacterial effects (56). Notably, a more recent case report of group A *Streptococcus* impetiginized atopic dermatitis treated with topical 1% GV and oral doxycycline was presented in the *Journal of the American Academy of Dermatology*. Not only did the GV successfully treat the infection and dermatitis, it offered almost instantaneous relief of the associated discomfort. The authors suggest that it is often difficult to distinguish whether or not the underlying process is inflammatory, infectious, or both, and GV is a viable option for treating both (57).

Evening primrose is a tall herbal plant with yellow flowers that bloom in the evening. It was first described by the Native Americans in 17th century as being used to treat "swelling in the body." Evening primrose oil (EPO) contains 8%–10% gamma-linolenic acid (GLA)—n-6 EFA—and can be used as an alternative or adjunctive treatment for atopic dermatitis. Senapati et al. carried out a randomized, placebo-controlled study assessing the efficacy of oral EPO in treating atopic dermatitis in an Indian population. Twenty-five subjects were enrolled in both the experimental and placebo arms and instructed to take age-dependent oral doses—in capsule form—of either EPO or sunflower oil, respectively. Patients were followed for 5 months and investigators evaluated participants' disease monthly, based on four parameters: extent, intensity, dryness, and itching. On average, subjects treated with EPO gradually and progressively improved in all parameters during the course of the study, while those in the placebo group on average demonstrated inconsistent improvement. Based on the four parameters, subjects were given a numeric score, and improvement was defined as receiving a score up to 75% of baseline. At the study's end, 96% of participants in the experimental arm and 32% in the placebo arm demonstrated improvement, which was statistically significant (58).

Despite the promising findings of PMO in the Senapati et al. study, a recent *Cochrane Review* suggests the contrary. This review of 19 studies, including two meta-analyses—one with seven studies and other with eight studies—concluded that there was no statistically significant difference in improvement between those treated with oral EPO and those treated with placebo. Additionally, side effects of EPO noted in the report were predominantly GI upset and EPO's anticoagulant effects. Therefore, Cochrane's recommendation is that EPO not be used in treating atopic dermatitis, and further studies evaluating the effects of EPO on atopic dermatitis "would be hard to justify" (59).

In addition, the *Cochrane Report* reviewed the efficacy of borage oil (BO) versus placebo in treating atopic dermatitis. While no meta-analysis was performed for the BO studies, *Cochrane* reviewed eight studies and concluded that BO is not efficacious in treating eczema (59).

Although the *Cochrane Review* suggests that oral supplementation with BO does not improve atopic dermatitis, the following study suggests that topical BO likely has a role. While EPO contains 8%–10% GLA, BO (also known as starflower oil) (60), contains 24% GLA, making it a suitable consideration for the treatment of atopic dermatitis. Kanehara et al. conducted a double-blind, placebo-controlled trial assessing the

\* Optimal n-3:n-6 is 1:3 to 1:4.

† 3 g of the oils, administered in capsule form; 6 capsules = 3 grams.

‡ Confirmed in this study as well.

§ Potent steroid.

¶ Tar solution.



efficacy of BO-coated undershirts in treating atopic dermatitis. Investigators had BO chemically bonded to cotton undershirts, which would be gradually released; BO remained on the shirts after 60 washings. Thirty-two pediatric subjects participated in the study, with 16 assigned to the BO-coated undershirt group and 16 to the uncoated group. Subjects were evaluated by a single clinician at baseline for a variety of signs and symptoms associated with atopic dermatitis,\* instructed to wear the undershirts daily, and were reassessed 2 weeks later for the same signs and symptoms. As evaluated by the clinician, participants in the BO group showed a statistically significant improvement in symptoms of “itch” and “erythema” after 2 weeks, while those in the placebo did not show statistically significant improvement in any signs or symptoms. Notably, 75% of parents of children in the BO group reported symptomatic improvement, while 56.2% in the placebo also reported symptomatic improvement. Authors speculate that the symptomatic improvement observed by parents in the placebo group may be due to high quality, pure, organic undershirts that their children were wearing. Of note, those in the BO group showed a statistically significant decrease in transepidermal water loss (TEWL) after 2 weeks of treatment, while the placebo group did not. Individuals with atopic dermatitis have defective epidermal function, leading to increased TEWL and subsequent dry skin and associated symptoms. Therefore, the implications of topical BO in treating atopic dermatitis are twofold: decreasing inflammation and restoring epidermal structure and function, thereby decreasing water loss (61).

Another topical agent studied for the treatment of atopic dermatitis is extract from *Mahonia aquifolium*, an evergreen shrub related to the barberry. In one study, 30 patients with atopic dermatitis were treated with three times daily application of Relieva cream, a homeopathic product containing a proprietary 10% *M. aquifolium* extract. Eczema area and severity index (EASI) scoring revealed significant improvement in both short-term (4-week) and long-term (12-week) improvement as compared to baseline (62). However, no placebo control was utilized in this study.

Traditional Chinese herbal medicine (TCHM), also known as the PentaHerbs (PTH) formulation, is a “widely used ancestral Chinese concoction” of five herbal extracts, which includes *Flos lonicerae*, *Herba menthae* (HM), *Cortex moutan*, *Rhizoma atractylodis*, *C. phellodendri* in the quantities of 2g, 1g, 2g, 2g, and 2g, respectively. It is quite commonly used in Asia and China to treat children with atopic dermatitis. Hon et al. conducted a randomized, double-blind, placebo-controlled trial assessing the efficacy of oral TCHM in treating atopic dermatitis. Eighty-five subjects were enrolled—42 TCHM, 43 placebo—and instructed to take three capsules twice daily for 12 weeks. Participants were assessed every 4 weeks† using the SCORAD scale and the Children’s Dermatology Life Quality Index (CDLQI),‡ as well as the Allergic Rhinitis Score (ARS)§. Subjects were instructed to continue using prior medications

and amount of topical corticosteroids—0.1% mometasone furoate—were weighed during visits. Analysis of the data after 16 weeks demonstrated statistically significant improvement in the SCORAD scores in both the TCHM and placebo groups. However, there was not a statistically significant difference between the two groups. While SCORAD values did not differ, the TCHM group showed a greater than 30% improvement in CDLQI scores, while the placebo group showed no improvement at the study’s end; TCHM’s improvement compared to baseline and compared to the placebo group were both statistically significant. In addition to an improvement in CDLQI scores, those in the TCHM group demonstrated statistically significant decrease in the number of days topical corticosteroids were used per month, when compared to baseline. Additionally, the quantity of steroid used by the TCHM group was statistically significantly less than both the amounts used at their baseline and the amount used by the placebo group during the study. Interestingly, prior studies evaluating the chemical makeup of the TCHM have shown it to have no chemical similarity to corticosteroids. Side effects of TCHM in this study were “generally mild and self-limiting” (63).

Mast cells are known to play a role in allergic processes. In response to triggers,† mast cells release pro-inflammatory mediators such as histamine, cytokines, and prostaglandin D<sub>2</sub> (PGD<sub>2</sub>). Subsequent to the 2007 Hon et al. study, several investigators from the same research group conducted an in vivo study exploring the effects of the five substances in the PTH formula on mast cells in an attempt to explain PTH’s modulatory effect on atopic dermatitis. In part one of the study, rat peritoneal mast cells (RPMCs) were incubated with each of the five herbal components and then triggered to release pro-inflammatory mediators. In part two of the study, Human mast cells (HMC-1) were incubated with either dexamethasone, PTH formula, or one of the five components, and then triggered,\*\* and cytokine†† production was measured. PHF as well as one component of PHF, namely *Cortex moutan* (CM) alone, demonstrated statistically significant decreases in histamine concentrations from RPMCs as compared to controls. Likewise, statistically significant reductions in PGD<sub>2</sub> concentrations were observed upon exposure to PHF, CM, and *Flos lonicerae* (FL). One component of PHF, namely *C. phellodendri* (CP) seemed to increase histamine response and PGD<sub>2</sub> release. While the aforementioned 2007 Hon et al. study demonstrated no improvement in the severity of atopic dermatitis when treated with TCHM, authors suggest in this report that proportions of the individual components may have been subtherapeutic or an optimal ratio of the components was not present. Researchers recommend that increasing the ratio of CM or HM and decreasing CP could potentially increase the efficacy of PTH in lessening the severity of atopic dermatitis (64).

## Psoriasis

Among dermatologic conditions, psoriasis carries significant psychological burden and is associated with widespread treatment dissatisfaction (65,66). Patients with psoriasis therefore often turn to alternative therapies to complement their medical treatments.

\* Specifically—erythema, itch, papules, erosion, scaling, and lichenification. These were rated as 0–3, correlating to “none,” “mild,” “moderate,” and “severe.”

† Study duration was 16 weeks. Patients were not taking TCHM or placebo from week 12 to week 16.

‡ CDLQI contains ten questions relating to six categories: symptoms and feeling, daily activities, leisure, work and school, personal relationships, and treatment.

§ ARS evaluates symptoms of sneezing, watery rhinorrhea, nasal congestion, itching nose, itching eyes, and watering eyes.

† Compound 48/80 is polycationic compound, similar to Substance P in that it is a secretagogue. Substance P serum levels have previously been shown to be associated with disease severity in atopic dermatitis.

\*\* Triggered with a substance known as A23187+PMA.

†† Cytokines that were measured included: GM-CSF, IL-6, IL-8, IL-10, TNF-alpha.

Aloe vera, a succulent plant that probably originated in Northern Africa, the Canary islands, and Cape Verde, is a commonly used herbal medicine. Conflicting results have been observed in the treatment of psoriasis. In one study by Syed et al., aloe vera cream applied three times daily to 60 patients with slight to moderate psoriasis showed improvement as compared to placebo. Improvement was determined by a "positive response," measured as a composite of decreased erythema, infiltration, reductions of lesions, desquamation, and lower psoriasis-associated severity index (PASI) score (67). A more recent investigation of aloe vera gel applied twice daily for 4 weeks to 41 patients with slight to moderate psoriasis vulgaris showed a greater improvement in placebo-treated patients as compared to those with aloe vera. Efficacy was measured utilizing a modified PASI score (68).

The Dead Sea, a salt lake bordering Jordan, the West Bank, and Israel, is the deepest hypersaline lake in the world, and with 33.7% salinity is one of the world's saltiest bodies. It is purported to have many health benefits and has been investigated in the treatment of psoriasis. In a double-blind, randomized control clinical trial by Cheesbrough et al., 24 patients were treated with either a 30% Dead Sea salt lotion or placebo for a total of 12 weeks. There was no statistically significant improvement in either the Dead Sea salt lotion or vehicle during the treatment period (69). A more recent study by Dawe et al. investigated Dead Sea salt (DSS) soaks plus NB-UVB

versus NB-UVB alone in the treatment of plaque psoriasis in a randomized, single-blind, controlled, right/left comparison study. Sixty-six patients pretreated one limb with DSS soaks and then underwent NB-UVB for 12 weeks. There was no significant difference between the DSS soaks plus NB-UVB when compared to NB-UVB alone (70).

Indigo naturalis, derived from the leaves of plants such as *Baphicacavthus cusia*, has been investigated in the treatment of psoriasis. A recent randomized, observer-blind, vehicle-controlled, inpatient comparison study by Lin et al. evaluated the efficacy of a topical indigo naturalis ointment versus vehicle in the treatment of recalcitrant plaque-type psoriasis in 42 patients. Statistically significant reductions in the sum of scaling, erythema, and induration scores were observed in indigo naturalis-treated patients as compared to controls (71). A follow-up study in the *Journal of Investigative Science* found that the antipsoriatic effects of indigo naturalis involve, at least partially, modulating the proliferation and differentiation of keratinocytes (72).

Finally, just as *M. aquifolium* has been studied for the treatment of atopic dermatitis, so too, it has been investigated for its use in treating psoriasis. The study consisted of 200 patients treating one plaque of psoriasis with Relieva twice daily for 12 weeks. PASI scores were significantly improved in the Relieva-treated patients as compared to placebo (73,74). See further Table 17.3.

**Table 17.3** Alternative Treatments for Atopic Dermatitis, Psoriasis, Pruritus

Alternative Medication	Study Type	Experimental Result	Source
Aloe vera	Double-blind, placebo-controlled (in vivo)	Slight to moderate improvement in psoriasis	(Syed, Ahmad et al. 1996)
Aloe vera	Double-blind, placebo-controlled (in vivo)	No improvement in psoriasis	(Paulsen, Korsholm et al. 2005)
Blackcurrant seed oil, oral	Double-blind, placebo-controlled (in vivo)	Improvement in atopic dermatitis in infants	(Linnaamaa, Savolainen et al. 2010)
Borage oil, oral	Cochrane Review (in vivo)	Improvement of atopic dermatitis is no greater than placebo	(Bamford, Ray et al. 2013)
Borage oil, topical	Double-blind, placebo-controlled (in vivo)	Symptomatic improvement in atopic dermatitis	(Kanehara, Ohtani et al. 2007)
Traditional Chinese herbal medicine, oral	Randomized, double-blind, placebo-controlled (in vivo)	Overall improvement of atopic dermatitis no greater than placebo Improvement in quality of life and decreased amount of topical steroid needed to treat	(Hon, Leung et al. 2007)
Traditional Chinese herbal medicine (PentaHerbs formula)	In vitro	Reduction in rat peritoneal mast cell release of pro-inflammatory mediators of atopic dermatitis	(Chan, Hon et al. 2008)
Colloidal oatmeal suspension	Alternating assignment, assessor-blinded (in vivo)	Improvement in pruritus in burn wound healing patients	(Matheson, Clayton et al. 2001)
Dead sea salt lotion	Placebo-controlled (in vivo)	No improvement in psoriasis	(Cheesbrough 1992)
Dead Sea salt soaks (with NB-UVB)	Randomized, observer-blinded, paired comparison (in vivo)	No additional improvement in psoriasis as compared to NB-UVB alone	(Dawe, Yule et al. 2005)
Evening primrose oil, oral	Cochrane Review and meta-analyses (in vivo)	Improvement of atopic dermatitis is no greater than placebo	(Bamford, Ray et al. 2013)

(Continued)

**Table 17.3 (Continued)** Alternative Treatments for Atopic Dermatitis, Psoriasis, Pruritus

Alternative Medication	Study Type	Experimental Result	Source
Evening primrose oil, oral	Randomized, placebo-controlled (in vivo)	Improvement in atopic dermatitis	(Senapati, Sabyasachi et al. 2008)
Gentian violet	Case report	Improvement of <i>Streptococcus</i> impetiginized atopic dermatitis	(Stoff, MacKelfresh et al. 2010)
Gentian violet	Placebo-controlled trial (in vivo; in vitro)	Reduces <i>S. aureus</i> skin burden and improves atopic dermatitis that is colonized with <i>S. aureus</i>	(Brockow, Grabenhorst et al. 1999)
Indigo naturalis ointment	Randomized, observer-blinded, vehicle-controlled (in vivo)	Improvement in psoriasis	(YK, CJ et al. 2008)
<i>Mahonia aquifolium</i>	In vivo	Improvement in psoriasis	(Bernstein et al 2006; Smith et al 2009)
Vitamin B12, topical	Double-blind, randomized, placebo-controlled (in vivo)	Improvement in atopic dermatitis	(Januchowski, 2011)

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# Section III

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## Non-Pathological Skin Treatments



پزشک آموز



پزىشک آموز

# Skin Care Products for Normal, Dry, and Greasy Skin

Christine Lafforgue, Céline Try, Laurence Nicod, and Philippe Humbert

## INTRODUCTION

Skin care products have biologically active ingredients with medicinal or druglike benefits. Furthermore, they satisfy the needs of beauty and health. Many substances, either chemically synthesized or extracted from plants or animals, can be used as functional ingredients. Nowadays, many products with biologically active ingredients have been developed and marketed. They are intended to carry out their functions as protection, whitening, tanning, antiwrinkling, deodorants, antiaging, and nail and hair care. Skin care products may, however, cause some unwanted problems. The common ones are irritability to the skin, contact dermatitis, photosensitivity, comedogenicity, hair and nail damage, hyper- or hypopigmentation, infectivity, carcinogenicity, and even systemic adverse effects.

Advances in electronics and computing have allowed the development of instruments for measuring certain parameters of the skin. Quantification of these parameters has enabled the evaluation, comparison, and hence improvement of the efficacy of skin care products.

## THE DIFFERENT TYPES OF SKIN

### Normal Skin

Normal skin is defined as having no visible lesions or sensations of discomfort. It results from an equilibrium of various continuous biologic processes including keratinization, desquamation, water loss, sebum excretion, and sweating. In situations of perfect balance between sebum production and requirements, the skin is comfortable, well moisturized, supple, elastic, clean, easy to decorate, and not so easy to irritate.

During aging, the skin undergoes certain changes arising from external causes, in particular ultraviolet (UV) radiation as well as the general aging process that affects the entire organism. According to certain authors, these changes with age are manifested as changes in the chemical structure, the quality and quantity of structural proteins, proteoglycans and hyaluronic acid (1). Also, during adult life, dermal collagen content progressively decreases, correlating with a reduction in skin thickness (2). A simultaneous decrease in the amount of proteoglycans and hyaluronic acid is also seen (1).

Elsewhere, hormonal changes occurring during adolescence and then aging account for the respective increases in sebum secretion seen in puberty, then its progressive decrease during adult life, declining considerably in postmenopausal women (3). With aging, a decrease in the rate of corneocyte desquamation is also seen (4).

### Dry Skin

The term dry skin or xerosis describes an integument with a dry, rough, or scaly appearance with the possible presence of reddening, cracking, or itching. The skin is less flexible than normal, contributing to the irregular feel to the touch (5,6).

Dry skin results from many factors:

- The water content of stratum corneum is reduced (13% of water for normal skin and less of 10% for dry skin).
- An abnormal keratinization modifying the stratum corneum equilibrium (between desquamation and proliferation) and corneocytes cohesion (abnormal lipid compartment).
- A decrease in barrier function, which increases in the passive rate of transepidermal water loss.

Two types of dry skin can be distinguished (7): acquired dry skin and constitutional dry skin. Acquired dry skin may arise from normal, or sometimes even greasy skin, which is rendered temporarily and locally dry by external factors such as solar radiation, exposure to extreme climate (cold, heat, wind, dryness), exposure to chemicals, or various therapeutic measures (lithium, retinoids). Constitutional dry skin can be nonpathologic (senile skin, fragile skin) or pathologic (ichthyoses, dry skin of atopic dermatitis, hypothyroidism, uremia).

### Greasy Skin

Greasy skin mainly involves the upper part of the body, where greater numbers of sebaceous gland are found. Simple greasy skin is common in adolescents and young adults. It is characterized by skin thickening and an increase in sebaceous excretion, giving the face a shiny appearance, especially on the nose and forehead, with an unclean appearance and rancid smell. In extreme cases, the follicular ducts are often dilated (kerosis). However, they can also plug by minuscule cornified spicules, which protrude and give a sensation of roughness to the touch.

Several parameters induce production of sebum by sebaceous glands, notably by the main stimulus, circulating androgen levels. In men, the sebum causal level is higher from birth and is temporarily inverted during prepuberty, which occurs earlier in girls. In females, a decrease during the estrogenic phase of the menstrual cycle and an increase during the luteal phase are observed (8).

Several types of complications can be associated with greasy skin, especially the following:

#### *Acne*

This complication is characterized by the presence of comedones (blackheads) and closed comedones (microcysts). Causes can include excessive proliferation of *Propionibacterium acnes*, which produces free acids by enzymatic hydrolysis of

triglycerides; irritation of the dermis by keratin and free fatty acids released by comedones; and immune reactions with sensitization of acne subjects to *P. acnes*.

#### Seborrheic Dermatitis

This is also a frequent problem, the cause of which is still unknown but may include *Malassezia* yeast (*Malassezia globosa* and *M. fufur*), chemical agents (detergents), or nervous factors (stress, anxiety). It is characterized by the presence of erythematous squamous plaques, made up of greasy squames, localized mainly on the hairline and eyebrows, nasal folds, chin, and presternal region. The scalp is often affected, with the formation of crusty plaques covering the bases of the hair shafts. These lesions are often slightly pruritic.

### METHODS FOR EVALUATING SKIN CHARACTERISTICS (BIOENGINEERING)

The main parameters of different skin types that can be used to evaluate products efficacy are those related to skin surface morphology, stratum corneum hydration, and sebum excretion. Because of parameter variation between different anatomical zones on the same subject and between different subjects, these techniques are used mainly to measure the change in a parameter with time, on the same zone. For example, a comparison can be made between the initial state (pretreatment) and the final state (post-treatment).

#### Evaluation of Skin Surface Morphology

D-Squames and Corneofix F20 (9,10) are used to detect and assess some damage of the horny layer and alteration of the viable epidermis behavior. The principles of measure consist of a special transparent adhesive tape, which collects corneocytes from the top layer of the skin for 5 seconds. The tape is removed, stuck on a glass slide, inserted into a microfilm viewer, and its optical density is measured. The latter is inversely proportional to the amount of scales per unit area.

With the probe, precise information regarding desquamation can be obtained: the number, size and thickness of the corneocytes or the roughness of the stratum corneum can be evaluated.

#### Other Probes

The Visioscan VC98 device (Courage and Khazaka, Köln, Germany) is a video camera that monitors the skin surface illuminated under a UVA light source. Interpretation of the image by the supplied software gives information about skin roughness, smoothness, scaliness, and wrinkles.

Optical coherence tomography (OCT) was originally used for ophthalmologic diagnosis but has found applications in dermatologic investigation (11). OCT is based on the principle of Michelson interferometry and uses a light source with a short coherence length. The light source is split into a reference, and a sample beam is focused on the skin. Structural information on morphology and thickness can be easily obtained, and information on water content or hydration is also available via the calculation of refractive index profiles.

Confocal laser scanning microscopy (Vivascope 1500) (12) enables instant visualization of skin structures at a histopathologic resolution and represents a new noninvasive approach for the in vivo study of physiologic and pathologic conditions of the skin.

The confocal microscope consists of a small source of light, which illuminates a small spot within the object; the illuminated spots are then imaged onto a detector through a small aperture. The source, illuminated spot, and detector aperture are placed in optically conjugate focal planes. The images are obtained with a defined horizontal layer of 5-mm thickness, thus eliminating reflected light from other skin layers as well as aberrations.

A near infrared laser wavelength (830 nm) is absolutely harmless for user and patient.

### Skin Hydration Measurements

Objective assessment of skin hydration remains the preoccupation of most cosmetic scientists and dermatologists. The methods and techniques used vary in complexity and have been fully described elsewhere (13).

#### Measurement of Electrical Properties of the Skin

The dielectric constants of keratin and epidermal lipids are very small as compared with that of water. Therefore, the dielectric constant of the stratum corneum is principally determined by its level of hydration: the greater the water content, the larger the dielectric constant.

The Corneometer CM 825 (Courage and Khazaka, Köln, Germany) (14) is an apparatus with a probe that is placed in contact with the skin. The probe acts as a capacitor. The capacitance thus measured is proportional to the dielectric constant of the skin, varies according to its state of hydration, and is expressed in arbitrary units. The device measures capacitance, which is proportional to the dielectric constant of the skin, and varies according to its state of hydration. On the forearm the following data are obtained (15):

- <75: dehydrated skin
- 75–90: skin with a tendency to dehydration
- >90: normal skin

Other instruments can evaluate the hydration state of the skin by assessing electrical properties (16):

- The DermaLab (Cortex Technology, Hadsund, Denmark) measures the impedance of the skin.
- The MoistureMeter SC-4 (Delfin Technology, Kuopio, Finland) is a novel capacitive device. The instrument shows arbitrary capacitance units.
- The Nova Dermal Phase Meter DPM 9003 (Nova Technology Corporation, Portsmouth, New Hampshire) measures impedance-based capacitive reactance of the skin. The final readout is given in arbitrary DPM units, which are related to the capacitance.
- The Skicon 200, based on the experimental device developed by Tagami and coworkers (ISBS Company, Hamamatsu, Japan) measures the conductance in micro-siemens.

### Infrared Spectroscopy (17)

Water absorbs infrared radiation. Because of this property, it's possible to quantify the water content of stratum corneum using attenuated total reflectance infrared spectroscopy. It has been shown that absorbance at 3400/cm characteristic of OH stretching vibrations increases in the deeper layers of the stratum corneum, suggesting a corresponding increase in water content as a function of stratum corneum depth.



## Measurement of Passive Transepidermal Water Loss (18,19)

Measurement of transepidermal water loss (TEWL) (18,19) determines the continual flux of water vapor diffusing across the stratum corneum. It does not measure skin hydration, but does allow evaluation of the barrier function and efficacy of hydrating products whose mode of action relies on occlusivity. TEWL is measured using an evaporimeter (Servo Med Evaporimeter, Kinna, Sweden). This device is applied on the skin surface, built as a cylindrical chamber open to the surrounding air, and determines the continual flux of water vapor diffusing across the stratum corneum. The process measures the partial water vapor pressure at two points 3 and 9 mm above the skin surface, respectively, with the aid of two pairs of humidity transducers and thermistors. Normal TEWL values are between 2 and 5 g/m<sup>2</sup>/hr.

## Measurement of Wettability (20–25)

Wettability results from the interactions between a fluid and the skin. A drop of water is placed on the skin and forms with the skin a semi-hydrophobic contact angle. The drop is observed with an operating microscope equipped with a slanted mirror, which gives a view of the profile of the drop, recording the image of the profile using a video camera connected to a computer. The water contact angle  $\gamma$  can be measured and is used as an indicator of hydrophobic or hydrophilic tendency of the skin or to see the effect of products in the greasy or dry skin. For normal skin,  $\gamma$  (forehead) is between 578 and 738. In the case of dry skin, the affinity with water decreases, and the contact angle between skin and water increases.

## Evaluation of Friction Coefficient

Friction is an interaction between two surfaces. Many factors influence the friction coefficient value as hydration or sebum. Hydration studies have correlated increases and decreases in skin hydration with the changes in the friction coefficient (26–28).

## Sebum Measurement

*Measurement of Sebum Excretion (29,30)*

The Sebumeter SM 810 (Courage and Khazaka, Köln, Germany) uses a plastic tape which is applied on the skin for 30 seconds. The sebum lipids are adsorbed on the film and render it transparent. Next, the probe is inserted into the Sebumeter, which shines a light beam onto the film. A reflective metal plate behind the film reflects the light back, passing a second time through the film before entering a photomultiplier. The device automatically determines the increase in film transparency as a function of skin surface sebum and gives the lipid index in micrograms per square centimeter.

The Lipometer (L'Oréal, Paris) functions in a similar way to the Sebumeter. Its principal differences are:

- The plastic film is replaced by a ground-glass disc mounted on a dynamometer to standardize the application pressure
- A series of standard calibrated values are used to convert readings into absolute data (e.g., mg lipid/cm<sup>2</sup>)

*Quantification of Sebum Output, Density, and Activity of Sebaceous Glands (31)*

The main component of Sebutape (Cutoderm, Dallas, Texas, U.S.) is a microporous hydrophobic polymer film, which becomes translucent and then transparent when impregnated with oil. It can be stuck on the skin as it has an adhesive coating, or simply applied (Sebuxif F16). The film absorbs the sebum originating from the follicular openings, which forms transparent spots that are easily visible on a dark background and whose area is proportional

to the collected volume. This method quantified the topographical distribution of functional glands and their output on the skin surface using computerized image analysis. The size of the spot is proportional to follicular excretion activity. With sustained application of Sebuxif F16, the progressive enlargement of sebum spots can be monitored using a UV light video camera (Visioscan VC98, Courage and Khazaka) and 3D images obtained at will.

## Skin Brightness Measurement

The glossmeter is a new instrumentation to measure the specularly reflecting light from the skin. It can be used to evaluate the skin care including the brightness of the skin. It could be used to evaluate the effect of matting products or hydrating products. Two glossmeters were developed in 2009: the Glossymeter GL 200 (Courage and Khazaka, Köln, Germany) and the Skinglossmeter (Delphin Technologies, Kuopio, Finland) (32).

## SKIN CARE PRODUCTS Care of Normal Skin (33)

The stratum corneum matrix is rich in ceramides, free fatty acids, and cholesterol, which serve to repel water and provide an effective barrier. The integrity of the stratum corneum is also promoted by substances in the corneocytes known as natural moisturizing factors (NMFs), a complex mixture of free amino acids, amino acid derivatives, and salts, which attract and hold water. This also helps to maintain skin flexibility and elasticity by absorbing water from the atmosphere, which enables the outermost layers of the skin to remain hydrated despite the drying action of the environment. Fundamental skin care consists mainly of cleansing, moisturizing, and protection.

### *Cleansing Products*

Washing removes debris from the skin surface, and soap and water are frequently used together for this purpose because of convenience and perceived cost-effectiveness. Soaps are made by hydrolysis of natural triglycerides and neutralization of fatty acids released by sodium. They are good emulsifiers, having emollient action and increased lathering power. However, two problems are associated with them:

- Their powerful detergent action may completely eliminate the protective surface lipid film, which helps maintain the skin's physiological balance, and thus may engender irritation.
- Soaps have highly alkaline nature (pH around 10). The repeated use of soap may shift the pH of the skin surface, making it more alkaline, thereby negating the protective influence of the acid mantle and upsetting the balance of resident flora on the skin (34).

All surfactants interact with skin lipids and also with cutaneous proteins, thus use of a mild or lipid-rich cleanser permits washing and avoids damage to the skin barrier (35).

### *Hydrating Products*

Current emollients are available in the form of sprays, lotions, creams, and ointments. Although the development and formulation of emollients has moved forward, the basic principle remains the same—they are all variations of an oil (lipid) and water emulsion. Technically these emulsions may take the form of oil-in-water or water-in-oil emulsions, with oil-in-water emulsions being the most common. Thus, modern emollients can not only help to maintain skin hydration, but can also help to replenish skin barrier lipids. The characteristics of emollients are described below in the section "Care of Dry Skin."

### Photoprotective Products

Ultraviolet radiation can cause inflammatory changes, erythema, and subsequently pigmentation, inducing premature skin aging and risk of cancer. Throughout the seasons, photoprotection has an important role for all skin types, including normal skin. The ideal photoprotector must effectively absorb, diffract, or reflect noxious radiation (UVB and UVA), be substantive to the stratum corneum and be water and sweat resistant, be stable in daylight and in air and to heat and water, and be totally innocuous.

A proper sunscreen product must provide protection against acute and long-term UV-induced skin damage and be stable to heat and UV radiation. The degree to which a sunscreen protects the skin from UV rays is given as its sun protection factor (SPF). The SPF can be used as a guide to select sunscreen to avoid sunburn. The SPF indicates the time a person with sunscreen applied can be exposed to sunlight before getting sunburn relative to the time a person without sunscreen can be exposed. The SPF value concerns protection in front of UVB and UVA radiation with a minimum balance of 1 UVA for 3 UVB for Europe legislation.

The photoprotective ability of sun products is determined by two types of substance: chemical filters and physical screens.

Chemical filters (36) are synthetic chemical substances with the following properties:

- Powerful absorption of UV radiation owing to the measure of double bonds
- Relative stability when excited, so the absorbed energy is released slowly

Physical screens are mineral particles that in addition to absorbing UV rays reflect and diffract them like a mirror. The main type used is ultrafine titanium dioxide (TiO<sub>2</sub>), made up of particles of 20–30 nm in size (37). The reduced size of ultrafine particles confers better reflection in the UVB and short UVA wavelengths, and better transparency in the visible wavelengths.

Consumers are more aware of unnatural chemicals and other toxins and are searching for natural products to use on their skin. Antioxidants can have profound effects on the intracellular signaling pathways involved in skin damage and thus may be protective against photodamage as well as may prevent wrinkles and inflammation. The body has physiologic antioxidants, such as vitamins, glutathione, ubiquinon 10 (CoQ10), and lipoic acid. The use of exogenous antioxidants can add to the body's physiologic reservoir of endogenous antioxidants and enhance the enzymatic clearance of reactive oxygen species (ROS) (38).

Following, there are many examples of natural components which have antioxidant properties. Some of them can be found in skin care.

Vitamins A, C, and E are naturally present in human skin. These vitamins are part of a complex system of enzymatic and nonenzymatic antioxidants that protect the skin from harmful ROS (39,40). However, the skin is subjected to substantial environmental free radical stress from sunlight, pollution, and smoking that can deplete dermal stores of naturally occurring antioxidants. To remedy vitamin deficiency, many topical products contain vitamins (41).

Soy contains active ingredients: phytosterols and small protein serine protease inhibitors (Bowman-Birk inhibitor and soybean trypsin inhibitor). Soy has antioxidant and anti-inflammatory effects (42,43). It stimulates collagen synthesis, initiates the skin elastin repair process, inhibits pigmentation, controls oil production, and moisturizes the skin.

White, green, oolong, and black tea are derived from the leaves and buds of the tea plants (*Camellia sinensis*), with the different varieties dependent on the type of processing and oxidation or fermentation. The main active ingredients are polyphenols that include catechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate. These polyphenols have very potent antioxidant, anti-inflammatory, and anticarcinogenic properties, making teas useful in the prevention and treatment of photodamage (44,45).

Pomegranate extract (*Punica granatum*) is primarily composed of alkaloids and polyphenols, the active constituent being ellagic acid. It has demonstrated a variety of beneficial functions including antioxidative and antiviral activity. Pomegranate seed oil fractions may facilitate epidermal regeneration (30).

Ectoin, a natural, vital substance, was developed for use in cosmetic applications. It was discovered in halophilic bacteria, which survive and grow under extreme conditions in salt lakes, sea water, and saline deserts. Efficacy studies in vitro demonstrated that ectoin counteracts the effects of UVA-induced and UVA-accelerated skin aging at different cell levels. Ectoin protects the skin from the effects of UVA-induced cell damage in a number of different ways and has the potential to protect mitochondria of human fibroblasts in vitro against UVA radiation-induced mutagenesis (46).

Sesamol (47) is a highly acclaimed antioxidant. An experiment on mouse skin showed that it has a good effect on prevention on photodamage, observed on biochemical and histopathologic changes.

Resveratrol, or polydatin, is now used because it promotes modulation of epidermal cytokines and proteins engaged in cell repair (48,49).

### Care of Dry Skin (50–52)

Treatment of dry skin is aimed at restoration of the epidermal water barrier. This is accomplished with moisturizing agents that are topically applied to the skin.

Excessive bathing or the use of hot baths or showers should be discouraged and the use of mild soaps rather than harsh soaps or detergents should be encouraged.

Soap substitutes such as cetyl alcohol preparations can be helpful for dry skin. Hydration of the stratum corneum by balneotherapy followed by either addition of oil to the bath water or rapid application of emollients (occlusive agents) to the skin on exit from the bath can improve hydration of the stratum corneum.

Hydration can be done in two ways:

- By the input of external water, which is retained in the stratum corneum by addition of humectants
- By the slowing down of stratum corneum water loss due to evaporation (reducing TEWL), by means of an occlusive lipid film

The lipids in the latter can combat the delipidization associated with dry skin and protect against external agents. Formulation of a hydrating product involves both of these principles, but with different emphasis placed on them depending on the type of skin (dehydrated skin or dry skin) and conditions of use envisaged.

### Humectant Moisturizing Ingredients

Humectants are compounds that attract water from the dermis into the stratum corneum. They are many and varied. When humidity is higher than 70%, humectants can also attract water from the atmosphere into the epidermis. Humectants can be

thought of as the cosmetic equivalents of NMF. NMFs are natural components including hygroscopic and hydrosoluble substances in the stratum corneum which are most likely to be enveloped by cell membrane lipids. These substances play an important role in water retention, since it has been shown that their extraction results in a 25% loss in stratum corneum water content and 66% loss in elasticity (53). NMF agents include the following:

Polyols are molecules with numerous hydroxyl groups (hydrophilic and hygroscopic substances). Glycerol and sorbitol are excellent humectants and are used at concentration between 2% and 10%. Propylene glycol has good hydrating ability at low concentrations (inferior to 10%) and keratolytic activity at high concentrations (superior to 40%). Other polyols are used like mannitol but there are less hydrating.

Pyrrolidone carboxylic acid is the one of principal components of NMF (about 12%). It is found in salt form and has a hydrating effect at concentration of 3% to 5%.

Urea hydrates at concentration at less than 10%. Over this concentration, urea has keratolytic power. This molecule has a high solubility in water and is rapidly hydrolyzed and decomposed.

Lactic acid and sodium lactate can capture a high concentration of water and participate in maintaining acid mantle and NMF efficacy on the skin surface. It has an effect from concentration of 3%.

Certain macromolecules of biologic origin, such as hydrolyzed wheat proteins, have a high content of hydrophilic groups, but with their large size, they cannot penetrate the stratum corneum and they form a hygroscopic film at the surface.

Certain macromolecules of biologic origin have a high content of hydrophilic groups, but with their large size, they cannot penetrate the stratum corneum and they form a hygroscopic film at the surface. This category includes the following:

- Glycosaminoglycans such as hyaluronic acid and chondroitin sulphate are polysaccharides. These compounds are found in the ground substance of all connective tissue and possess a considerable water sorption and retention, owing the large number of hydroxyl groups.
- Collagen and elastin are the two main structural proteins of the connective tissue and have hygroscopic property. With water, they form an aqueous gel. These proteins are generally used in denatured or hydrolyzed form.
- DNA has a large number of phosphate groups and possesses a good hygroscopic property. It is used in a denatured and partially hydrolyzed form in cosmetology.

#### *Occlusive Moisturizing Ingredients (51)*

Occlusives increase the water content of the skin by slowing the evaporation of water from the surface of the skin. These ingredients are often greasy and are most effective when applied to damp skin. Mineral oil is often used because of its favorable texture, but it is not as effective at preventing evaporation of water as many other occlusives.

Actually, filmogenic products compose the emulsion. Water-in-oil emulsion is very occlusive. Petrolatum, an hydrocarbon oil, is the most effective occlusive moisturizer.

Other hydrocarbons include mineral oil, paraffin, and squalene. In occlusive moisturizing ingredients, other categories of compounds exist and include vegetable fats, fatty alcohols, wax esters, vegetable waxes, phospholipids, sterols, silicones, and oils rich in polyunsaturated fatty acids (PUFAs).

Oils rich in PUFAs (52) occupy a particular place in cosmetology. They are occlusive but their potential lies in their

high PUFA content. PUFAs are long-chain fatty acids, unsaturated at o-3 or o-6, of which some are classed among the essential fatty acids (linoleic acid, arachidonic acid, linolenic acid). These PUFAs are found in large quantities in certain animal oils (fish oil) and plants (evening primrose, borage, grape seed). They are involved in several important physiologic functions such as metabolism of prostaglandins and leukotrienes, inflammation, and hence the maintenance of stratum corneum hydration.

#### *Additives to Moisturizer*

Other agents are now included in moisturization products. These agents have different functions than simply reducing TEWL or reconstituting the lipid components of the stratum corneum. Special moisturizing agents such as  $\alpha$ - or  $\beta$ -hydroxy acids can help promote corneocyte desquamation and decrease roughness.  $\alpha$ -Hydroxy acids (52) (lactic, glycolic, malic, tartaric, citric, gluconic, and mandelic acids) have been shown to improve the appearance of photodamaged skin and are an effective keratolytic in concentration as low as 10%, and have a tendency to reduce corneocyte cohesion at the base of the stratum corneum. At high concentration (30%–70%), their keratolytic action predominates: they act on the deeper epidermal layers, and even the papillary and reticular dermis. At these concentrations they are suggested for the treatment of hyperkeratosis.

Salicylic acid is the only  $\beta$ -hydroxy acid. Its mechanism of action is supposed on the dissolution of the intercellular cement between adjacent corneocytes, reducing corneocyte adhesion. It is unique in that it can enter the pilosebaceous unit and increase exfoliation in the oily areas of the face. Because of its exfoliating effects, salicylic acid is beneficial in aging skin because of increased desquamation of the stratum corneum.

Urea (51) can also be added to moisturizers and enhances the water-binding capacity of the stratum corneum by disrupting hydrogen bonding. Urea exposes water-binding sites on corneocytes and promotes desquamation by decreasing the intercellular cementing substance between the corneocytes. Also, long-term treatment with urea has been demonstrated to decrease TEWL.

### **Care of Greasy Skin (54)**

The essential requirement is to reduce excess skin surface sebum without total delipidization. Severe degreasing treatment can lead to an exacerbation of sebaceous secretion.

Facial washing may be carried out with either a mild soap or a lipid-rich soap. This must be followed by copious rinsing. In cold weather, the protection afforded by a continuous aqueous phase, light emulsion (oil in water) suffices. However, products used for this type of skin must be noncomedogenic.

### **Acne Treatment**

#### *Comedolytic Agents*

Salicylic acid, lactic acid, glycolic acid, and benzoyl peroxide may all decrease follicular impaction and have been proved in both human and animal use. Of these compounds, salicylic acid is the most widely used. It has been shown to be safe and effective in reducing comedones when applied in 0.5% and 2% solutions.

The use of topical benzoyl peroxide does have some limitations. It is not effective as a monotherapy for severe acne. Leave-on benzoyl peroxide creams and gels have the potential to bleach clothes and bed linens, and like all topical medications have the risk of causing an allergic contact dermatitis.



Some more recent innovations in benzoyl peroxide formulations include microsphere technology, aqueous-based gel, liposomal delivery, and microemulsion. The latest innovation involves the use of micronization to produce solubilized benzoyl peroxide particles, which allows the smaller-sized particles to penetrate down into the follicle.

Moreover, irritation associated with benzoyl peroxide can be minimized, while maintaining equal efficacy, by using the 2.5% formulation of benzoyl peroxide and vehicle such as the dimethyl isosorbide-containing hydrophase base and 10% urea (55).

As benzoyl peroxide treatment technology advances, it should become an even more useful treatment, as monotherapy or adjunct, for mild to moderate acne vulgaris.

#### Topical Antibiotics

The use of topical antibiotics (macrolide as clindamycin or erythromycin) is frequent. Its bactericidal action on *P. acnes* inhibits bacterial protein synthesis.

The problem of the emergence of antibiotic-resistant *P. acnes* encountered with topical clindamycin monotherapy is markedly reduced by the addition of benzoyl peroxide as a combination formulation. Fixed combination products of clindamycin 1% and benzoyl peroxide 5% are commonly used in the treatment of acne vulgaris. Although any given topical acne product may be therapeutically effective, signs and symptoms of cutaneous tolerability may lead to missed applications by the patient, thus limiting adherence to therapy. Benzoyl peroxide can cause cutaneous irritation and dryness, which are dose-dependent. Recently, clindamycin and benzoyl peroxide 2.5% gel appears to provide efficacy comparable to that of higher concentration (5%) fixed clindamycin-benzoyl peroxide combination products and should optimize patient compliance as a result of the reduction in cutaneous tolerability reactions, including signs of skin irritation or dryness (56).

#### Azelaic Acid

This is a naturally occurring dicarboxylic acid produced by yeasts of the *Pityrosporum* genus. It is a compound with significant direct anti-*P. acnes* activity and some anticomedonal activity. The compound has no effect on sebaceous gland size, sebum production, or sebum composition, even after long-term application. Azelaic acid is bacteriostatic at low concentrations and bactericidal at higher levels.

#### Topical Tretinoin

Tretinoin, which is the acid form of vitamin A (also known as all-trans retinoic acid), works both by comedolysis and by normalizing the maturation of follicular epithelium so that comedon formation ceases. It has no antiseborrheic or bactericidal activity. All concentrations of tretinoin are useful in comedonal and inflammatory acne (0.05% or 0.1% cream is the maximal benefit).

Despite its benefits, tretinoin, as with other topical retinoids, has the potential to cause localized irritation. To minimize this response while maintaining clinical efficacy, tretinoin has been formulated in sponge-like polymeric microspheres that encapsulate the active ingredient and deliver gradually and relatively selectively to the follicle. In comparison with a standard 0.025% cream, a tretinoin microsphere gel 0.1% formulation has been shown to be less irritating to normal skin and to cause significantly less erythema and dryness in patients with mild to moderate facial acne over a period of 12 weeks (57,58).

## Sebum Regulator

### Intradermal Botulinum Toxin

The use of intradermal botulinum toxin type A is a new technique. The purported mechanism of action of botulinum pore reduction can be postulated on the basis of its known neuromodulatory effects. A. Shah shows that intradermal botulinum toxin can be injected safely in the area of the forehead, medial cheek, and nose without complications. A treatment of intradermal botulinum toxin subjectively reduced skin oiliness and pore size in 17/20 patients at 1 month after injection (59).

The role of botulinum toxin in targeted therapy without systemic consequences may eventually make it an alternative to isotretinoin in the treatment of oily skin and acne-prone skin.

### Seborrheic Dermatitis Treatment

Essentially, treatment consists of daily application of topical antifungals (topical imidazoles), which act on *Pityrosporum*. Treatments using products with 5- $\alpha$  reductase inhibitors (acetamide and unsaponifiable plant oils) may also be used to combat seborrhea. In parallel, treatments for greasy skin will also be used.

## CONCLUSION

In recent years, considerable progress has been made in the cosmetics industry, which was originally mainly concerned with perfume and makeup. Today the emphasis is much more on the development of veritable medical cosmetics of increasingly high performance, specifically adapted for all kinds of skin types, and backed up by rigorous scientific testing procedures for efficacy and tolerance. Whether one considers the various protective roles of all these products or looks more specifically at, say, the corrective properties of treatment for dry or greasy skin, all have the common aim of maintaining or restoring the skin to its "normal" state. At the same time, these products can also be used as complementary treatments for more classical therapy, particularly where side effects occur.

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## Self-Tanning Products

Stanley B. Levy

### INTRODUCTION

With improvements in formulation and aesthetics, sunless or self-tanning products have become a viable alternative to UV tanning. Public awareness as to the hazards of both natural and artificial UV tanning has facilitated self-tanners becoming a significant component of the overall sun care market. Ten to over 20% of adolescent and young adults in both the United States and Australia reported using these products (1–3). Individual users were also more likely to have sunburn consistent with higher use of these products in fairer caucasians. In other studies, exclusive users of sunless tanners were more likely to practice overall sun protection (4,5) and decrease their use of UVL tanning beds (6,7). A randomized trial at the beach for skin cancer prevention promoting sunless tanning reduced sunbathing and sunburns (8).

Dihydroxyacetone (DHA), a keto-sugar, is the active ingredient in sunless or self-tanners, and is responsible for darkening the skin by staining. DHA is classified in the *International Cosmetic Ingredient Dictionary and Handbook* (9) as a colorant or a colorless dye. Other similar sugars such as erythrose and glyceraldehyde are occasionally used. Products containing DHA should not be confused with bronzers intended to produce a darker color on the skin by the use of water-soluble colorants or dyes. Other agents that have been used to enhance skin pigmentation with and without stimulation by UV with varying degrees of success and toxicity (Table 19.1) will not be discussed in detail here (10,11).

### CHEMISTRY

Dihydroxyacetone ( $C_3H_6O_3$ ) is a white, crystalline hygroscopic powder. This 3-carbon sugar forms a dimer in freshly prepared aqueous solution (Figure 19.1). With heating to effect a solution in alcohol, ether, or acetone it reverts to the monomer. The monomeric form is less stable but more important in the browning reaction leading to the skin color change (12). DHA is stable between pH 4 to 6, but above pH 7 efficacy is lost with the formation of brown colored compounds. A buffered mixture at pH 5 is most stable. Heating above 38° C for long

periods of time will also effect stability. DHA needs to be stored in a cool, dry place, ideally 4° C and low atmospheric humidity (13). Glyceraldehyde, the isomer of DHA, is also present in solution. Glyceraldehyde may degrade into formaldehyde and formic acid. In acidic solution (pH 4), this isomerization and therefore these latter undesirable ingredients are minimized. Commercially available formulations generally contain 2.5% to 10% DHA (Figure 19.2).

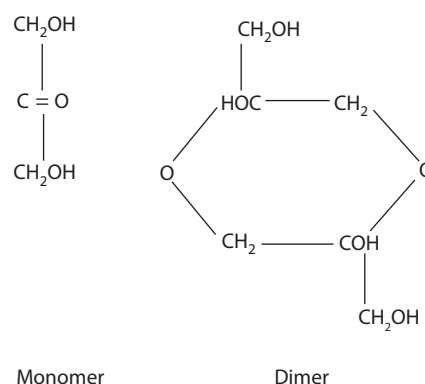
The Maillard or browning reaction has been defined as the reaction of an amino group of amino acids, peptides, or proteins with the aldehydic hydroxyl group of sugars. DHA in the context of this reaction may be considered a 3-carbon sugar, reacting with free amino groups available as amino acids, peptides, and proteins supplied by the keratin to form products or chromophores referred to as melanoidins (14). Melanoidins have some physicochemical properties similar to naturally occurring melanin (15). Electron spin resonance has shown that free radicals are produced in vivo by the Maillard reaction (16).

### MECHANISM OF ACTION

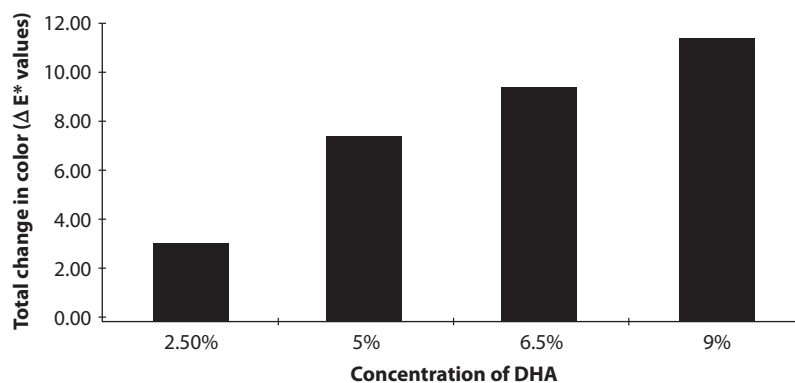
The site of action of DHA is the stratum corneum (17). Tape stripping of the skin quickly removes the color (18), as does mechanical rubbing. Deeper staining in areas with thicker stratum corneum and no staining of mucous membranes without a stratum corneum is also consistent with this being the site of action. DHA may be used as a substitute for dansyl chloride as a measure of stratum corneum turnover time (19,20). Microscopic studies of stripped stratum corneum and hair reveal irregular pigment masses in the keratin layers (21) consistent with melanoidins. These melanoidins are formed

**Table 19.1** Tanning Product Types

Product Type	Active Ingredient
Sunless or self-tanner	Dihydroxyacetone
Bronzer	Dyes
Tanning simulator	Melanins
Tanning preparation	UVB sunscreens
Tan accelerator	Tyrosine
Tanning promoter	5-Methoxypsoralen
Tanning pill	Canthaxanthin
Hormone	Synthetic analogues $\alpha$ -MSH



**Figure 19.1** Chemical structure of DHA.



**Figure 19.2** Degree of skin darkening with concentrations of DHA.

via the Maillard reaction with DHA as a sugar reacting with the amino groups supplied by the keratin.

## APPLICATION

Following application of a typical DHA containing self-tanning lotion, color change may be observed within an hour (22). This color change may be seen under Wood's light (black light) within 20 minutes. Maximal darkening may take 8 to 24 hours to develop. Individuals can make several successive applications every few hours to achieve their desired color. Color may last as long as 5 to 7 days with a single application. Depending on anatomical application, the same color can be maintained with repeat applications every 1 to 4 days. The face requires fewer applications but more frequent reapplication to maintain the color than the extremities. Depth of color varies with the thickness and compactness of the stratum corneum. Palms and soles stain deepest necessitating washing of hands after application to avoid staining. Hair and nails will color but not mucous membranes lacking a stratum corneum or keratin layer. Rougher hyperkeratotic skin over the knees, elbows, and ankles will color more unevenly as will older skin with keratoses and mottled pigmentation. Color will also be maintained longer in these areas.

As in the formulation, the pH of the skin before application may have an effect on the tonality of the skin color (12). Alkaline residues from soaps or detergents may interfere with the reaction between DHA and the amino acids on the skin surface, resulting in a less natural (more yellow) appearing color. Wiping the skin surface with a hydroalcoholic, acidic toner just prior to DHA application may improve results. Ex-vitro epidermal studies suggest that skin hydration (23) and relative humidity (24) influence the development of coloration.

Careful application is key with using these products (Table 19.2). The skin may be prepared with a mild form of exfoliation. Even application is required, with lighter application around elbows, knees, and ankles to avoid excessive darkening in these areas. Care also needs to be taken around the hairline where lighter hair may darken. Hands need to be washed immediately after use to avoid darkening of the palms, fingers, and nails. Skill and experience are necessary with using these products resulting in greater user satisfaction.

Spray-on tanning formulations may aid in providing an even application. Larger air-operator assisted delivery units are available for airbrushing on by a technician (25). Tanning

booths using sprays are now commonplace in spas and salons. This form of application introduces the potential hazard of inhalation of sprayed material.

Some formulations include colorants as used in bronzers, including dyes and caramel, to achieve an immediate effect. Similarly tinting with iron oxides or titanium titanium can provide immediate color and allow the user to more easily visualize the evenness of application. Metal oxides may, however, induce degradation of DHA (26). Vitamins, botanical extracts, antioxidants (27), anti-irritants, and even alpha hydroxy acids may be added to broaden the manufacturer's claims made with a given product. The addition of sunscreen ingredients to self-tanners warrants a more detailed discussion in the next section.

## SUNSCREEN ACTIVITY

DHA itself has at most a modest effect on SPF (28), providing perhaps SPF 3 or 4 protection. SPF increases with DHA concentration and number of applications (29). Low level SPF persists for several days, decreasing with loss of color (30). The brown color obtained on the skin does absorb in the low end of the visible spectrum with overlap into long UVA and may provide some UVA I protection (31). Melanoidins can act as free-radical scavengers as they demonstrate an electron spin resonance signal (32). Superficial skin coloration induced by frequent topical application of DHA in high concentrations delays skin cancer development in hairless mice irradiated with moderate UV doses (33).

Individuals using DHA-containing tanning products need to be cautioned that despite visible darkening of their skin, these products provide minimal sun protection. Confusion may be compounded by the addition of UV filters to the formulation providing significant sun protection. The stated SPF for the product is applicable for a few hours after application, but not for the days during which the skin color change may remain perceptible.

## INDICATIONS

Even with recent improvement in DHA formulations, the color achieved remains dependent on skin type. Individuals of medium complexion with skin phototypes II or III (34), as opposed to those who are lighter or darker, will obtain a more pleasing color. Individuals with underlying golden skin tones



**Table 19.2** Application Instructions For Self-Tanners

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Prepare skin with mechanical exfoliation
Spot test
Wipe skin with hydroalcoholic acidic toner
Apply carefully and evenly
Apply less to thicker skin
Allow to dry
Reapply regularly
Remember sun protection

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will achieve better results than individuals with a rosy, sallow, or olive complexion. Older consumers with roughened, hyperkeratotic skin or mottled pigmentation with freckling may be less pleased with their use.

Dermatologists regularly recommend these products for tanning as a safe alternative to UV exposure. They may be used to camouflage some skin irregularities such as leg spider veins. Light to medium complected patients with vitiligo who show increased contrast with the vitiliginous areas with natural or unavoidable tanning in their normal skin may also benefit (35,36). DHA provides some protection for individuals with certain photosensitivity disorders (37). Protection of uninvolved skin by DHA during psoralen-UVA (PUVA) treatment allows higher UVA exposures to be tolerated, with fewer treatments resulting in faster clearing, known as Turbo-PUVA (38).

## SAFETY

The visible color change associated with the use of artificial tanning products might suggest to some users that these products are hazardous. Based on the chemistry of DHA and its toxicological profile, it can be considered nontoxic. It reacts quickly in the stratum corneum, minimizing systemic absorption. The acute toxicity of DHA was investigated for diabetics in the 1920s, with oral intake well tolerated (39). The phosphate of DHA is found naturally as one of the intermediates in the Krebs cycle. Toxicity based on inhalation in closed spray-on tanning booths is unknown.

Contact dermatitis to DHA has only rarely been reported (40,41). As with other topical products with active ingredients, such as sunscreens, much of the reported sensitivity is secondary to other ingredients in the vehicle (42). Adverse reactions are more likely to occur on the basis of irritation and not true allergy. Ultimately all claims related to product safety are based on testing the final formulation.

Although not approved by regulatory agencies, some of the alternative agents for increasing skin pigmentation (Table 19.1) are available to individuals. Tanning pills containing carotenoids such as canthaxanthin have been reported to cause retinopathy, urticaria, hepatitis, and aplastic anemia (43). More recently, injections of analogues of melanocyte-stimulating hormone may be gaining in popularity (44). Of potential benefit to individuals with photosensitive disorders (45), synthetic analogues of  $\alpha$ -MSH may drive proliferation of neoplastic melanocytic cells in the nevi of predisposed individuals (46).

## CONCLUSION

Increasing awareness as to the hazards of UV light should fuel ongoing interest in self-tanning products. It is incumbent on dermatologists to be familiar with this category. The benign toxicologic profile of DHA reinforces the notion that these

products represent a safe alternative to a UV-induced tan. The results obtained are dependent on the final formulation, individual application technique, and the consumer's skin type. Greater experience in formulation combined with increasing sophistication on the part of the consumers has led to increasing satisfaction with the use of these products.

Users need to be clearly informed that these products do not offer significant protection against UVB. If formulated with standard sunscreens, individuals should be cautioned that the duration of UV protection is more short-lived than the color change.

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# Astringents, Masks, and Ancillary Skin Care Products

Zoe Diana Draelos

## INTRODUCTION

Ancillary skin care encompasses products designed to supplement basic cleansing and moisturizing of the skin. Frequently, these products are recommended as part of a skin care routine designed to impart benefits above and beyond basic hygiene needs or maintenance of the skin barrier. Ancillary skin care products include astringents, exfoliants, facial scrubs, epidermabrasion, textured cloths, mechanized skin care devices, and face masks. This chapter discusses these ancillary skin care products and their dermatologic value.

## ASTRINGENTS

Astringents are liquids applied to the face following cleansing. They comprise a broad category of formulations known by many terms: toners, clarifying lotions, controlling lotions, protection lotions, skin fresheners, toning lotions, T-zone tonics, etc. Originally, astringents were developed to remove alkaline soap scum from the face following cleansing with lye-based soaps and high-mineral content well water. The development of synthetic detergents (syndets) and public softened water systems greatly decreased the amount of post-washing residue. A new use for astringents was found when cleansing cream became a preferred method of removing facial cosmetics and environmental dirt. The astringent then became an effective product for removing the oily residue left behind following cleansing cream use.

Astringent formulations are presently available for all skin types (oily, normal, dry, sensitive, photoaged, etc.), with a variety of uses (1). Oily skin astringents contain a high concentration of alcohols, water, and fragrance functioning to remove any sebum left behind following cleansing to produce a clean feel and to possibly deliver some treatment product to the face. For example, 2% salicylic acid or witch hazel may be added for a keratolytic and drying effect on the facial skin of acne patients. Clays, starches, or synthetic polymers may be added to absorb sebum and minimize the appearance of facial oil. Astringents for normal skin are generally formulated to give the skin a clean, fresh feeling without much dryness. They may contain propylene glycol to function as a humectant, a water-attracting mild moisturizing agent.

Products formulated for dry or sensitive skin are alcohol-free and are based on lightweight occlusive moisturizers, such as silicone (dimethicone, cyclomethicone). In addition, soothing agents such as allantoin, guaiazulene, and quaternium-19 may be added. The newest type of astringents are those designed for photoaged skin that contain salicylic acid ( $\beta$ -hydroxy acid) or glycolic acid ( $\alpha$ -hydroxy acid) to aid in keratinocyte exfoliation and achieve smoother, more evenly pigmented skin.

## EXFOLIANTS

Exfoliants are solutions, lotions, or creams applied to the face following cleansing designed to hasten stratum corneum exfoliation. They are similar in function to the antiaging astringents previously discussed. Their exfoliant effect is based on the use of  $\alpha$ -, poly-, or  $\beta$ -hydroxy acids, thus inducing chemical exfoliation.

Exfoliants containing hydroxy acids produce both epidermal and dermal changes. The epidermal changes are immediate and occur at the junction of the stratum corneum and stratum granulosum. They consist of a reduction in the thickness of the hyperkeratotic stratum corneum due to decreased corneocyte adhesion (2). The dermal effects, which are delayed, consist of increased glycosaminoglycan synthesis (3). These effects are most pronounced with the  $\alpha$ -hydroxy acids (glycolic acid, lactic acid, malic acid), which rapidly penetrate the epidermis to enter the dermis. Individuals with sensitive skin may not be able to tolerate the low pH of 3 required to cause this epidermal renewal (4). This has led to development of polyhydroxy acids (gluconolactone, lactobionic acid, ferulic acid), which are larger molecular weight hydroxy acids that do not penetrate as rapidly to the dermis. This produces less irritation allowing polyhydroxy acid use by persons with sensitive skin, eczema, and atopic dermatitis.

Another mechanism for reducing the irritation of chemical exfoliants is through neutralization or buffering. Irritation can also be minimized by raising the exfoliant pH through sodium hydroxide neutralization; however this also reduces the exfoliation produced. The use of buffering agents, such as phosphoric acid or monosodium phosphate, is preferable since the buffer maintains the product at a desired pH (5). Ideally, the pH of an exfoliant solution should not be lower than 3. More exfoliation is induced with lower pH, since the hydroxy acid concentration is increased, but more irritation in the form of stinging and burning is also expected.

$\beta$ -Hydroxy acids, such as salicylic acid, may also be used, but do not produce dermal penetration. Salicylic acid is technically not a  $\beta$ -hydroxy acid, but rather a phenolic compound, but the marketing nomenclature has popularized this terminology. Salicylic acid is an oil-soluble acid, as compared to the  $\alpha$ -hydroxy acids that are mainly water soluble, and remains on the skin surface. Since exfoliation occurs on the skin surface, this is a desirable characteristic that minimizes irritation. Salicylic acid is also able to exfoliate within the follicular ostia, making it the exfoliant of choice in acne patients as well as sensitive skin patients. Salicylic acid is listed on the acne monograph and functions as a keratolytic in many over-the-counter acne preparations.

## FACIAL SCRUBS

Facial scrubs are mechanical exfoliants, as opposed to the chemical exfoliants previously discussed, employing small granules in a cleansing base to enhance corneocyte desquamation. The scrubbing granules may be polyethylene beads, aluminum oxide, ground fruit pits, or sodium tetraborate decahydrate granules aiding in the removal desquamating stratum corneum from the face (6). Sibley et al. considered abrasive scrubbing creams effective in controlling excess sebum and removing desquamating tissue (7). However, they can cause epithelial damage if used too vigorously. This view is held by Mills and Kligman, who noted that the products produced peeling and erythema without a reduction in comedones. Aluminum oxide and ground fruit pits provide the most abrasive scrub because of their rough-edged particles, followed by polyethylene beads, which are smoother and produce less stratum corneum removal. Sodium tetraborate decahydrate granules become softer and dissolve during rubbing, providing the least abrasive scrub.

A currently popular trend in facial exfoliant scrubs is the production of warmth. These products are labeled as “self-heating” scrubs. The heat is produced as part of an exothermic reaction resulting in the heat by-product. The heat does not increase exfoliant efficacy but is added for consumer comfort and marketing purposes. Sometimes these heated exfoliant scrubs are preceded by a self-administered hydroxy acid peel, thus combining both chemical and physical exfoliation into one kit.

## Epidermabrasion and Textured Cloths

Another mechanical method of enhancing stratum corneum exfoliation has been labeled epidermabrasion by Durr and Orentreich, who examined the use of a nonwoven polyester fiber web sponge for the removal of keratin excrescences and trapped hairs in pilosebaceous ducts (8–10). Other epidermabrasion implements include rubber puffs, sea sponges, loofahs, and the most recent addition, textured fibered face cloths. The fibered face cloths have become a large segment of the current epidermabrasion marketplace and therefore are discussed in detail.

Fibered cloths are extremely versatile dermatologic devices. They can be premoistened and impregnated with surfactants to cleanse the face, be perfumed containing volatile solvents to freshen the face, be packaged dry with lipids and detergents to clean the face, be covered with a plastic film pouch with microscopic holes to time-release an active ingredient onto the skin surface, and be textured with patterns to physically exfoliate the skin. Even though many of the facial uses of fibered cloths are new, the cloths have been around for 30 years.

The first fibered cloths were introduced as baby wipes. They were made from carded rayon fibers that were held together by adhesive binders. They exhibited wonderful strength for their thickness, but were rather coarse and a frequent cause of irritant contact dermatitis due to both the surfactants employed and the rough cloth texture. The need for a strong but soft cloth led to development of air-laid nonwoven fibered cloths in the mid-1970s. These cloths were composed of wood pulp, polyester, and adhesive binders. They were thicker and softer. The technology was further developed in the 1980s by adding both cotton and rayon fibers to improve strength.

Modern fibered cloth technology focused on creating a soft wipe with excellent strength to prevent tearing. The fibers used are a combination of polyester, rayon, cotton, and cellulose held together via heat through a technique known as thermobonding. Additional strength is imparted to the wipe by

hydroentangling the fibers. This is achieved by entwining the individual rayon, polyester, and wood pulp fibers with high-pressure jets of water. Thermobonding and hydroentangling have eliminated the use of adhesive binders, thereby creating a soft, strong cloth suitable for facial use.

Face cloths are available both dry and moist. The dry packaged cloths are impregnated with a cleanser that foams modestly when the cloth is water moistened. The type of cleanser in the cloth can produce aggressive sebum removal for oily skin or minimal sebum removal for dry skin. Humectants and emollients can also be added to the cloth to decrease barrier damage or to smooth xerotic skin scale. Thus, face cloths can be designed for normal to oily skin, normal to dry skin, or sensitive skin.

In addition to the composition of the ingredients preapplied to the dry cloth, the weave of the cloth will also determine its cutaneous effect. There are two types of fiber weaves used in facial products: open weave and closed weave. Open weave cloths are so named because of the 2- to 3-mm windows in the cloth between the adjacent fiber bundles. These cloths are used in persons with dry and/or sensitive skin to increase the softness of the cloth and decrease the surface area contact between the cloth and the skin, yielding a milder exfoliant effect. Closed weave cloths, on the hand, are designed with a much tighter weave and are double-sided. One side of the closed weave cloth is textured and impregnated with a synthetic detergent cleanser designed to optimize the removal of sebum, cosmetics, and environmental dirt while providing an exfoliant effect. The opposite side of the cloth is smooth and designed for rinsing the face and possibly applying skin conditioning or antiaging agents. Some of the newer cloths contain a central petrolatum strip designed to leave behind a moisturizer on the skin during the rinse process.

The texture of the cloth provides gentle mechanical exfoliation that may be valuable in the patient who cannot tolerate chemical exfoliation with hydroxy acids. The mechanical exfoliation can be achieved on the skin surface and around the follicular ostia because of the ability of the textured cloth to traverse the irregular topography of the skin more effectively than the hands or a washcloth. The degree of exfoliation achieved is dependent on the cloth weave, the pressure with which the cloth is stroked over the skin surface, and the length of time the cloth is applied.

Figure 20.1 provides a demonstration of the amount of facial foundation left behind on the skin after cleansing with a variety of different techniques. The pads contain unremoved cosmetic. Notice that there is a small amount of cosmetic on the pad after using either a closed weave or an open weave cloth. More cosmetic is left behind after soap cleansing and even more after using a lipid-free cleanser. This illustration demonstrates the cleansing attributes of a face cloth.



**Figure 20.1** Cleansing cloth: facial foundation removal; (a) normal/dry; (b) normal/oily; (c) syndet bar; (d) lipid free.





**Figure 20.2** An example of a currently marketed handheld reusable cleansing pad for facial use.

### Handheld Reusable Textured Cleansing Pads

The new environmental concerns regarding disposable cleansing cloths has led to a revival of the old-fashioned cleansing pad. Originally, these were textured flat rubber brushes with a ring on the back for middle finger insertion. The pad had numerous tiny rubber bristles to which soap was applied and the face was scrubbed with finger pressure on the back of the pad. Similar devices were in vogue for cleansing the male scalp. These pads have seen a revival in the form of a flexible silicone pad with numerous carefully designed fingers that are stroked over the face (Figure 20.2). The back of the pad contains a suction cup to adhere the pad to the mirror or shower door for easy use. These pads are reusable and designed to allow the cleansing fingers to adequately clean the skin in and around the pores without injury.

### MECHANIZED SKIN CARE DEVICES

Mechanization of the epidermabrasion process is known as microdermabrasion. This is a procedure performed by estheticians and paramedical personnel where small particulates, such as aluminum, silica, and baking soda, are sprayed against the skin surface and simultaneously removed with a vacuum. Microdermabrasion simply represents another technique to induce stratum corneum exfoliation, a natural body process that slows with advancing age.

A variety of devices are available to exfoliate the facial skin. These include rotary brushes that drag synthetic bristles across the skin surface to physically remove the stratum corneum. These devices are sold with a special cleanser to remove sebum and clean the bristles simultaneously. A variant of this technology used scrubbing pads of various roughnesses to produce exfoliation. The scrubbing pads were held on the device head with adhesive and could be replaced when worn. These devices vibrated instead of rotating to remove skin scale.

A third type of facial cleansing device produces a sonicating motion, similar to the sonicating electric toothbrushes. The handheld device runs on a rechargeable battery that is attached

to a miniaturized motor creating an oscillatory motion of the brush head. This oscillatory sonic motion allows the brush bristles to traverse the dermatoglyphics, facial pores, and facial scars more adeptly than other mechanized cleansing methods.

### FACE MASKS

Face masks are the last ancillary skin care product discussed and consist of substances applied to the face for an extended time period for therapeutic and/or esthetic purposes. Masks are available for home purchase and professional use. They may be packaged in a jar or bottle for immediate application to the face or as dry ingredients in a pouch for mixing with water. Some premixed masks are applied to a disposable cloth, similar to the cleansing cloths previously described. The cloth is shaped to fit over the face or body area and is removed from the pouch moistened with the ingredients and ready for application. Once the liquids have evaporated from the mask, it is removed and discarded. These instant masks are very popular in the Orient, but are quickly gaining popularity around the world for their easy use and efficiency. Typically, a mask is applied on a weekly basis to provide a time for relaxation, an esthetically pleasing sensation, and skin benefits. There are four basic mask formulations: wax-based, vinyl or rubber-based, hydrocolloid, and earth-based.

#### Wax Masks

Wax masks are popular among women who visit professional spas for their warm, esthetically pleasing feel. They are composed of beeswax or, more commonly, paraffin wax to which petroleum jelly and cetyl or stearyl alcohols have been added to provide a soft, pliable material for facial application with a soft brush. The wax is heated in a pot placed in a water bath to control the temperature and prevent burning. Sometimes the wax is dipped from the pot and painted over the face and other times it may be brushed over thin cotton gauze draped over the face. Gauze is commonly used to enable the facial technician to remove the wax in one piece (11). Gauze also prevents the wax from sticking to the vellus hairs on the face, which may be painfully epilated as the wax is peeled from the face.

Wax-based face masks temporarily impede cutaneous transepidermal water loss. This effect is limited only to the time the mask is in direct contact with the face, unless a suitable occlusive moisturizer is applied immediately following mask removal. For this reason, they are popular in persons with dry skin.

#### Vinyl and Rubber-Based Masks

Vinyl and rubber-based masks are popular masks for home use, since they are easily squeezed from a pouch onto the face and removed in one piece. Rubber-based masks are usually based on latex, while vinyl-based masks are based on film-forming substances, such as polyvinyl alcohol or vinyl acetate. Because of the concern over latex allergy, there are no true rubber-based masks for home use.

Vinyl masks are squeezed premixed from a tube or pouch and applied with the fingertips or a wooden applicator to the face. Upon evaporation of the vehicle, a thin flexible vinyl film remains behind on the face. The mask is generally left in contact with the skin for 10 to 30 minutes and then peeled in one sheet by loosening the edges from the face.

Vinyl and rubber masks are appropriate for all skin types. The evaporation of the vehicle from the wet mask creates a cooling sensation, and the shrinking of the mask with drying may give the impression that the skin is actually tightening.



These masks can temporarily impede transepidermal water loss while they are in contact with the skin.

### Hydrocolloid Masks

Hydrocolloid masks are used both in professional salons and at home. Hydrocolloids are substances such as oatmeal that are of large molecular weight and thus interfere with transepidermal water loss. These masks are formulated from gums and humectants and enjoy tremendous popularity since many specialty ingredients are easily incorporated into their formulation. They are marketed in the form of dry ingredients in a sealed pouch that must be mixed with warm water prior to application. The resulting paste is then smeared over the face with the hands or a wooden blade and allowed to dry (12).

Hydrocolloid masks leave the skin feeling smooth and create the sensation of skin tightening as the water evaporates and the mask dries. Temporary moisturization can occur while the mask is on the skin. Specialty additives such as honey, egg whites, chamomile flowers, aloe vera, almond oil, zinc oxide, sulfur, avocado, and witch hazel may be used to customize the mask. Many spas have their own special concoction. By varying the ingredients, masks can be created for all skin types. In addition, herbal medicine can be practiced by combining various healing plants into a poultice for facial application. These hydrocolloid ingredients are well suited for the instant mask application previously described for home use.

### Earth-Based Masks

Earth-based masks, also known as paste masks or mud packs, are formulated of absorbent clays such as bentonite, kaolin, or china clay. The clays produce an astringent effect on the skin making this mask most appropriate for oily-complexed patients. The astringent effect of the mask can be enhanced through the addition of other substances such as magnesium, zinc oxide, and salicylic acid.

### SUMMARY

This chapter has discussed the various ancillary skin care products for purchase in the current marketplace. Astringents represent a broad category and may impart both cleansing and moisturizing effects to the skin, depending on formulation and skin type. Exfoliants, which became popular when glycolic acid was introduced to the antiaging marketplace, can contain both chemical and physical exfoliating ingredients to enhance desquamation of the stratum corneum. Physical exfoliating agents are commonly packaged as particulate facial

scrubs, woven sponges, or textured cloths. Textured cloths and reusable silicone cleansing pads are the newest introduction and can function like washcloths or may leave behind ingredients on the skin surface. Mechanized skin care devices attempt to deliver at-home microdermabrasion with rotary, vibrating, or sonicating motors. Finally, face masks deliver both esthetic and skin care benefits in a professional or home environment.

Ancillary skin care is an interesting area that combines devices and products to offer innovative solutions to skin care. These products can be used alone or in combination to deliver exfoliant, antiaging, moisturizing, astringent, or aesthetic benefits. Most of the new introductions in the commercial skin care market have occurred in this arena, since the profit margin is high and consumers are now focusing on cleansing as a way of enhancing the skin appearance. While cleansing has traditionally been a method to obtain good skin hygiene and minimize infection, a new concept in skin care is the expansion of cleansing beyond soap and water to include astringents, exfoliants, facial scrubs, epidermabrasion, textured cloths, mechanized skin care devices, and face masks.

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## Regulatory Overview of Cosmeceuticals

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Cosmeceuticals are an intriguing and burgeoning field within dermatology and the skin care market. This industry was estimated to have generated approximately \$8.2 billion in sales in 2012 (1). Furthermore, researchers focusing on cosmeceutical products highlight strong growth perspectives in the coming years with a rapid compound annual growth rate of 7.7% and the global cosmeceutical market reaching \$42.4 billion by 2018 (2). While consumers in the United States spend more on cosmeceutical products than all of Europe combined, consumers in France and Germany spend the most within Europe (3). Although there is no strict definition for cosmeceuticals within the skin care industry or dermatology realm (4), they are typically considered cosmetic products with components that have “drug-like” benefits and properties. Examples of cosmeceuticals include moisturizers, serums, topical antioxidants, retinoids, peptides, and botanicals. As a category, they are believed to contain either one or a mix of ingredients that improve skin condition and appearance without making an explicit assertion on skin health. In addition to their aesthetic properties which make them desirable for both consumers and patients, development of cosmeceutical products has several economical advantages, as the process of marketing them is simpler and they require less monetary investment than a drug, which may require from \$800 million to upward of \$1 billion (5).

### REGULATORY OVERSIGHT

The term “cosmeceuticals” is derived as an amalgam of cosmetics and pharmaceuticals. The Food and Drug Association (FDA) does not formally acknowledge the term “cosmeceutical” but notes that the term is used by the cosmetic industry to refer to cosmetic products that have medicinal or drug-like benefits (6).

It is necessary to distinguish between cosmetics, drugs, and cosmeceuticals. Cosmetics are defined as either (1) articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, or (2) articles intended for use as a component of any such articles. Cosmetics are recognized by the FDA, however approval is not required for marketing (6).

Drugs are defined as (1) articles recognized in the official United States Pharmacopoeia, official Homoeopathic Pharmacopoeia of the United States, or official National Formulary, or any supplement to any of them, (2) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals, (3) articles (other than food) intended to affect the structure or any function of the body of man or other animals, and (4) articles intended for use as a component of any article specified in

clause (1), (2), or (3). Drugs are recognized by the FDA and unlike cosmetics, approval is required for marketing (6).

Cosmeceuticals are cosmetic products that have medicinal or drug-like benefits. They are not recognized by the FDA and approval is not required for marketing if pharmaceutical claims are not made (5).

In the United States, regulatory oversight for the cosmeceutical industry is primarily provided by the FDA. As long as no claims that would meet criteria for evaluation as a drug are met, cosmeceuticals are regarded as cosmetics for regulatory purposes. The FDA does not possess any legal authority in the approval of cosmetic products before they are marketed, but if a cosmetic product is determined to be misbranded or adulterated, they are able to take legal action. Thus, while cosmeceuticals may contain drug-like benefits, there is no regulatory approval mechanism insofar as the cosmeceutical does not make a claim that would qualify it as a drug.

The regulation of cosmeceuticals is not well defined outside the United States. For example, in Europe, regulatory authorities consider many cosmeceuticals as cosmetics (3) while in Japan, they are treated as quasi-drugs (3). Even more obscured is the process in India, where multiple regulatory bodies may be involved without guidelines on product aims for cosmetics and the term “cosmeceutical” is not included in official legal definitions (3).

### THE ROLE OF BOTANICALS

Botanicals have an expanding role in cosmeceuticals due to the rapidly growing demand for the use of complementary and alternative medical therapies (7). Specifically, several studies have evaluated the ability of whole botanical extracts and specific phytochemicals to modulate cellular functions (8–10). In this regard, botanicals may have valuable properties in addition to a cosmetic purpose. Yet despite these encouraging data, official claims need to remain guarded to be marketed as cosmeceuticals rather than a drug.

Some botanical products have blurred the boundaries of cosmeceuticals and drugs. For instance, two botanically derived dermatological preparations have received FDA approval as prescription drugs. One is an extract of *Camellia sinensis* (green tea) composed of sin catechins that is used for the topical treatment of external genital and perianal warts (11). The second is a purified proanthocyanidin extracted from the South American tree *Croton lechleri* for the treatment of diarrhea associated with anti-HIV drugs (12).

In recent years, there has been greater demand for “organic” cosmeceuticals as a result of the growing demand for natural and botanical alternatives. In the United States, the standards for “organic” are not set by the FDA but by the United States Department of Agriculture (USDA). The FDA

does not have a role in regulating, reviewing, or enforcing these standards. Depending on the country of origin, there are several regulatory bodies that provide oversight on the “organic” distinction, as outlined in Table 21.1.

While the FDA regulates cosmetics under the authority of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and the Fair Packaging and Labeling Act (FPLA), the term “organic” is not defined in either of these laws. Rather, the Agricultural Marketing Service of the USDA, which oversees the National Organic Program (NOP), regulates the term “organic.” As outlined in Table 21.1, the NOP regulations include a definition of “organic” and provide for certification that agricultural ingredients have been produced under conditions that would meet the definition.

The USDA requirements for the use of the term “organic” are separate from the laws and regulations that FDA enforces for cosmetics. Cosmetics, body care products, and personal care products labeled with organic claims must comply with both USDA regulations for the organic claim and FDA regulations for labeling and safety requirements for cosmetics.

Importantly, cosmetics made with “organic” ingredients are not necessarily safer for consumers than those made with ingredients from other sources. Under the FD&C Act, all cosmetic products and ingredients are subject to the same safety requirement: they must be safe for consumers under labeled or customary conditions of use. Companies and individuals who market cosmetics have a legal responsibility to ensure that their products and ingredients are safe for the intended use.

Notably, the USDA has no authority over the production and labeling of cosmetics, body care products, and personal care products that are not made up of agricultural ingredients or do not make any claims to meeting USDA organic standards (13).

In Europe, the Soil Association is the principal body involved in the inspection and organic certification of health and beauty products. With regard to the certification process of non-food items such as health and beauty products, the Soil Association issues an annual certificate of registration, a trading schedule, and license to use the Soil Association organic symbol once the producer or operator has fulfilled the necessary standards of product safety and integrity (14).

The standards set forth by the Soil Association apply to health and beauty products made from organic ingredients, and include herbal products, natural and herbal medicine-like products, toiletries, body care products, and cosmetics and perfumery (14). In addition, organic health and beauty products are required to fulfill the following principles: be fit for their purpose, have as high as possible proportion of organic ingredients, be clearly identified, traceable and separate from non-organic products at all stages of manufacturing, not be tested on animals, not be harmful to human health and the environment in manufacture and use, be produced in line with ethical trade standards, and be labeled to give clear and accurate information to the consumer (14).

The Soil Association, along with several other European organizations, collaborated and introduced a new unified standard for organic health and beauty products, the COSMOS standard, effective since 2015. This standard introduced the concept of “green chemistry,” defined as a desire within the cosmetics sector to contribute to sustainable development. This principle extends to the requirements expected of manufacturers who produce the chemically processed ingredients used in cosmetic products. For example, such manufacturers are restricted to a certain list of chemical processes, must use renewable resources, and must comply with specific quantitative requirements for their ingredients (14).

## COSMETIC LABELING STANDARDS

The FDA does not have the resources nor authority under the law for pre-market approval of cosmetic product labeling. It is the manufacturer's and/or distributor's responsibility to ensure that products are labeled properly. Importantly, organic cosmetics cannot be labeled with any therapeutic claims. Any assertion that a product treats or prevents disease or affects the structure or function of any part of the body may cause the product to be considered a drug.

Labeling information that is required to appear on the principal display panel (the part of the label most likely displayed or examined under customary conditions of display for sale) includes an identity statement and an accurate statement of the net quantity of contents. An identity statement indicates the nature or use of the product (by means of either

**Table 21.1** Organic Labeling Standards of International Regulatory Bodies

Regulatory body			
<b>United States Department of Agriculture National Organic Program (United States)</b>	<b>Label</b>	<b>Requirement</b>	<b>May Display USDA Organic Seal?</b>
	100% Organic	100% organic ingredients	YES
	Organic	95% organic ingredients	YES
	Made with organic ingredients	70% organic ingredients	NO
	No organic distinction allowed	<70% organic ingredients	NO
<b>COSMOS (Europe)</b>	<b>Label</b>	<b>Requirement</b>	
	ORGANIC	1) 95% of a product's “agro-ingredients” and 20% of the entire product must be organic 2) For products that are less than 95% organic, the label may make reference to individual organic ingredients	
	NATURAL	1) No minimum level of organic ingredients required but the label must report organic ingredients on the ingredient list 2) May report % of organic ingredients to total product or total product minus water and minerals 3) Must not make any organic claim of products or ingredients on the front packaging label	

the common or usual name, a descriptive name, a fanciful name understood by the public, or an illustration) and the net quantity of contents can be displayed in terms of weight, measure, numerical count, or combination of numerical count and weight or measure.

Additionally, the following must appear on the information panel (a panel other than the principal display panel that can accommodate label information where the consumer is likely to see it) of the product: name and place of business (e.g. manufacturer, packer, or distributor), distributor statement, material facts (e.g. directions for safe use if a product could be unsafe if used incorrectly), warning and caution statements, and ingredients.

## CONCLUSION

While cosmeceuticals are extensively used and marketed and their popularity will undoubtedly continue to soar, with regulatory overview still evolving they do not yet have a widely accepted definition (4). Hence it is the responsibility of the skin care provider as well as the consumer to understand and critically evaluate the evidence (15). Additionally, because organic regulatory distinctions may be especially confusing for consumers, skin care providers should be familiar with the topic.

For today's consumer, cosmeceuticals offer numerous new potentially exciting and favorable skin care products and options, and the interest and expansion in botanically derived products has significantly increased these choices. However, despite this surge in enthusiasm, the multitude of choices can also be confusing. As the cosmeceutical industry continues to expand, including those products that are "natural" and "organic," there will be a greater need to monitor marketing claims and the evidence behind them. Furthermore, with the well-being of the consumer and patient in mind, the safety of these products will need to be evaluated closely (16).

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بزشک آموز



# Photodamage: Protection

Laurent Meunier

## INTRODUCTION

The spectral distribution of the solar energy at the sea level comprises roughly 3%–7% of ultraviolet radiation (UVR) (290–400 nm), 44% of visible light (400–700 nm), and 53% of infrared (IR) radiation (700–1440 nm). Terrestrial solar UVR comprises approximately 5% UVB (280–320 nm) and 95% UVA (320–400 nm), the majority (75%) of which is UVA1 (340–400 nm). Seventy percent of UVB radiation that reaches the skin is absorbed by the stratum corneum, 20% reaches viable epidermis, and only 10% penetrates the upper part of the dermis. On the other hand, UVA radiation is partly absorbed by the epidermis, but 20%–30% of it reaches deep dermis. The major chromophores that determine the depth of penetration are nucleic acids, aromatic amino acids, and melanin. Energy from the shorter-wavelength UVB is absorbed in greater amounts by the epidermis and by keratinocyte DNA, compared with the energy from UVA, which penetrates more deeply into the dermal layers of the skin. Visible and IR light wavelengths penetrate deep into the dermis and produce heat following absorption.

## UVR AND PHOTODAMAGE MECHANISMS

UVR photodamage occurs through direct and indirect mechanisms. Direct absorption of UV energy drives biochemical reactions that result in molecular changes and production of reactive oxygen species (ROS). UVA wavelengths, which penetrate deeply into skin, induce formation of ROS, which act as mediators of indirect photodamage resulting in oxidative stress and adduct formation of biomolecules. DNA has absorption maxima in UV wavelength range and is therefore a direct target of UV damage, forming cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs). These DNA photoproducts are mainly induced by UVB, but with some contribution from UVA (1,2). They interfere with biological processes and induce signals that inform cellular decisions for entry into one of two main pathways: cell survival through initiation of cellular repair processes or cell death by apoptosis (3). ROS produced in the skin following UV irradiation are key mediators of oxidative damage to the skin. Cell damage from UV also occurs through peroxidation of membrane lipids via generation of lipid peroxides. UV irradiation results in the rapid depletion of several endogenous skin enzymes and antioxidants such as glutathione reductase and catalase. Exposure to UV radiation suppresses the immune response, and UV-induced immune suppression is a major risk factor for skin cancer induction (4). Multiple mechanisms are involved depending upon the doses and the type of irradiation (acute or chronic): DNA damage, production of cytokines, and inflammatory mediators (5). UV exposure affects antigen-presenting cell function and induces immune tolerance in part through the induction of regulatory T cells (6).

## SOLAR EXPOSURE: SHORT- AND LONG-TERM EFFECTS

Erythema (sunburn) is the most familiar symptom associated with UVR overexposure. The erythema reaction to UVR depends on the wavelength range, and increasing wavelength decreases the erythema effectiveness considerably. UVB, particularly at 307 nm, is the most effective waveband for eliciting a sunburn reaction, while UVA radiation is 1000-fold less potent in producing skin erythema. UVB-induced erythema reaches a peak at 6–24 h depending on the dose, and fades over a day or longer. UVA-induced erythema contributes to at least 15% of total sun-induced erythema. The minimal erythema dose (MED) is defined as the smallest UV dose that produces perceptible redness of the skin with clearly defined borders at 16–24 h after UV exposure.

UVA-induced changes in color begin with an immediate pigment darkening (IPD) of the skin due to photooxidation of preexisting melanin, and a partial fading occurs rapidly within 1 h after the end of exposure. Following exposure to UVA, a stable residual pigmentation is observed after the transient part of IPD has faded out. This pigmentation (persistent pigment darkening or PPD), due also to melanin photooxidation, remains detectable for a few days or weeks, depending on the UVA dose. The delayed pigmentation is characterized by a visible brown pigmentation in UV-exposed skin, which represents an increase in epidermal melanin content. It becomes visible after about 72 h. An acute erythemogenic dose of UVB is necessary to induce delayed pigmentation. Both UVA and UVB can cause tanning, but UVA is less effective. UVB-induced pigmentation can bring some protection but UVA pigmentation is not protective.

The hallmarks of long-term exposure to solar UVR are photoaging and photocarcinogenesis. Photoaging is characterized by the induction of extracellular matrix-degrading proteolytic enzymes (matrix metalloproteinases [MMPs]), a family of zinc-dependent endopeptidases secreted by epidermal keratinocytes and dermal fibroblasts, without a parallel induction of inhibitors of proteolysis (tissue inhibitor of metalloproteinases). The resulting pathological remodeling process involves the degradation of collagen and the accumulation of abnormal elastin in the superficial dermis, resulting in the characteristic changes of solar elastosis. Exposure to UV has been shown to induce proinflammatory cytokines, such as IL-1 $\alpha$ , and MMPs in keratinocytes and fibroblasts. The mechanisms by which UVR induces MMPs are poorly understood. This may occur via the generation of ROS or via the formation of CPDs. Induction of inflammatory cytokines by UV, leading to overall skin inflammation is another significant contributor to the photoaging process in skin. It is widely considered that UVA has a larger role than UVB in photoaging, both because of the

deeper penetration of UVA into the dermis and because of the sensitivity of fibroblasts to UVA-induced MMPs.

Occupational and recreational exposure to solar radiation represents a major environmental risk factor for the development of skin cancers. Epidemiological studies have shown associations between melanoma incidence and intermittent exposure to solar light during childhood and adolescence. In contrast, squamous cell carcinoma (SCC) is related to cumulative solar exposure. Similar to melanoma and in contrast to SCC, sporadic basal cell carcinoma (BCC) may occur in individuals with intermittent extreme UV exposure behavior (7). However, published epidemiological literature indicates that outdoor workers are at significantly increased risk for BCC (8).

Sunlight overexposure is involved in increasing the risk of skin cancer since DNA represents one of its biological targets. Indeed, DNA alteration can affect many cellular functions and can lead to mutations and genetic instability. Unlike UVB, which directly impacts DNA, UVA toxicity mainly depends on indirect mechanisms in which ROS are generated through the activation of endogenous photosensitizers present in skin, triggering the genotoxic effects.

Malignant melanoma of the skin (cutaneous malignant melanoma [CMM]) is associated with solar exposure, but the mechanisms involved are still unclear. By using an animal model, Noonan and colleagues (9) recently found that melanoma induction by UVA requires the presence of melanin pigment and is associated with oxidative DNA damage within melanocytes, while UVB initiates melanoma in a pigment-independent manner associated with direct UVB DNA damage.

## PREVENTION OF PHOTODAMAGE

### Sunscreens

The use of sunscreens should be considered as a part of photoprotection and should always be associated to appropriate physical measures (shade, clothing, hats, sunglasses) especially in children. An ideal sunscreen should provide uniform protection across the range of UVB and UVA, a property which assures that the natural spectrum of sunlight is attenuated in a uniform manner. It should also have aesthetically pleasing composition that enhances compliance (10). Sunscreens are composed of excipients and filters that are categorized as organic or inorganic (chemical or physical). Organic (chemical) UV filters act by absorbing UV radiation and most of them are photostable under the conditions of use. The sun protection factor (SPF) and the UVA protection factor (UVA-PF) are the two common indices used to quantify the efficacy of sunscreens. The measurement of SPF is performed *in vivo* using a panel of volunteers. Sunscreen is applied to the protected test sites at a density of 2 mg/cm<sup>2</sup> and SPF is then calculated as the ratio of MED on sunscreen-protected skin to that on unprotected skin. Methods for assessment of UVA protection vary by country. Persistent pigment darkening (PPD) measures the minimal UVA radiation dose required to induce the first perceptible pigmentation changes (ie, minimal pigmenting dose) in sunscreen-protected skin compared to unprotected skin. The EU requires UVA-PF to be at least one-third of the labeled SPF. For example, a sunscreen with a SPF of 30 must have a UVA-PF of at least 10. In the UK, the ratio of UVA absorbance to mean UVB absorbance is measured *in vitro*; a star rating system is used. Australia adopted the *in vitro* test procedure ISO 24443:2012 for determining broad-spectrum performance, which is similar to the European assessment. In Europe and

in the United States, recommendations include the determination of the critical wavelength (CW). The CW test is conducted by applying the test product to polymethylmethacrylate plates and then by measuring UV transmittances 290–400 nm. CW is defined as the wavelength at which 90% of the total area under the absorbance curve occurs. The broad-spectrum status is admitted when the CW is superior to 370 nm.

Correct use, appropriate amount of sunscreen applied, and reapplication frequency are important factors for the effectiveness of sunscreens (11). In practice, most people often apply only 25%–50% of the amount used for SPF testing. This results in an effective SPF that is less than 33% of the labeled SPF. Recently, a modification of the “teaspoon rule” for sunscreen application has been proposed (12). Namely, to achieve 2 mg/cm<sup>2</sup> of density, the following should be done: 1 teaspoon of sunscreen to the face/head/neck, 1 teaspoon to each upper extremity, a total of 2 teaspoons to the front and back torso, and 2 teaspoons to each lower extremity. The reapplication frequency is also an important factor. Two applications of sunscreen, the first 15–30 minutes before sun exposure, followed by another application 15–30 minutes later, is recommended to obtain adequate amounts of sunscreen on the skin.

### Protection Afforded by Sunscreens

The protective property of sunscreens against acute and chronic effects of UV has now been well established (10) and only well-balanced UVA-UVB sunscreens, absorbing over the entire UV spectrum are able to prevent or significantly reduce the associated biological damage (13).

DNA damage is an important part of the photoaging process. By using *in vitro* skin models, Dehaven et al. (14) demonstrated that sunscreen application prior to full-spectrum solar exposure protects from CPD and SBC formation. *In vitro* experiments showed that UVA-activated synthesis of MMPs is prevented by a broad-spectrum sunscreen (15). This is important since progressive skin darkening in response to repeated low-dose UVA1 exposures does not prevent UVA1-induced collagenolytic changes (16). A recent randomized controlled trial that was conducted in 903 adults younger than 55 years showed that regular sunscreen use retards skin photoaging (17). There is also evidence suggesting that sunscreens can diminish the appearance of premature aging and prevent exacerbations of photodermatoses such as polymorphous light eruption (17–19).

UVB and UVA are both immunosuppressive, and broad-spectrum sunscreens provide protection against the reduction of human cutaneous immunity induced by solar exposure (20,21). Several studies have explored the effect of sunscreen use on the development of actinic keratosis (AK). Thompson and colleagues (22) conducted a randomized, controlled trial of the effect on solar keratosis of daily use of an SPF 17 broad-spectrum sunscreen in 588 subjects. The sunscreen group had fewer new lesions and more remissions than the base-cream group. Darlington and colleagues (23) found that daily use of a broad-spectrum, SPF 16 sunscreen resulted in a 24% reduction in the average rate of AK development. Thus, promoting regular sunscreen use can be a safe and cost-effective approach to prevent AKs and SCC (24). Indeed, Green and colleagues (25) found that the routine use of a SPF 16 sunscreen for 4.5 years resulted in a 38% reduction in the incidence of SCC, while there was no beneficial effect on BCC prevention. The same investigators reported similar protective effects of daily sunscreen use after further follow-up of 8 years (26). The relationship between UV light and BCC development is complex and these tumors have a long latency period from the time of UV

damage to the clinical onset of disease. Thus longer periods of follow-up may be needed to observe a protective effect and further studies are needed. Nevertheless, currently available results do not support a role of sunscreen use in prevention of BCC growth.

The use of sunscreen in the prevention of melanoma has been the subject of much controversy but analysis of case-control studies revealed neither a protective nor a harmful association between sunscreen use and melanoma (27,28). However, a randomized controlled study demonstrated that regular sunscreen use reduces the risk of developing melanoma (29). Moreover, by using a BRAF (V600E) mouse model, Viros and colleagues (30) demonstrated that sunscreen delayed UV-driven melanoma in susceptible mice. These data validate public health campaigns that promote sunscreen protection for individuals at risk of melanoma.

### Sunscreen and Vitamin D

Solar UVB converts 7-dehydrocholesterol in the skin to previtamin D<sub>3</sub> and is the major source of vitamin D (31). Sufficient levels of vitamin D are essential for bone development and maintenance and may reduce the risk for certain cancers and influence other diseases. Although sunscreens can significantly reduce production of vitamin D under strict photoprotection, their normal usage, most likely due to inadequate application (0.5 mg/cm<sup>2</sup>), does not result in vitamin D deficiency (31).

### PHYSICAL PHOTOPROTECTION

Physical methods of photoprotection include glass, window films, sunglasses, and clothing (32). Almost all glass blocks UVB radiation, regardless of type or properties of the glass. However, the transmission of UVA radiation varies according to the type, thickness, and color of the glass (33). Mean UVR exposure to a car passenger is 3%–4% of the ambient UVR, with the highest UV exposure to the left arm and lateral head of the driver. Although windshields (laminated glass) block the majority of UVA radiation, side and back windows (tempered glass) block only 20% of UVA radiation. This partial protection is not sufficient to protect patients with severe photosensitive disorders. The thickness of the glass was shown to have a limited effect on the protection. On the other hand, the color of the glass plays an important role in UVR protection and it has been shown that a gray color offered the highest protection. The addition of window film to glass used in side and back windows results in 99% reduction in the transmission of UVR and represents an excellent way to increase UVA protection. Several data provided arguments supporting a role of driving in UV-related skin damage and cutaneous carcinogenesis. A recent population-based study of 279 head and neck melanomas found a sex-related distribution of these tumors that may be related to driving (34). The general population should be aware that a car window does not totally protect against sun-related damage and that protection by clothes and sunscreens is still relevant even in a car, especially on sunny days. However, results of a retrospective survey reveal poor patient awareness and compliance with sun-protection measures while in an automobile (35).

Clothing may offer constant level of protection throughout the day but several factors affect the UV protection factor (UPF) afforded by textiles. The *in vitro* method using spectrophotometry is most commonly used to determine the UPF of textiles. UPFs measured with this method reflect mainly the protection against UVB and are usually smaller than those

occurring in real life. Several fabric characteristics can affect the UPF, such as porosity, weight, thickness, type of fabric, laundering, hydration, stretch, fabric processing, UV absorbers, color, and fabric-to-skin distance. Porosity of the fabric is the most important factor that affects UV transmission and fabrics, with large space between the yarns having generally lower UPF values. Cotton and rayon (cellulosic fibers) have the least UV-absorbing capacity (UPF<15), whereas polyester has the highest. Wool, silk, and nylon lie between these two groups. Colored fabrics have better UV protection than white fabrics, and darker colors have better UV protection than lighter colors, because of increased UV absorption. Hydration or wetting decreases the UPF for most clothing, because the presence of water eliminates the scattering of UV light at the fabric/air interface (36). Adding broadband UVR absorbers (e.g., Tinosorb FD) during laundry enhances UVR blocking properties of fabrics even after several washes. Hats and visors provide variable sun protection. Wide-brimmed hats (7.5 cm) provide SPF 7 for the nose, 3 for cheek, 5 for neck, and 2 for chin, whereas narrow-brimmed hats provide only SPF 1.5 for the nose, and minimal, if any, protection for other areas (32). Exposure to UVA and UVB can affect the eyes, and in contrast to the time of maximum UV exposure to the skin (10 a.m. to 2 p.m.), the time of maximum UV exposure to the eyes is from 8 to 10 a.m. and 2 to 4 p.m., when rays of the sun are parallel to the eyes. Sunglass standards have been developed to ensure quality, performance, and adequate protection to consumers. While in Australia and Europe standards are mandatory, the U.S. standard is voluntary and not followed by all manufacturers. The U.S. standard (ANSI Z80.3) requires less than 1% of the wavelengths below 310 nm to be transmitted. The ideal sunglass should substantially reduce UV transmission to cornea and lens and protect the retina against violet/blue light. Size, style, and position of the sunglasses are other factors that should be considered. Small lenses increase the probability of UVR reaching the eyes from the side of the lens. Based on this factor, the Australian standard has a minimum requirement of lens size, which is 28 mm for adult and 24 mm for children (37). Moving the sunglasses a small distance (6 mm) away from the forehead results in more than 20% increase in the amount of UVR reaching the eyes. Excessively dark tinted sunglasses can cause the pupil to dilate, making the eye structures more susceptible to UV. The public should be aware to choose sunglasses that are in compliance with one of the three national standards, choose a wraparound style or sunglasses with side shields, and position the sunglasses as close as possible to the forehead (37).

### ANTIOXIDANTS AND DIETARY AGENTS

Orally consumed substances, either in the diet or as supplements, can modulate cutaneous responses to UV and provide additional photoprotection (38).

### Nonsteroidal Anti-Inflammatory Drugs

Regular users of nonsteroidal anti-inflammatory drugs (NSAIDs) appear to have lower risks of SCC and lower counts of AKs than nonusers. The use of NSAIDs in the year prior to skin cancer diagnosis may reduce the risk of SCC but the effect on BCC is weak (39,40). COX-2 produces prostaglandins such as prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) from arachidonic acid. UV-induced PGE<sub>2</sub> has been shown to contribute to UV-induced immunosuppression and to enhance proliferation of keratinocytes (38). Selective oral cyclooxygenase (COX-2) inhibitors such as celecoxib are likely to



be useful for protection from sunlight-induced skin cancer and recent data indicate that celecoxib may prevent the appearance of cutaneous SCCs accelerated by BRAF inhibitors (41). A double-blind placebo-controlled multicenter randomized trial suggests that celecoxib could potentially reduce new non-melanoma skin cancers (NMSCs) in patients with extensive actinic damage (42). Currently, oral COX-2 inhibitors cannot be recommended on a wide population level because of concerns regarding cardiovascular toxicity, and it would be better to evaluate topical inhibitors (43). Diclofenac, a nonselective NSAID that inhibits both COX-1 and COX-2, is available topically and has been successfully used in the treatment of AK (44).

## Dietary Components

Several studies in mice showed that fruit or plants rich in antioxidants might have photoprotective effects (45). Flavonoids are well known for their antioxidant (or free radical scavenging) properties and some of them absorb UVB rays, hence contributing to their photoprotective effect by behaving as UV filters. Quercetin, which is the most abundant flavonol in the human diet, has been reported to block UVB induced oxidative stress and DNA damage. Epigallocatechin-3-gallate (EGCG) is a member of the group of flavonoids that is mainly found in tea, red wine, strawberry, and cacao products. Many studies showed that this compound could prevent the skin from UV-induced damage. Polyphenols (green tea polyphenols, pomegranate, fruit extract, grape seed proanthocyanidins, resveratrol, silymarin, genistein, and delphinidin) are found in a wide variety of fruits and vegetables. Molecular targets of these compounds include (i) regulation of anti-inflammatory activity, (ii) modulation of immunosuppression, (iii) prevention of DNA damage and regulation of DNA repair, and (iv) modulation of cell-signaling pathways critically involved in different stages of photocarcinogenesis (46,47).

Goji berries (*Lycium barbarum*) that have been used in traditional Chinese medicine reduce UV-induced immunosuppression and lipid peroxidation (48). Pomegranate (*Punica granatum*) fruit possesses strong antioxidant, anti-inflammatory, and antiproliferative properties (49,50).

Carotenoids are ingested with food components, notably fruits and vegetables, but also from animal sources. Most of human intervention studies provided evidence that an elevated intake of carotenoids ameliorates UV-induced erythema, the efficacy depending on the duration of treatment before exposure and on the dose (51). However, beta-carotene supplementation has not been shown to have any beneficial effect on photodamage and skin cancer prevention (17,25). Conversely, it was associated with increased risk not only of lung cancer but also of gastric cancer in smokers and asbestos workers. Thus, nutritional prevention of skin cancer through beta-carotene supplementation should not be recommended (52). Lycopene is a carotenoid found at high concentrations in tomatoes. A randomized controlled study showed that women whose diet is supplemented with tomato paste had reduced UV-induced erythema and MMP-1 production. The mitochondrial DNA deletion following an acute UV-exposure was also significantly reduced after supplementation with tomato paste (53). Antioxidant mixture may exert in vitro protective effects against UV-induced lipid peroxidation and antioxidant nutritional supplement containing lycopene, beta-carotene, and *Lactobacillus johnsonii* may provide protection against the development of UVA-induced protein-losing enteropathy (PLE) lesions (54).

Resveratrol (RES), a phytoalexin antioxidant found in red grapes, has been shown modulate transcription factors and to have protective effects against UV radiation-mediated oxidative stress and cutaneous damage, including skin cancers (55–57). RES suppresses UV-induced malignant tumor progression in mice and acts in part through the Akt-mediated downregulation of TGF-beta2 (58).

The leaves of the fern *Polypodium leucotomos* (PL) are rich in phenolic compounds such as ferulic and caffeic acids, and PL extracts have powerful antioxidant properties (59,60). Their short-term effects include inhibition of ROS production induced by UV radiation, DNA damage, isomerization and decomposition of transurocanic acid, prevention of UV-mediated apoptosis, and necrosis (61). PL has also been shown to accelerate removal of UV-induced photoproducts, highlighting its anticarcinogenic role. By reducing UV-induced inflammatory responses and inhibiting extracellular matrix remodeling, PL demonstrates some protective effects against photoaging and PUVA-induced phototoxicity. A randomized clinical trial showed that in healthy volunteers oral PL may result in a decrease of UVA-induced mitochondrial common deletion (62). PL may decrease UV-induced erythema and sunburn cells (63, 64) and oral PL treatment might be beneficial for the prevention of PLE (65).

Caffeine has been shown to be chemopreventive in non-melanoma skin cancer in mice and to promote UVB-induced apoptosis in HaCaT keratinocytes (66,67). However, the results from observational studies performed in humans are contradictory (38).

Dietary fish oils that contain omega-3 polyunsaturated fatty acids (PUFAs) may modulate the lipid content of the cell membrane and modify the cell behavior in response to UV exposure. These compounds may alter membrane lipid peroxidation and signal transduction pathways that are implicated in UV-induced erythema and immunosuppression. A community-based study in Queensland, Australia, examined the diets of 1119 adults and found that a moderate intake of oily fish may decrease the acquisition of actinic keratosis (68). A randomized controlled study performed in nickel-allergic women found that supplementation with omega-3 fatty acids resulted in a reduction of UV-induced immunosuppression (69). The results of a cross-sectional study conducted on 2919 subjects suggest a possible benefit of PUFAs on skin photoaging (70).

## Vitamins and Hormones

Nicotinamide (or niacinamide) is an amide form of vitamin B3 that is highly photoprotective. Nicotinamide partly prevents the loss of cellular energy that occurs after UV exposure and patients suffering from a vitamin B3 deficiency (pellagra) frequently have a photosensitive dermatitis (38,71). Nicotinamide enhances repair of UV-induced DNA damage in keratinocytes and melanocytes (72,73), protects against UV-induced immunosuppression (74,75), and prevents photocarcinogenesis in animal studies (71).

Activation of the melanocortin 1 receptor (MC1R) by alpha-MSH stimulates eumelanin synthesis and enhances repair of UV-induced DNA damage (76). Potent alpha-MSH analogues increase the tanning response after UV light exposure and reduce thymine dimer formation in subjects exposed to UV light (77). However, serious concerns exist regarding the public's unrestricted use of untested alpha-MSH analogues because of their potential to induce malignant changes in melanocytes (78).



## PROTECTION FROM VISIBLE LIGHT AND INFRARED RADIATION

At least 50% of the total energy that is being emitted by the sun and that reaches human skin is in the IR range. In addition, within the IR range, IRA rays (770–1400 nm), which represent one-third of the total solar energy, are capable of penetrating human skin and directly affecting cells located in the epidermis, dermis, and subcutis. More than 65% of IRA reaches the dermis and there is now increasing evidence that IRA, similar to UVB or UVA, significantly contributes to photoaging of human skin (79). Recent works demonstrate that IR and heat exposure each induce cutaneous angiogenesis and inflammatory cellular infiltration, disrupt the dermal extracellular matrix by inducing MMPs, and alter dermal structural proteins (80). The recent analysis of IRA-induced transcriptome in primary human skin fibroblasts identifies IRA as an environmental factor with relevance for skin homeostasis and photoaging (81). Exposure of human skin fibroblasts in vitro and human skin in vivo to physiologically relevant doses of IRA causes an increase in MMP-1 without a concomitant upregulation of tissue inhibitor of metalloproteinase-1 expression (82,83). The underlying mechanisms responsible for UVB-, UVA-, and IRA-induced MMP-1 expression markedly differ. The major chromophores for UVB appear to be nuclear DNA and cytoplasmic-free tryptophan, whereas the UVA stress response is controlled by the lipid composition of specialized membrane microdomains (rafts) (84). IRA radiation is strongest absorbed by mitochondria and the earliest biological event following IRA irradiation of human skin fibroblasts is an increase in mitochondrial production of ROS (85). IRA exposure induces similar biological effects to UV radiation, but the underlying mechanisms are substantially different since the cellular response to IRA irradiation mostly involves the mitochondrial electron transport chain. Thus, effective sun protection may require specific strategies to prevent IRA-induced skin damage, and mitochondrial-targeted antioxidants may be used to protect human skin against IRA radiation-induced damage (86). Visible light exposure can induce ROS, which can lead to the release of proinflammatory cytokines and MMPs in the skin, similar to the effects of UV, and therefore visible light may contribute to the signs of premature aging in the skin. However, commercially available sunscreens were found to have minimal effects on reducing visible light-induced ROS, suggesting that UVA/UVB sunscreens do not protect the skin from visible light-induced responses (87).

## EDUCATION AND PREVENTION

Indoor tanning is associated with an increased risk of developing melanoma and cutaneous carcinoma. Exposure to indoor tanning is common in the United States and Western countries, especially among young persons and high school students (88,89). A recent meta-analysis shows that use of indoor tanning increases the odds of melanoma after 10 tanning sessions (90). Public health efforts are needed to change social norms regarding tanned skin and to increase awareness, knowledge, and behaviors related to indoor tanning among adolescents. Clinicians should continue to educate patients on the harms of indoor tanning and encourage its cessation. Prevention efforts should be also associated with population-based interventions in the form of legislation to discourage and decrease access to indoor tanning facilities (91).

Although most vacationers use sunscreen at sunny holiday destinations, an alarmingly high proportion experiences

sunburn, and skin cancer prevention campaigns should emphasize the significance of covering up and seeking shade (92). Clinicians and public health agencies should discourage people from prolonging sun exposure through sunscreen use in order to acquire a suntan (93). Campaigns designed to promote sun protection should focus on specific populations, and a large nationwide cross-sectional study showed that factors associated with low adherence to sun protection behavior were age below 20 or over 64 years, male sex, and lower knowledge about accurate sun protection recommendations and UV-associated risks (94).

Photoprotection, including seeking shade when outdoors and using sunscreens and protective clothing, is the first line of defense against photoaging. Primary prevention also includes avoidance of artificial sources of UV exposure, including tanning beds, and judicious use of therapeutic light boxes for medical treatment of dermatoses. Topical retinoids are the mainstay of treatment of patients with mild to moderate photoaging. These compounds increase collagen production, induce epidermal hyperplasia, decrease keratinocyte and melanocyte atypia, and have been shown to reduce CPDs. Oral retinoids likely prevent SCCs, as well as possibly preventing BCCs and reducing AKs. However, because of their side effects, their use is limited to selected high-risk patients only.

## CONCLUSION

Sunscreen use alone will not reduce skin cancer and photoaging, but it should be considered as an effective ancillary to wearing protective clothing and seeking shade (93). The positive perceptions of a suntan as healthy and attractive remains a challenge, and in a world where fashion is more powerful than health it will not be easy to change these perceptions, unless fashion changes (95).

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# Photodamage and Skin Cancer: How Successful Are Sunscreens as a Means of Prevention?

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## INTRODUCTION

Sunscreens were first developed in the 1930s as a means of protection from sunburn, as light-skinned individuals demonstrated increasing sun-seeking behaviours in the face of tanned skin becoming fashionable (1). However, despite this long history sunscreen uptake has been temperamental, its use sometimes controversial, and the incidence of skin cancers within the western hemisphere continues to rise (2). This now represents an impending public health crisis, as there is an ever-increasing demand on already stretched dermatological and oncological services.

Sunscreens have been widely promoted as a successful preventative method, for example in Australia where they have campaigned to “slop on sunscreen” as part of the “slip, slop, slap, seek & slide” SunSmart campaign (3). However, despite decades of such public health messages, skin cancer remains on the rise overall. So is it fair to say that sunscreen has “failed” as a preventative measure against photodamage and skin cancer?

This chapter looks at the scientific evidence behind ultraviolet (UV) radiation and carcinogenesis and the mechanisms of photoprotection provided by sunscreen, and investigates the evidence base and controversies surrounding its effectiveness as a photoprotective agent. Why is it that some studies show, in direct contradiction to scientific logic, that sunscreen use is associated with increased skin cancer rates, in particular of malignant melanoma (4)? The key behavioral factors so integral to sunscreen use are thus also analyzed.

It is evident that clear communication and high-quality patient education is an integral part of preventing sunlight-induced skin aging and more importantly, carcinogenesis. This chapter looks at the future of preventing photocarcinogenesis and the role of sunscreen within this, and therefore how we may best advise our patients on the key issue of prevention.

## ULTRAVIOLET RADIATION AND PHOTOCARCINOGENESIS

Epidemiological studies from nearly 50 years ago first demonstrated the link between sun exposure and dermatological malignancies. This was deduced from observations that melanoma is proportionally more common in those living closer to the equator, where UV exposure is higher (5). Secondly, those who have a reduced ability to absorb UV light due to possessing less melanin, such as those with Fitzpatrick skin type I, are statistically more likely to develop skin cancer (6). Thirdly, non-melanoma skin malignancies such as basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) are

commonly found on sun-exposed areas such as the face and upper chest (7).

Sunlight represents a broad range within the electromagnetic spectrum; the two types of radiation we notice are visible light (wavelength 400–760 nm) and infrared (760–10<sup>6</sup>nm) in the form of heat. The form of radiation that we are concerned with, however, is ultraviolet B (UVB) (290–320 nm) and ultraviolet A (UVA) (320–400 nm), which are invisible but biologically active components of sunlight. UVB, for example, interacts with 7-dehydrocholesterol in the skin to produce cholecalciferol (vitamin D3).

Both forms of UV are thought to induce DNA damage and thus genetic mutations, which could lead to the so-called “hallmarks of cancer” (8). UVB is thought to cause direct damage to cellular DNA, leading to the formation of pyrimidine dimers (Figure 23.1). The importance of this is highlighted by the condition xeroderma pigmentosum: the resulting defect in the nucleotide excision repair pathway that repairs these abnormal pyrimidine dimers leads to a skin cancer risk 2000 times greater than that of the general population (9).

UVA, on the other hand, is thought to penetrate deeper into the dermis due to its longer wavelength, and then to cause DNA damage by the production of circulating free radicals. However, more recent research has shown that this view is overly simplistic and the damaging effects of UVA and UVB are probably more synergistic than previously understood (10). Furthermore, there is evidence that radiation within different areas of the electromagnetic spectrum also have a role of photodamage and photoaging, in particular infrared (11).

It has also become evident that immunity is a key player in dermatological malignancies. The use of immunotherapies has changed the landscape of both malignant melanoma and solid tumor malignancies. There is now evidence that combination treatment with the immunomodulatory agents ipilimumab (an anticytotoxic T-lymphocyte-4 antigen monoclonal antibody) and nivolumab (an antiprogrammed death-1 receptor antibody) may provide the best outcomes in advanced melanoma. Such agents work by augmenting antitumor immunity by blocking checkpoints in the immune pathway by acting on antigens and receptors such as CTL4 and PD-1 (12). The relationship between UV radiation and immunity has also been studied, and both UVA and UVB are known to cause immunosuppression (13). In addition to this, the role of UVB in generating vitamin D3, which has a multifactorial impact on immunity that is yet to be fully understood, must also be considered (14).

As sun-seeking behaviors and so exposure to UV radiation have increased over the 20th century, so have incidences

of all types of skin cancer (15). Broadly speaking, these are divided into melanoma and non-melanoma skin carcinomas. SCCs and BCCs tend to present in sun-exposed areas, with SCCs thought to be largely associated with cumulative UVB exposure and BCCs with episodes of sunburn. Although there is not a significant risk of mortality (it is greater with SCCs than BCCs), they are locally invasive and damaging, as well as cosmetically concerning to patients. Melanomas are further subdivided into different subtypes with varying degrees of invasiveness and aggression. Nodular melanomas are associated with a poorer prognosis than the more common superficial spreading melanomas. Melanomas tend to present in non-sun-exposed areas such as the back and lower leg (16). Mutations such as the oncogene *braf*, which are found in around 50% of melanomas, have been suggested to be caused by free radical damage caused by UVA (17). The revolutionary impact of *braf* inhibitors on metastatic melanoma highlights the importance of such a mutation. Melanoma has a significant mortality rate, and is the cancer which causes the most deaths of young adults worldwide (18). Figure 23.2 summarizes the rising incidence of malignant melanoma between 1975 and 2011.

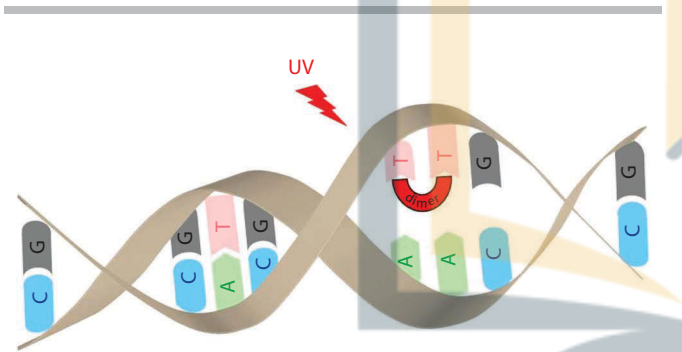


Figure 23.1 UV light-induced thymine dimer.

## SUNSCREEN: HOW DOES IT WORK AND HOW IS THIS MEASURED?

The ideal sunscreen would provide complete UV protection, be long-lasting, and be cosmetically acceptable. However, the reality is that most sunscreens provide relatively limited protection across the UV spectrum, require regular reapplication, and can be cosmetically unappealing due to shine and comedogenic properties. Furthermore, sunscreens are costly and the large area to which they need to be applied can mean they are unacceptably expensive for individuals to use as their primary sun protection measure (19).

Sunscreens are divided into organic and inorganic agents, which act by providing chemical and physical barriers to UV radiation, respectively. The protection given is divided into UVB, UVA, or “broad-spectrum” coverage.

Organic barriers work by absorbing UV radiation; historically they were manufactured to absorb UVB only as this was once thought to be the sole “harmful” wavelength. The most commonly used organic agents are cinnamates such as octyl methoxycinnamate, which still provide predominantly UVB absorption (20). However, more recently developed agents provide cover for a broader wavelength including UVA. The most commonly used UVA-protective agents are benzophenones such as oxybenzone. However, oxybenzone, for example, is limited by a number of factors including its preponderance toward causing allergic dermatitis, and its skin penetration, as evidenced by its presence in the urine and bloodstream of individuals using the product. Another challenge in the production of effective broad-spectrum sunscreens is being able to successfully combine both UVA- and UVB-protective products in an acceptable lotion (21).

Inorganic agents such as titanium dioxide and zinc oxide form a physical barrier of inert metal particles, which are able to reflect and scatter UV radiation but also visible light. They have been assumed to be safer than organic agents, and have been advocated for children. Inorganic agents provide varying degrees of protection, with titanium dioxide being inferior to zinc in providing protection against UVA (22). These tend to form thick, shiny lotions that can be cosmetically unappealing

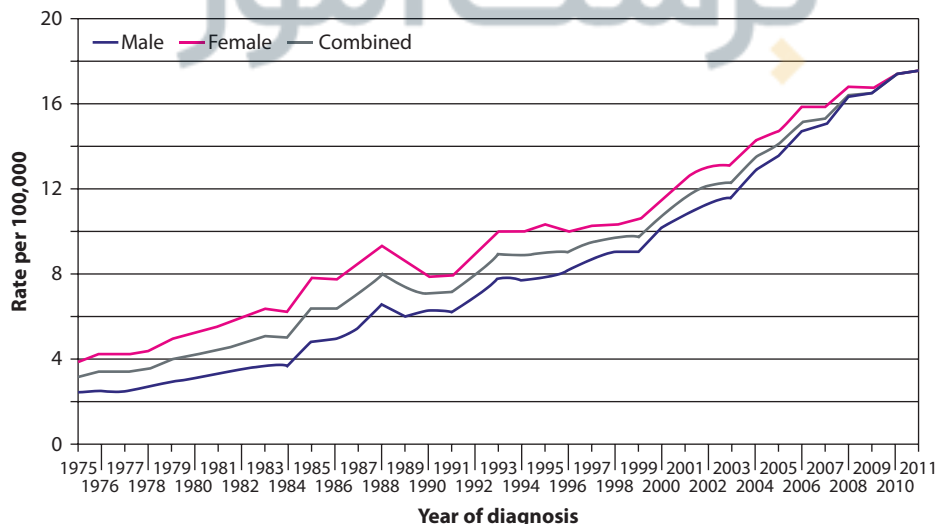


Figure 23.2 Malignant melanoma 1975–2011 European age-standardized incidence rates per 100,000 population, by sex, Great Britain. (From Cancer Research UK. <http://www.cancerresearchuk.org/cancer-info/cancerstats/types/skin/incidence/>. Accessed 2015.)

to use. However, new developments in sunscreen technology have resulted in the formulation of micro-ionized particles, which are significantly smaller and therefore scatter less visible light, reducing undesirable shine (23). However, there have been suggestions that these micro-ionized particles would be absorbed more easily and so result in toxicities.

Over the years, safety concerns have been raised regarding active ingredients within sunscreen, leading to delays in consumers being able to access theoretically more effective products. The proposed dangers include possible carcinogenicity, and that rather than reducing free radical production, the active products in sunscreens may even produce free radicals (24). So far, studies analyzing toxicities related to sunscreen ingredients such as retinyl palmitate, oxybenzone, zinc oxide, and titanium dioxide have failed to demonstrate they have such harmful effects (25).

A quirk of American legislation means that sunscreen products are classified as drugs, as opposed to cosmetic products as they are in Europe. This means that in order for them to be available to consumers, they have to pass far more stringent safety regulations via the Food and Drug Administration (FDA). Subsequently, FDA hasn't approved an over-the-counter sunscreen ingredient since 1999 (26). Therefore, individuals using sunscreen in the United States have yet to benefit from newer products which provide greater protection against UVA, some of which have been available in Europe and Japan for quite some time. However, recent updates in the law may see this change (27).

The protection sunscreens provide against UVB is quantified by its sun protection factor (SPF). This is the ratio of the amount of UV radiation required to cause an erythematous reaction with a filter compared to without. For example, applying SPF2 sunscreen enables an individual to have exposure to UV radiation twice as strong as they would otherwise without protection, before erythema is induced. The absorption of UVB rays is also influenced by the strength of SPF, but there is an exponentially decreasing return on increasing SPF. For example, whilst SPF2 applied at a rate of 2mg/cm<sup>2</sup> absorbs 50% of rays, SPF15 absorbs 93%, SPF 30 absorbs 97%, and SPF 50 absorbs 98%. Therefore, higher SPF sunscreens may provide a negligible difference in UV absorption while encouraging the user to feel disproportionately more protected against UV. As discussed below, this is reflected in some studies of high SPF sunscreen use, which suggest that they are not providing greater sun protection as one would assume (28). Furthermore, in calculating the SPF, FDA uses a dose of 2.2mg/cm<sup>2</sup> of exposed skin. However, this estimated dose is thought to be up to four times higher than what individuals use in reality (29). There is also the big question of whether SPF is even a useful measure of long-term UV damage, as there is evidence that photoaging, immunosuppression, gene mutations, and pyrimidine dimer formation is induced by suberythematous UVB radiation (30).

UVA measurement techniques vary in not only methodology, but also in the generation of rating scales produced. A variety of in vivo and in vitro methods are used in Japan, Europe, the United Kingdom, the United States, and Australia. FDA, for example, employs an in vitro method which measures the UV transmittance after radiation is administered to a test product. The critical wavelength is determined by the wavelength at which 90% of the total area under the absorbance curve occurs. Sunscreens which include a critical wavelength of greater than or equal to 370 nm are defined as "broad spectrum." In contrast, Japan uses an in vivo method with

persistent pigment darkening (PPD) as the primary clinical endpoint, where the minimal UVA radiation dose required to induce pigmentation is compared when using UVA protection versus without. This enables generation of a UVA protection grade from PA + to PA +++. The EU also uses PPD as the clinical endpoint but also requires UVA protection as a proportion of UVB protection to be present, which then generates a star rating (31).

It can be seen, therefore, that there is considerable variation in both the ingredients in the products available and the protection they subsequently provide. This makes comparing studies difficult, and applying the results of many such studies, often using outdated agents, unhelpful. Therefore, analyzing the efficacy of sunscreen use can be difficult.

## SUNSCREEN: DOES IT REALLY WORK AND WHAT EVIDENCE IS OUT THERE?

Effects of photoaging, including pigmentation, collagen loss, and blood vessel dilatation have been shown to be reduced through the appropriate use of sunscreen (32). In fact, an Australian randomized-controlled trial of 903 participants examined the effects of daily application of broad-spectrum SPF 15+ sunscreen on a measure of dermal elastosis. After 4 years of follow-up, the trial concluded that skin aging was significantly less in those using regular sunscreen (33). Although sunscreen use logically ought to also reduce the incidence of skin cancers, data from clinical trials has been far more conflicting in nature.

Trials have demonstrated that sunscreens reduce the incidence of solar keratosis (34) and SCCs, while its effects on BCCs have been insignificant or only somewhat effective. The primary example is from another Australian randomized-controlled trial. This involved 1621 participants, beginning in 1992, and encouraged those in one arm of the study to apply SPF 15+ sunscreen to specific areas at least every morning. Follow-up of these participants over 4.5 years led to this same conclusion—that such sunscreen use was effective in preventing SCCs but not BCCs (35).

However, the major controversy undoubtedly surrounds the effect that sunscreen use has on the incidence of malignant melanoma, the most lethal skin cancer. A case control study of 571 melanoma cases and 913 healthy controls, published in 2000, found a positive association between sunscreen use and trunk melanoma formation (4). Of note, the median SPF used in this study was 6, and those using sunscreen were also found to spend more time in the sun.

A quantitative review of 18 case-control studies did not corroborate these results, finding no increase in melanoma associated with sunscreen use (36). Similarly, a meta-analysis of 9067 patients from 11 observational studies found a relative risk for melanoma formation of 1.01, when comparing sunscreen use to no use (37). While this may be reassuring to a certain extent, they do not show a benefit of sunscreen use in terms of melanoma incidence. To this end, a randomized-controlled trial has shown that sunscreen use decreases the development of melanocytic nevi in children, which could be considered as a surrogate marker for melanoma risk (6). The highest-level evidence available is from a 10-year follow-up of the randomized-controlled trial mentioned above, involving 1621 participants in Australia (38). In this study, there was a marginally statistically significant protection afforded by the use of regular sunscreen against the occurrence of invasive melanoma. The secondary endpoint outcomes are, however, small in number: with 3 melanomas classified as invasive out



of 812 individuals on the “treatment” arm (and 11 new primary melanomas) versus 11 of 809 in the comparison group (22 new primary melanomas).

Numerous suggestions have been posited for explanations regarding this rather equivocal data. As mentioned above, the protection afforded especially against UVA by sunscreen has historically been poor, such that many of the early studies were conducted using sunscreens with only UVB cover. Newer trials with modern sunscreens are therefore warranted.

Vitamin D has also been proposed to have a significant effect. Chronic sun exposure, which would raise vitamin D levels, has been associated with melanoma risk reduction (39), and it has also been found that greater weekend sun exposure (but not burning) may be protective against melanoma (40). Despite this, current research does not demonstrate that sunscreen use decreases levels of vitamin D significantly, nor has supplementation been shown to reduce risks of melanoma (41). A further case-control study has concluded that increased sun exposure is correlated with increased melanoma survival (42). These results certainly raise the issue that all sunscreen studies should be looking not only at melanoma incidence, but aggressiveness and patient survival as well. Overall, however, there is no evidence to establish a clear relationship between vitamin D and melanoma risk, and there is no consensus as to whether individuals ought to change their sun exposure behavior in regard to this (43).

As discussed, it has also been suggested that some of the chemical products in sunscreen may be counteracting the benefits it affords in UV protection. Retinyl palmitate, for example, has previously been linked with carcinogenesis, although there is currently no evidence that it increases the risk of skin cancer (44). The formation of damaging free radicals from chemicals in sunscreen has also been posited as negating the benefits of reduced free-radical formation from UV exposure. If sunscreens are not being applied effectively (as discussed below), the balance may tip in favor of excess production of reactive oxygen species and thus carcinogenesis (45).

In summary, there is evidence that sunscreen use prevents photoaging, solar keratoses, and SCC formation but no evidence of any benefit (or harm) for BCC. For melanoma, the water is a bit murkier, but again there is no clear evidence of harm—in fact, the strongest evidence is of a mild benefit. This all being said, behavioral factors are the most important factor in the outcome of sunscreen use, so they must be carefully considered in the interpretation of all results.

## HUMAN FACTORS IN SUNSCREEN USE

The use of sunscreen (whether adequate or not) has been suggested to provide individuals with a sense of protection, enabling them to indulge in sun-seeking behaviours. There is also an (incorrect) assumption that a lack of sunburn is equitable to lack of DNA damage, resulting in a false sense of security. Importantly, it has been found that application of sunscreen is associated with increased intentional sun exposure (46). Similarly, the use of high SPF sunscreens in particular is associated with an increase in sun-seeking behaviors (47), most likely due to complacency when using “stronger” sunscreens. Sunscreen dosage and use, as discussed above, is also frequently inadequate. A survey of 423 individuals found that less than a third of respondents knew to apply sunscreen 30 minutes before sun exposure, and a similar minority knew how to reapply the sunscreen appropriately. A paltry 18% knew the correct dose

of sunscreen to apply (48). Other studies have drawn similar conclusions. This usage of inadequate volumes of sunscreen has been shown to significantly reduce the effective SPF—with an exponential relationship. When the more commonly used amount of 0.5 mg/cm<sup>2</sup> is used, SPF 4 becomes 1.4, and SPF 16 becomes just 2 (49). These behavioral factors along with others, including the fact that fairer-skinned individuals are both more likely to use sunscreen and also to develop skin cancer, are strong confounding factors in the outcome of the above-discussed trials.

For many, tanned skin is considered to be a sign of health, wealth, and beauty. Some individuals continue to pursue this cosmetic ideal despite awareness of health risks of sun exposure. Even in the face of well-established public health messages about these serious risks, and thus the need for minimizing UV exposure, both tanning beds and holidays in the sun remain widely popular. This persistent belief is reflected in the results of numerous surveys in different countries. One international survey of sun protection behaviors of 8178 individuals showed that a substantial proportion of their study group reported “inadequate sun protection behaviours resulting in severe sunburn.” Even individuals who have previously suffered with skin cancers and melanomas continued to experience sunburn due to inadequate protection (50). Another recent survey of 148,869 Norwegian women found that although sunscreen use is increasing among Norwegian women, this increase has not been accompanied by a decrease in sunburn. Results from this survey also show that more than two-thirds of Norwegian women are also not protected with recommended SPF (51).

Overall it appears that sunscreen use has been increasing over the last two decades with relatively poor improvement in rates of sunburn. Sunscreen persists in being a popular method despite the high cost of regular sunscreen use and the relative low cost of purchasing a wide-brimmed hat or avoiding the sun altogether. Sunscreens are highly profitable and aggressively marketed by the cosmetic industry, and advertisements frequently show children or adults at a sunny beach in swimwear, with no other sun protection. In reality, such advertisements are promoting a behavior more harmful to the skin than any sunscreen can realistically counteract. Magazines and other media continue to frequently show both male and female beauty ideals as sporting a tanned appearance. Unsurprisingly, “positive tan appeal” remains high and use of indoor tanning has also increased. Worryingly, UV exposure from indoor tanning has also been suggested to be addictive (52). Indeed, vanity plays a key role in sunscreen use, with one study demonstrating that individuals reported a more positive response to a sunscreen product when it was promoted as preventing skin aging instead of preventing cancer (53).

Despite our deeply ingrained cultural appreciation of an attractive and “healthy” tan in Western culture, there is some encouraging evidence that the tide may be turning. Recent epidemiological studies of incidence of melanoma in children and young adults show some positive changes. One study found that overall trends have been decreasing in the pediatric population in the United States between 2000 and 2010, and another found that there is a stabilization or decreasing trend in the age group between 25 and 44 more recently (54,55). This may well be attributable to public health initiatives advocating sun protection and avoidance, and perhaps also represents a subtle shift in our



sociocultural beliefs and expectations surrounding tanning and sun exposure.

## CONCLUSION

This chapter has summarized (i) the epidemiology of skin cancers, (ii) the role of ultraviolet light in skin aging and carcinogenesis, (iii) the aims of topical sunscreens as a photoprotective tool, (iv) the (somewhat limited) data on outcomes of sunscreen use, particularly in regard to melanoma, and (v) the proposed reasons for variations within this data, with particular focus on the behavioral aspects of sun exposure and sunscreen use. But what might one conclude from all this?

Continued sun-seeking behaviour and a desire for a “fashionable” or “healthy” tan has (at least partly) driven the rates of both non-melanoma and melanoma skin cancers ever higher in the last 50 years. Currently used methods of photoprotection are proving to be wholly inadequate. However, as the formulations of topical sunscreens continue to improve, the main limiting factor of protection is likely to be behavioral.

The public need to be educated on proper sunscreen use and that, as stated by the International Agency for Research on Cancer, “sunscreens should not be the first choice for skin cancer prevention, and should not be the sole agent for protection against the sun” (56). Further public health initiatives to enforce the messages outlined in Table 23.1, while at the same time tackling the images and misconceptions promoted by sunscreen marketing and the fashion industry.

**Table 23.1** Key behaviors for protection

**Seek the shade**, especially between 10 a.m. and 4 p.m.  
**Do not burn.**  
**Avoid tanning and never use UV tanning beds.**  
**Cover up** with clothing, including a broad-brimmed hat and UV-blocking sunglasses.  
**Use a broad spectrum (UVA/UVB) sunscreen** with an SPF of 15 or higher every day. For extended outdoor activity, use a water-resistant, broad spectrum (UVA/UVB) sunscreen with an SPF of 30 or higher.  
**Apply 1 ounce** (2 tablespoons) of sunscreen to your entire body 30 minutes before going outside. Reapply every 2 hours or immediately after swimming or excessive sweating.  
**Keep newborns out of the sun.** Sunscreens should be used on babies over the age of 6 months.  
**Examine your skin** head-to-toe every month.  
**See your physician every year** for a professional skin exam.

Source: Skin Cancer Foundation, <http://www.skincancer.org/prevention/sun-protection/prevention-guidelines/preventing-skin-cancer>. With permission.

**Table 23.2** Useful tools or protection

SunSmart app to check the daily sun protection times for your location each day on the SunSmart app for iPhone, iPad, and Android. The app lets you know when you do and don't need sun protection, making it easier than ever to be smart about your sun exposure all year.

- Sunscreen calculator to find out if you are using enough sunscreen today.
- Vitamin D tracker to find out how your UV exposure today compares with recommended UV exposure for vitamin D.

Source: SunSmart, <http://www.sunsmart.com.au/tools/interactive-tools>. With permission.

Further large-scale trials with modern sunscreens are needed before we can understand more closely their precise effects. As newer products become available, the overall picture may become more clear. However, while there remains no convincing evidence of harm, appropriate sunscreen use should be encouraged along with covering up and other sun-avoiding behaviors.

## HELPFUL TIPS AND RECOMMENDATIONS

The Skin Cancer Foundation recommends the behaviors in Table 23.1 as being key to preventing skin cancer. Other useful tools to help individuals avoid dangerous UV radiation and protect themselves adequately include the items in Table 23.2. The Cancer Council Victoria's SunSmart program has produced a free smartphone application which helps calculate the above and personalizes sun protection recommendation according to skin type and exposure risk.

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## Photodamage: Protection and Reversal with Topical Antioxidants

Karen E. Burke

### INTRODUCTION

More than ever, our generation enjoys the luxury of travel and leisure time for outdoor sports, markedly increasing our exposure to solar ultraviolet (UV) radiation. Exposure is increased at high altitudes and with reflection from surfaces covered with snow, sand, water, or concrete. Our skin suffers the greatest damage—both acutely, with erythema and sunburn, and chronically, resulting in photoaging with mottled pigmentation, wrinkling, dryness, and precancerous keratoses as well as the severe consequence of skin cancer.

Certainly sunscreens are essential for protection, but they are not enough. The most significant inherent limitations are inadequate application (too little, too infrequently) and incomplete spectral protection from both UVA and UVB radiation. The skin naturally uses nutritional antioxidants to protect itself from photodamage, and when effective formulations are applied topically, sun protection is enhanced. Topical application of these antioxidants can give far higher concentrations in the skin than even maximal oral intake. The challenge is to make formulations that are stable and give effective transcutaneous absorption to deliver effectively high concentrations of the active forms to the dermis as well as the epidermis.

This chapter describes the necessary concentrations, molecular forms, and requirements for stability and topical delivery of several antioxidants that have been proven effective: vitamin C, vitamin E, and selenium. New research on topical application of genistein (a soy isoflavone) is also presented.

### VITAMIN C

Vitamin C (L-ascorbic acid) is the body's major aqueous-phase antioxidant and is vital for life. All animals make their own vitamin C except for humans and other primates, one species of Indian fruit-eating bat, and the guinea pig—all of which lack the enzyme L-glocono- $\gamma$  lactone oxidase. In fact, a 59 kg (130 lb) goat synthesizes 13 g of vitamin C per day, almost 200 times the US Food and Drug Administration (FDA) requirement (1). Not only do other animals make hundreds of times the vitamin C we ingest but also, when under stress, they can make more than ten times their normal amount of vitamin C. Unfortunately, we humans do not have that capability (1).

Our skin is the organ that suffers most from environmental free-radical stress from exposure to sunlight, pollution, and smoking. This contact, furthermore, depletes the level of vitamin C in the skin. Even minimal UV exposure of 1.6 minimal erythema dose (MED) decreases the level of vitamin C to 70% of the normal level, and exposure to 10 MED decreases the vitamin C to only 54% (2). Exposure to 10 parts per million of

ozone in city pollution decreases the level of epidermal vitamin C by 55% (3).

That was the bad news. The good news is that the most effective way to increase the skin level of vitamin C significantly is by topical application. To optimize percutaneous absorption and full activity of vitamin C, the precise formulation is of the utmost importance (4). Since L-ascorbic acid is an excellent antioxidant, making it an inherently unstable molecule, creation of an effective topical delivery system is crucial. Many products contain stable esterified derivatives (such as ascorbyl-6-palmitate or magnesium ascorbyl phosphate) which are not metabolized by the skin to the active ascorbic acid, so they have no activity (5). Furthermore, the ester molecules are not absorbed percutaneously (5). Some of these esterified derivatives have been reported to give contact allergy (6,7). Other formulations do not result in measurable absorption of vitamin C because they are not at the correct pH. Delivery of L-ascorbic acid ( $pK_a = 4.2$ ) depends upon removing the ionic charge—achieved optimally at a pH of 3.5 (5). New formulations stabilizing the vitamin C in microcapsules (8), liposomes (9,10), and microemulsions (10) are being investigated for stability, absorption, and activity.

Topical absorption was proven to be effective by radioactive labeling studies in pigs. After treatment with 10% vitamin C cream, 8.2% was found in the dermis and 0.7% was in the blood (4). Formulations containing 5%, 10%, 15%, 20%, or 25% vitamin C were tested. After 24 hours, 20% resulted in the highest skin levels, with maximized concentration in the skin after 3 days (5). In these experiments, levels of vitamin C after topical application of 15% serum were shown to be a factor of about 27 times that which could ever be attained by even very high oral intake. If topical application is discontinued after skin saturation is achieved, high levels remain in the skin for more than 3 days (5).

Topical vitamin C protects against solar damage primarily as an antioxidant that deactivates the UV-induced free radicals (11). Recent experiments with human epidermoid carcinoma cells demonstrate that vitamin C further protects against UV-induced apoptosis through reactivating silenced tumor suppressor genes p21 and p16 (12). Vitamin C is itself not a sunscreen, although applying vitamin C decreases the degree of redness and sunburn suffered even when applied after sun exposure. Protection can be confirmed by histologic examination. Treatment with topical 10% vitamin C decreases the number of abnormal "sunburn cells" by 40%–60% (5) and reduces the UV damage to DNA by 62% (4).

Topical vitamin C is also directly anti-inflammatory. More aggressive techniques of laser resurfacing used in the



past caused redness for at least 3–4 months. With vitamin C applied before and after laser surgery, redness was decreased after only 2 months (13). Topical vitamin C can also be used effectively to treat the inflammation of rosacea (14). This anti-inflammatory action has been researched with human cells in vitamin C-enriched media demonstrating decreased activation of the transcription factor nuclear factor  $\kappa$ B (NF- $\kappa$ B), the factor responsible for many pre-inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukins IL-1, IL-6, and IL 8 (15).

The main action of vitamin C on the skin is direct stimulation of collagen synthesis. Vitamin C is an essential cofactor for the two enzymes required for collagen synthesis: prolyl hydroxylase (which makes the collagen molecule stable) and lysyl hydroxylase (which cross-links the collagen to give structural strength) (16). Recent research has further demonstrated that vitamin C acts directly on DNA to increase the transcription rate and to stabilize the procollagen messenger RNA, thus regulating and maintaining the intercellular amount of collagen (17).

Exciting experiments have demonstrated that vitamin C also has anti-aging effects. Studies compared newborn with elderly (80–95 year-old) fibroblasts (18). Elderly cells proliferate at only one-fifth of the rate of newborn cells. However, when vitamin C is added to the culture medium, the elderly cells proliferate better than normal newborn fibroblasts. Even the newborn fibroblasts proliferate almost four times better when exposed to vitamin C (18). Not only do fibroblasts increase proliferation in the presence of vitamin C, but they also synthesize more collagen. Newborn fibroblasts synthesize a larger percentage of collagen than elderly cells, but again, when elderly cells are exposed to vitamin C, they produce more collagen than the normal, newborn fibroblasts (18). Surprisingly, even the newborn cells double the amount of collagen synthesized (18).

Vitamin C further reverses the adverse appearance of photoaging by inhibiting tyrosinase (19), thereby fading unattractive solar lentigines. By directly decreasing inflammation, post-inflammatory hyperpigmentation can also be reduced. Melasma and solar lentigines fade after only 2 months of daily application of topical vitamin C (15%) (personal observation, KEB). Melasma treated by QS-Nd:Yag laser (20) or fluorescent pulsed light (2) resolved more rapidly and effectively with application of topical vitamin C.

Because L-ascorbic acid may inhibit elastin biosynthesis (22), it may reduce the solar elastosis of photoaged skin. Also, in a histologic study of solar elastosis adjacent to skin cancers, patients with high oral vitamin C intake showed less solar elastosis (23). Topical vitamin C has also been shown to enhance collagen production in human skin. Postmenopausal women who applied 5% vitamin C to one arm and half of the neck with placebo to the other side showed an increase in mRNA of collagens I and III (24). Tissue levels of the inhibitor of metalloproteinase-I (MMP-I) were also increased, thus decreasing UV-induced collagen breakdown. However, mRNA levels of elastin, fibrillin, and tissue inhibitor of MMP-2 remained unchanged. Clinically, a significant decrease was observed in deep facial furrows and substantiated by silicone replicas. Histology showed decreased inflammation as well as deeper collagen and elastic tissue repair (24). Other studies demonstrated a decrease in the crepey laxity of forearm skin with restoration of a younger skinfold pattern after 6 months of once-daily treatment with 15% vitamin C.

This remarkable reversal of photoaging can be appreciated clinically in Figure 24.1. After 1 year of once-daily treatment with 15% topical vitamin C, wrinkles were clearly

reduced and mottled pigmentation resolved in both of the subjects shown. The skin acquired a healthy, more youthful glow.

Another important action of vitamin C on the skin is that topical vitamin C increases the synthesis of several specific lipids of the skin surface (25). Not only does this mean that vitamin C helps the natural moisturization of the skin, but it also enhances the protective barrier function of the skin (26). Interestingly, topical vitamin C significantly enhances the deposition of the sunscreen nanoparticles of zinc oxide and titanium dioxide on the skin without increasing their permeation (27). Thus the sunscreen efficacy is optimized without danger of absorption.

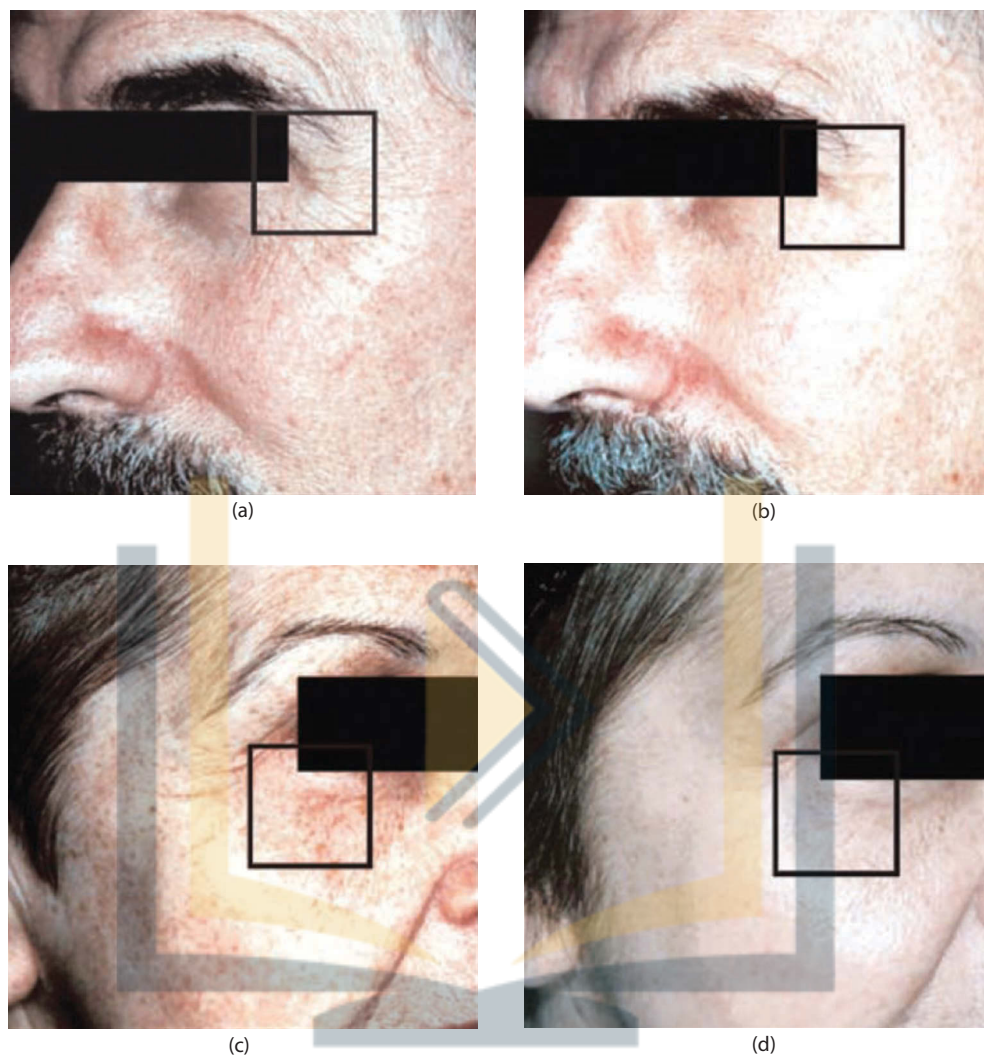
## VITAMIN E

Natural vitamin E is the most important lipid-soluble, membrane-bound antioxidant in the body. Vitamin E is especially abundant in the stratum corneum, delivered there by sebum (28,29). Its concentration is highest at the lower levels of the stratum corneum at the dermal depth of the sebaceous glands, with a decreasing gradient outward. As the outermost defense of the body, the stratum corneum is first to absorb the oxidative stress of sunlight and pollution. Vitamin E is depleted in the process, so topical application can be particularly advantageous, especially since the lipophilic structure makes it cosmetically attractive for application and absorption.

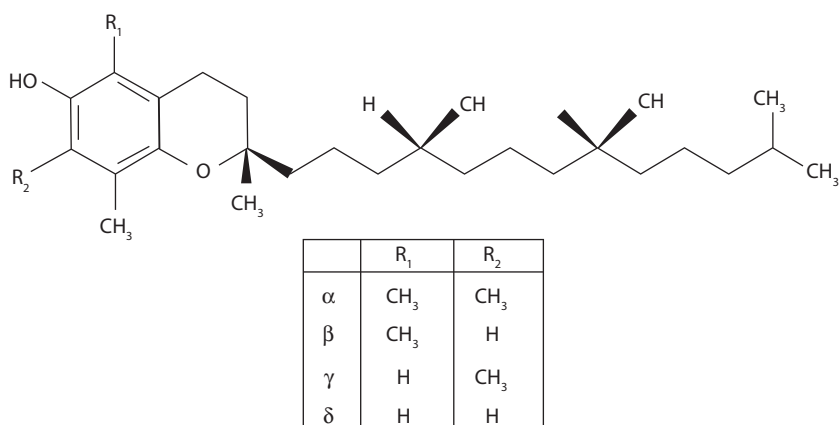
Several forms of vitamin E exist in natural dietary sources. The form that is found in mammalian tissues and which has by far the greatest biologic activity is pure, non-esterified RRR- $\alpha$ -tocopherol (or d- $\alpha$ -tocopherol) (30,31), which has three methyl groups on the 6-chromal ring (Figure 24.2). Humans use predominantly  $\alpha$ -tocopherol because a specific  $\alpha$ -tocopherol transfer protein selectively transfers  $\alpha$ -tocopherol into lipoproteins (32). The other natural forms are  $\beta$ ,  $\gamma$ , and  $\delta$  which contain only one or two methyl groups on the 6-chromal ring. Relative to the  $\alpha$  form, the  $\beta$ ,  $\gamma$ , and  $\delta$  RRR-tocopherols give only 42%, 72%, and 40%, respectively, of the protection against post-UV edema (33). The synthetic form is “dl” or “all-*rac*,” a mixture of eight stereoisomers. The synthetic isomers are esterified (to acetates and succinates) for use in commercial vitamins and some topical formulations because the esters are far more stable. This ester must be hydrolyzed before there is any biologic activity, a reaction which readily occurs in the stomach after oral ingestion or in cell and organ culture, but is very slow after topical application.

The skin has only a limited capacity to cleave the esterified forms of vitamin E to the active free tocopherol form, so the antioxidant potential of the esters is minimal (34,35). Furthermore, the all-*rac* form of vitamin E has been reported to cause allergic contact dermatitis (36) and erythema multiforme (37) when applied topically. No such adverse reaction has been reported with d- $\alpha$ -tocopherol. After application of topical d- $\alpha$ -tocopherol, the concentration of vitamin E increases by a factor of 10.6 (38), far higher than can be achieved by oral supplementation. To enhance stability and absorption, several formulations are being investigated, including liposomal encapsulation (10,39), microemulsions (10), and nanostructured lipid carriers and emulsions (40).

Previous studies have demonstrated protection from the acute (41–47) UV-induced damage of inflammation (erythema, “sunburn”) and hyperpigmentation (tanning) as well as protection from the chronic UV-induced damage of skin cancer (46–51) even by the various forms of vitamin E which are less metabolically potent when applied topically than the non-esterified Eol. Topical d- $\alpha$ -tocopherol was shown to be



**Figure 24.1** Correction of photoaging after 1 year of once-daily treatment with 15% vitamin C serum (SkinCeuticals). Notice the improvement of periorbital wrinkles and lightening of solar lentigines. (Photographs courtesy of SkinCeuticals, Dallas, TX, USA).



**Figure 24.2** Molecular structures of tocopherols.

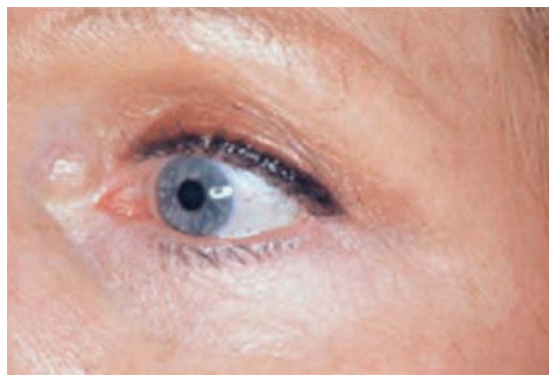
far more effective in protecting against all acute and chronic UV-induced damage than topical d- $\alpha$ -tocopheryl succinate in mice (38). In a model of chemically-induced skin cancer in mice, topical  $\alpha$ -tocopherol was further shown to protect against skin cancer and against lipid peroxidation with enhancement of several protective enzymes such as glutathione peroxidase and SOD (52). Studies showed decreased chemically-induced cell proliferation and decreased activation of p38 and NF-K $\beta$  signalling in HaCaT cells (52). In other mouse studies, topical  $\alpha$ -tocopheryl succinate and  $\alpha$ -tocopheryl acetate not only failed to inhibit UVB-induced immunosuppression and carcinogenesis, but also at higher concentrations were ineffective (53) or even appeared to enhance carcinogenesis (53–55). Topical  $\alpha$ -tocopheryl acetate was less effective than  $\alpha$ -tocopherol in protecting against UV-induced erythema in rabbits (54,56) and against UV-induced photoaging in mice (57).

Vitamin E has been demonstrated to reverse photoaging dramatically. A recent study showed that tocopherol enhanced collagen synthesis and inhibited collagen degradation by downregulating MMP gene expression in human fibroblasts stressed by exposure to hydrogen peroxide (58,59). This model further demonstrated that  $\gamma$ -tocopherol decreased stress-induced premature senescence and apoptosis by downregulating caspase-9 and caspase-3 (60). Also, vitamin E significantly stimulated collagen and fibrinectin synthesis in cultured human foreskin fibroblasts (61).

Figure 24.3 (62) shows the dramatic decrease in periorbital rhytides in a 48-year-old woman after 4 months of



(a)



(b)

**Figure 24.3** Correction of periorbital wrinkles after 4 months of once-daily treatment with 0.05% d- $\alpha$ -tocopherol cream.

daily application of d- $\alpha$ -tocopherol (5%). This correction of UV-induced damage was confirmed by histologic examination. Mice were exposed to UVB for six weeks, after which epidermal hypertrophy with thickened stratum corneum, an increased incidence of damaged “sunburn cells” in the basal layer, disruption of dermal collagen and degradation of dermal elastin, and dermal inflammation were noted, as shown in Figure 24.4 (62). Each group was then treated for 8 weeks with either vehicle cream, 0.05% retinoic acid, 5% d- $\alpha$ -tocopherol cream, or 0.05% L-selenomethionine cream (see following section), after which multiple biopsies from each mouse were evaluated (blind) by two experienced dermatopathologists. The histologic improvement in all parameters of photoaging can be appreciated in Figure 24.4, with a marked decrease in hyperkeratosis and epidermal hypertrophy, repair of damaged dermal collagen and elastin, and clearing of dermal inflammation after treatment with retinoic acid or with vitamin E. Each of these parameters were graded on a scale of 0 (no damage) to 4 (maximal damage) (62). As shown in Figure 24.5, in overall gradation, d- $\alpha$ -tocopherol cream was even more effective at reversing UV-induced damage than retinoic acid, the “gold-standard” of topical antiaging formulations. (KE Burke, L Ricotti, EG Gross, unpublished observation). Further electron microscopic analysis confirmed correction of collagen and elastin fiber damage and demonstrated repair of UV-induced disruption of the basement membrane anchoring fibrils.

## VITAMIN C WITH VITAMIN E

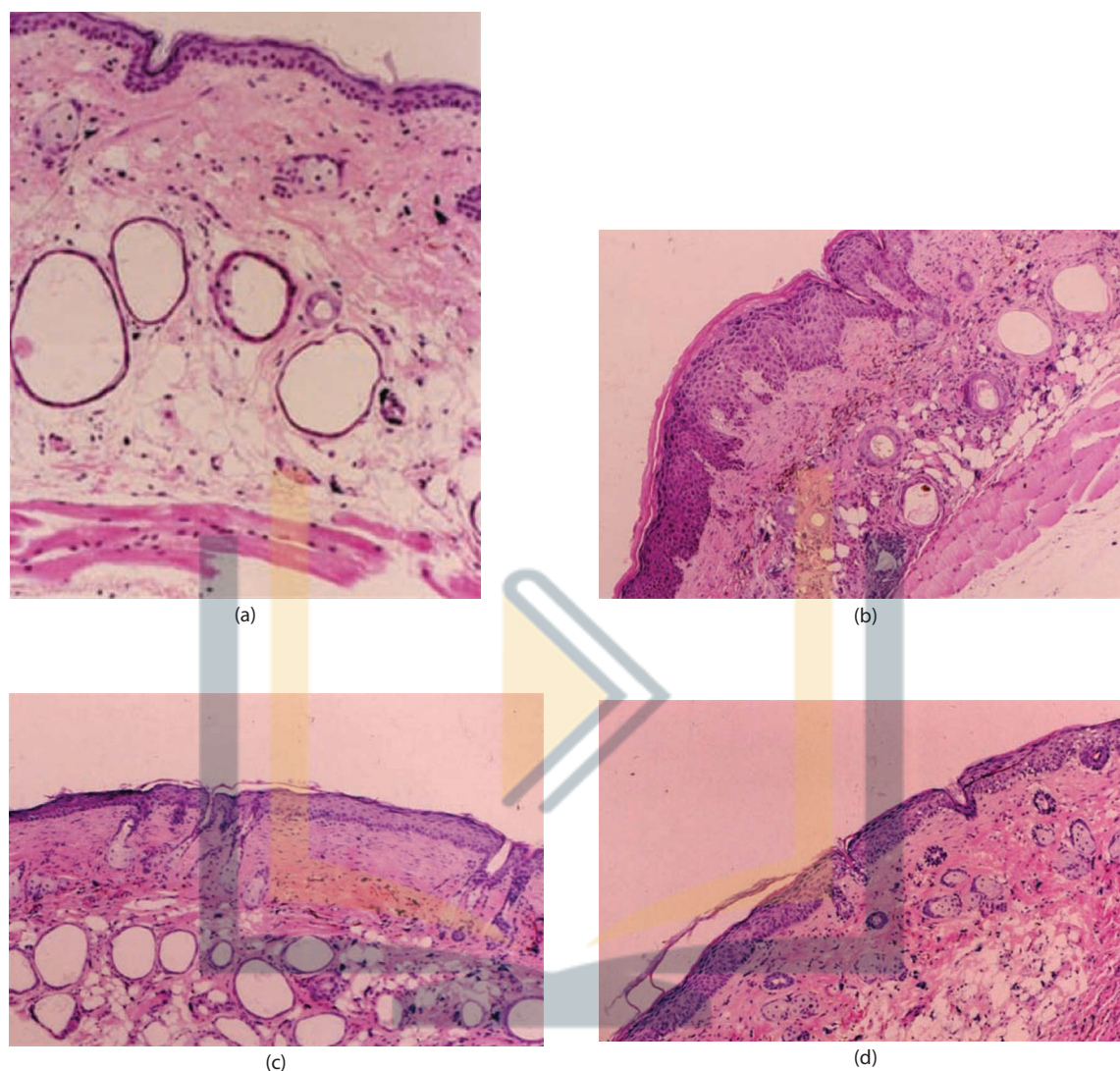
As presented above, the skin uses predominantly vitamin C to protect the aqueous environment and vitamin E to protect membranes from lipid peroxidation. Since vitamin C is naturally present intracellularly in relatively high concentrations, L-ascorbic acid not only acts directly as an antioxidant and as an essential cofactor in the synthesis of collagen, but also regenerates oxidized membrane vitamin E, so that the vitamin E need not be replaced (63). Oral vitamin C with E in high doses protects against UV-induced erythema in humans (64,65), whereas either vitamin alone is ineffective (65). Topical L-ascorbic acid (15%) with d- $\alpha$ -tocopherol (1%) gives four-fold protection against UV-induced erythema and thiamine dimer formation in porcine skin (66). This protection from UV-induced erythema (67) and tanning (68) by vitamins C and E combined with melatonin was further demonstrated in humans. Fortunately, mixing these hydrophilic and lipophilic antioxidants in a topical formulation stabilizes each (66) for a cosmetically attractive application.

## VITAMIN C WITH VITAMIN E AND FERULIC ACID

Ferulic acid is found ubiquitously and at high concentrations in plants (69–71) where it cross-links polysaccharides and proteins during lignin cell wall synthesis (72). A potent antioxidant, ferulic acid protects membranes from lipid peroxidation and neutralizes alkoxyl and peroxy radicals. It has also been shown to have synergistic interactions with ascorbic acid (73). Furthermore, ferulic acid itself minimally blocks UVB to give some sunscreen protection (see Figure 24.6).

After testing the effectiveness of a series of low molecular weight antioxidants that are available in chemically pure form, Zielinski and Pinnell (74) demonstrated that ferulic acid provides stability of more than 90% for L-ascorbic acid and 100% for  $\alpha$ -tocopherol. Addition of ferulic acid (optimally 0.5%) to the formulation of vitamin C (15%) + vitamin E (1%)





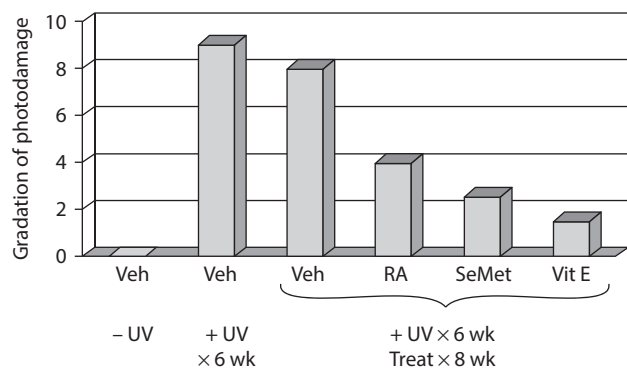
**Figure 24.4** Histologic correction of photodamage by topical antioxidants. Four groups of 10 Skh:2 mice were exposed to UVB 3 days per week for 6 weeks to photodamage the skin. Then each group was treated 5 days/week for 8 weeks as follows: (i) vehicle, (ii) 0.05% retinoic acid, (iii) 5.0% d- $\alpha$ -tocopherol, (iv) 0.05% L-selenomethionine (discussed in following section). (a) Biopsies taken before UVB exposure show a thin, outer keratin layer, normal epidermis, normally aligned, fine fibrillar dermal collagen and elastin, and no dermal inflammation. (b) Biopsies after UV exposure show a markedly thickened keratin layer, epidermal hypertrophy, degradation of dermal collagen and elastin, and prominent dermal inflammation. After treatment for 6 weeks with (c) topical 0.05% retinoic acid or (d) topical 5.0% d- $\alpha$ -tocopherol, there is reversal of hyperkeratosis and epidermal hypertrophy and repair of damaged dermal collagen and elastin with clearing of dermal inflammation (62). (The special stains that corroborate the degradation of collagen and elastin are not shown.)

doubles photoprotection to solar-simulated irradiation of skin from fourfold to approximately eightfold as measured by both erythema (as shown in Figure 24.6) and sunburn cell formation (75,76). Enhanced photoprotection was further demonstrated immunohistochemically by inhibition of UV-induced formation of thymine dimer mutations and of UV-induced p53 (76), both of which are associated with skin cancer (75–77). This formulation of vitamin C + E + ferulic acid did in fact decrease the tumor number and the tumor burden in mice when applied topically after UVB exposure but before the onset of the UVB-induced skin cancers (54) and when applied to mice before, during, and after UVB-exposure (10). However, other experiments did not confirm this protection (78). Evaluation by real-time polymerase chain reaction demonstrated suppression

of UV-induced cytokine mRNA formation (for inflammatory cytokines interleukin (IL)-1 $\alpha$ , IL-6, IL-8, and tumor necrosis factor (TNF)- $\alpha$ , as well as for the immunosuppressive cytokine IL-10) (76).

### VITAMIN C WITH FERULIC ACID AND PHLORETIN

Phloretin is another potent plant-derived phenolic antioxidant (found in both the flesh and peel of apples) which can be absorbed by the skin. When combined with vitamin C and ferulic acid, phloretin enhances photoprotection to UVA-induced erythema and pigmentation (79). Biopsies from human volunteers after UV exposure (89% UVA and 11%



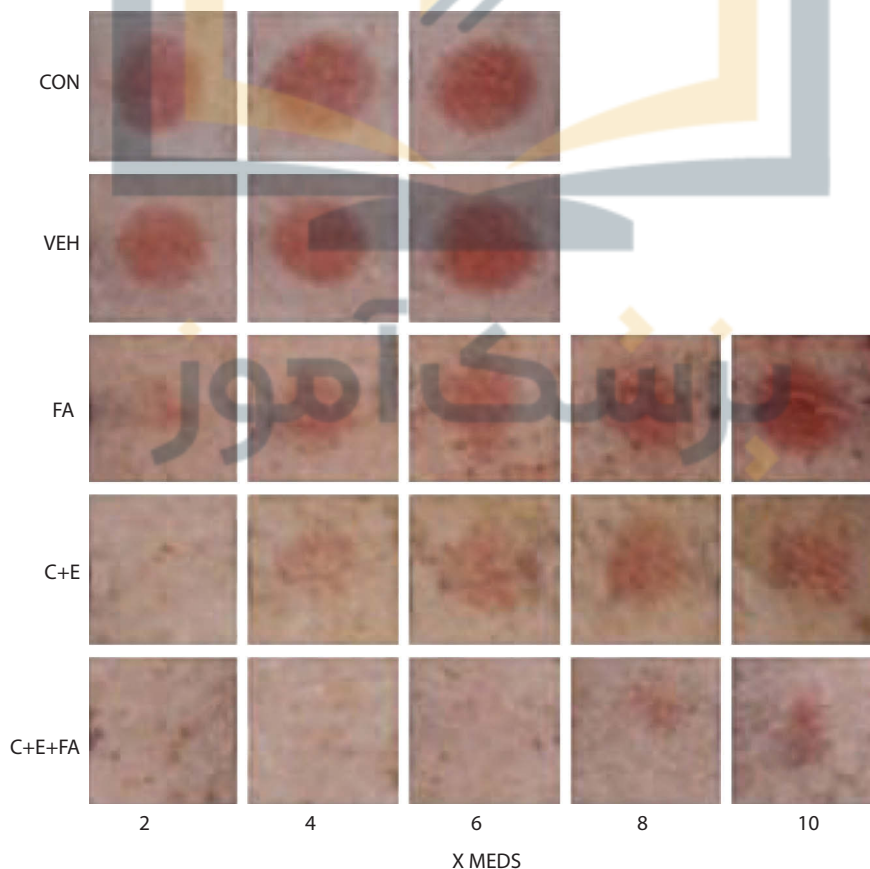
**Figure 24.5** Gradation of histologic correction of photodamage by topical antioxidants. Each of four histological parameters for photodamage (hyperkeratosis, epidermal thickness, collagen disruption, and solar elastosis) were graded on a scale of 0 (no damage) to 4 (maximal damage) and evaluated before exposure to UVB, after exposure to UVB, and after treatment for 8 weeks with vehicle (veh), 0.05% retinoic acid (RA), 0.05% L-selenomethionine (SeMet), or 5.0% d- $\alpha$ -tocopherol (vit E). The average gradation of all parameters of damage is shown (62).

UVB) and treatment with a formulation containing vitamin C (10%), ferulic acid (0.5%), and phloretin (2.0%) at pH = 2.5, demonstrated significant photoprotection as measured by decreased thymine dimer formation, decreased matrix metalloproteinase-9 expression, and decreased p53 expression (79). This topical serum also protected against UV-induced immunosuppression. There was no UV-induced decrease in CD1a-expressing Langerhans cells when skin was pretreated with vitamin C + ferulic acid + phloretin (79).<sup>7</sup>

## SELENIUM

Selenium, an essential trace element in humans and animals, is required by 25 selenoproteins and selenium-dependent enzymes. Most important to skin care are the intracellular antioxidant enzymes glutathione peroxidase and thioredoxin reductase (80,81). Selenium has been shown to have other protective effects that may not directly involve selenium-dependent glutathione peroxidase activity (82), such as protecting (83) and repairing DNA (84,85), reducing the DNA binding of carcinogens (86), inhibiting neoplastic transformation (87), and suppressing gene mutations at the lysine and histidine loci (88).

For decades, selenium has been implicated in reducing carcinogenesis. In animal tumor models, moderate selenium supplementation at levels above the dietary requirements



**Figure 24.6** Photoprotection by topical antioxidant formulations. Skin was (a) untreated or pretreated with (b) vehicle, (c) 0.5% ferulic acid, (d) 15% vitamin C + 1% vitamin E, or (e) 15% vitamin C + 1% vitamin E + 0.5% ferulic acid and irradiated with solar-simulated radiation (2×–10× minimal erythema dose [MED]). Visual erythema 1 day later is shown. (From Murray JC, Burch JA, Streilein RD, et al., *J Am Acad Dermatol* 2008; 59:418–25. With permission.)

decreases the number of tumors induced by several chemical carcinogens (89) and viruses (90), and reduces the incidence of spontaneous mammary tumors (91). In addition, selenium supplements inhibit the growth of human tumor cell lines (92) as well as the growth of transplanted tumors in mice (93), and decrease the mutagenic activity of several known carcinogens (94–96).

Recent research has elucidated precise anticarcinogenic mechanisms, as excellently researched by Jackson and Combs (97). Selenium metabolites have been shown to impair angiogenesis by tumors (98,99) and to promote cell cycle arrest and apoptosis of tumor cells (100), as well as inhibit local invasion and migration (99,101), even inhibiting pulmonary metastases of melanoma and melanoma migration (101,102) and metastases in mice, possibly by down-modulation of IL-18 expression (103). That Se can inhibit malignant spreading was demonstrated by Yan and colleagues for several oral forms of the element: methylseleninic acid (99), selenite (101), and high-Se soy protein (102). Their work showed that this effect involves reducing adhesion of cancer cells to extracellular matrix (104), inhibiting the urokinase plasminogen activator (uPA) system (99) and reducing angiogenesis (99). Also, two FDA-approved inhibiting histone deacetylase compounds with selenium prevent early melanocytic lesion development in skin by inducing apoptosis (105). These are currently being investigated as potential melanoma chemopreventative agents, as is selenium incorporated into the DNA alkylating agent temogolomide for treatment of melanoma and glioma (106). Selenium metabolites further enhance metabolism of foreign compounds including carcinogen-detoxifying enzymes (107).

Some, but not all, epidemiological studies have found a reduced risk for several kinds of cancer associated with a higher blood concentration of selenium (108–110). A decreased selenium concentration and glutathione peroxidase activity in blood, and interestingly, an increase of these parameters in malignant tissue was found in lung cancer patients (110). Patients with malignant melanoma were found to have significantly lower levels of serum selenium, and patients with more advanced disease (stage III disseminated melanoma) had the lowest levels (111). In a transgenic mouse model, topical treatment with L-selenomethionine resulted in a significant delay in the time required for melanoma development (though established tumors grew more rapidly) (112). Patients with cutaneous lymphoma (Sezary syndrome as well as mycosis fungoides) were found to have decreased serum titers of selenium, and stages III and IV had significantly lower levels than the earlier stages I and II (111). Also, the patients with Sezary syndrome or mycosis fungoides who responded less well to therapy were found to have lower levels of selenium (111).

A study of 240 non-melanoma skin cancer patients in good general health demonstrated a significantly lower mean plasma selenium concentration than control subjects without skin cancer (113). In fact, those patients whose blood concentrations were in the lower decile had 4.4 times the incidence of skin cancer as those in the highest decile (113).

This interesting correlation led to a 10-year prospective study of 1312 patients with a history of at least two basal cell carcinomas and/or one squamous cell carcinoma of the skin as well as at least one skin cancer within one year before entering the trial, selenium treatment did not initially protect against further development of such skin cancers; however, it did reduce total cancer incidence and the incidence of lung, colorectal, and prostate cancer significantly (to 42%, 42%, and 37% incidence, respectively) as well as total cancer mortality

(to 50%)(114). The lack of protection against skin cancer during the first two years of supplementation may suggest that the degree of damage at the onset of the trial was sufficiently severe to preclude reversal of oncologic potential by selenium supplementation. This initial minimal increase in skin cancer (by about 10%) was not observed for skin cancers diagnosed after the first two years of treatment.

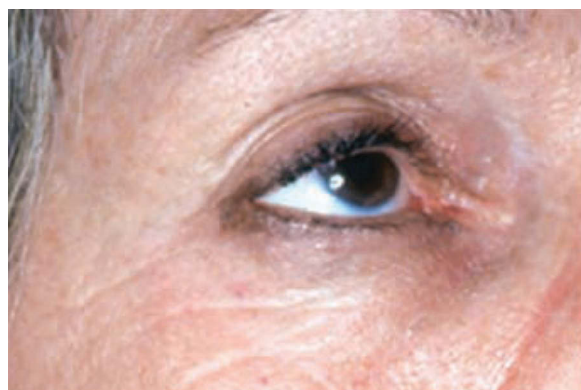
Topical preparations containing selenium sulfide are frequently used for the treatment of tinea versicolor (115), seborrheic dermatitis (116), and dandruff (117). However, the selenium from these preparations is not absorbed by the skin (117). Selenium can be absorbed transdermally in guinea pigs when applied as L-selenomethionine (118). After topical application of 0.02% L-selenomethionine to mice, skin levels of selenium increase by a factor of two to five over comparable oral doses (119), and after application of 0.05% L-selenomethionine by a factor of 8.0 (62). This formulation increased the MED in humans (120) and decreased UV-induced skin damage, as demonstrated by a decrease in post-UV tanning and skin cancer in Skh:2 mice (119).

With topical application of L-selenomethionine, selenium levels were also shown to increase significantly ( $\times 5$ ) within tumor tissue. Thus, there was a possibility that the timing of L-selenomethionine application could affect the degree of inhibition of UVB-tumorigenesis (or maybe even enhance tumorigenesis at some stage). Thus an investigation was undertaken applying topical L-selenomethionine (a) before, during, and after UVB exposure, (b) before UVB-exposure but discontinuing when tumors were first clinically detected, or (c) only after tumors were first detected and continuing thereafter (121). Optimal inhibition of skin cancer was achieved by application of topical L-selenomethionine before, during, and after exposure. Unfortunately, statistically significant protection was not seen with L-selenomethionine application only prior to tumor detection. The good news is that significant protection was also attained with application only after the onset of tumors. Thus even beginning L-selenomethionine supplementation late in the process of tumorigenesis can help protect from UV-induced photodamage and skin cancer (121).

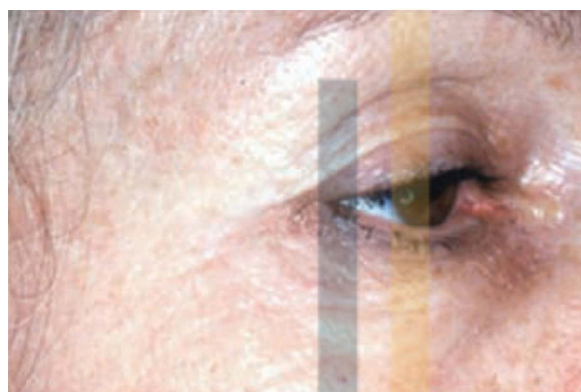
Topical L-selenomethionine is highly effective not only in preventing but also in reversing photoaging (62). Because topical L-selenomethionine penetrates transdermally, both the epidermis and dermis are protected, so previous damage can be repaired. As a cofactor for the glutathione peroxidases, selenium quenches ROS, thus decreasing inflammation and preventing activation of mediators which induce the metalloproteinases (MMPs) that would otherwise degrade collagen and elastic tissue. Also, selenomethionine decreased malondialdehyde (MDA, a measure of ROS) and protects normal human skin fibroblasts from UVA-induced metalloproteinases MMP-1 and MMP-3 which break down collagen (122), directly causing wrinkles. As a cofactor for thioredoxin reductase, melanin synthesis is inhibited and solar hyperpigmentation is corrected (123,124).

The significant decrease in periorbital rhytides in a 56-year-old woman after 4 months of daily application of L-selenomethionine (0.05%) can be seen in Figure 24.7. This correction of UV-induced damage was similar to that of topical vitamin E as discussed previously and confirmed by histologic examination as shown (for topical vitamin E) in Figure 24.4. All histologic parameters of photodamage demonstrated reversal after treatment with topical L-selenomethionine (62). Transmission electron microscopy further confirmed repair of dermal photoaging, as shown in Figure 24.8 (62). Normal, non-UV-damaged dermis (Figure 24.8a) consists of





(a)



(b)

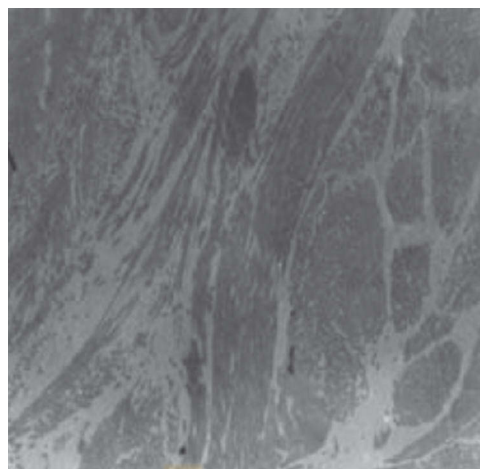
**Figure 24.7** Correction of periorbital wrinkles after 4 months of once-daily treatment with 0.05% L-selenomethionine lotion.

dense collagen with homogeneous bundles of uniformly dispersed fibers aligned parallel to the basal lamina above. The dermal-epidermal junction shows anchoring fibrils of dense collagen. After exposure to UV (Figure 24.8b), the dermal collagen is sparse; bundles are not uniform and are irregularly dispersed; fibers are disoriented and fragmented. There is an increase in extracellular mucopolysaccharide matrix. The dermal-epidermal junction has sparse collagen with a decrease in anchoring fibrils. Treatment with topical L-selenomethionine did indeed reverse this UV-induced damage (Figure 24.8c): dermal collagen was repaired, fibers became oriented and more regular. As collagen became denser, the increase in extracellular mucopolysaccharide matrix resolved. Collagen was replaced in the dermal-epidermal junction with repair of the anchoring fibrils. Clearly topical L-selenomethionine is effective in reversing photodamage of the skin (62).

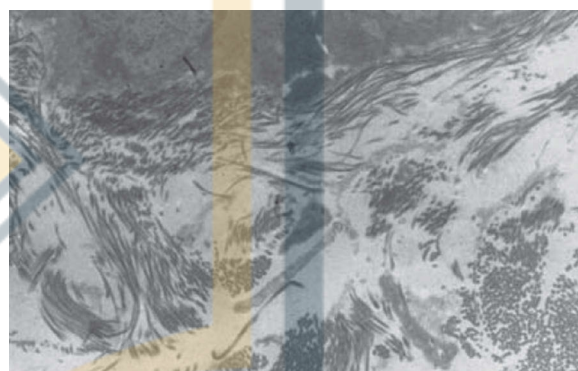
### SELENIUM WITH VITAMIN E

In many biologic systems, vitamin E and selenium often act synergistically. Borek et al. (125) demonstrated that selenium and RRR- $\alpha$ -tocopheryl succinate (natural vitamin E succinate) act alone by different mechanisms to prevent radiogenic and chemically induced transformation. They further showed that there was additive protection when both were used together (125).

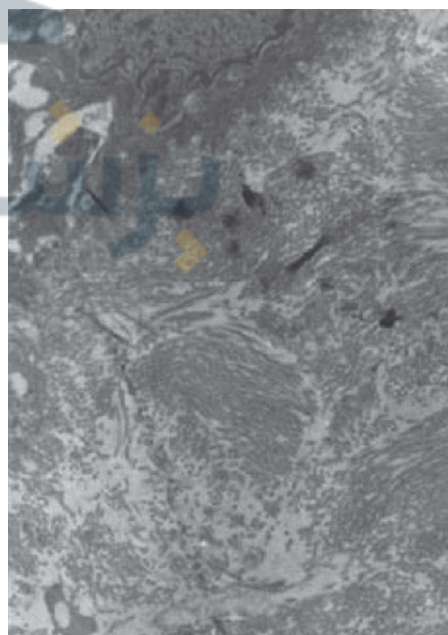
In experiments in mice comparing and combining topical L-selenomethionine with topical d- $\alpha$ -tocopherol (126), the



(a)



(b)



(c)

**Figure 24.8** Reversal of photodamage by topical antioxidants: transmission electron microscopy. (a) Before UV exposure, (b) after 6 weeks of UV exposure, (c) after treatment for 8 weeks of UVB-exposed skin with topical 0.05% L-selenomethionine (62).

topical combination was less effective than topical vitamin E alone—both in prolonging the onset and in decreasing the incidence of UV-induced skin cancers (126). However, topical L-selenomethionine with oral vitamin E was more effective than either alone. Both forms of vitamin E alone were equally effective and more effective than topical L-selenomethionine alone (126). Topical L-selenomethionine (alone or in combination with each form of vitamin E) was most effective in preventing the UV-induced inflammation of sunburn (100% effective!) (126). In reducing UV-induced pigmentation, topical L-selenomethionine with topical or with oral vitamin E was more effective than either one of these antioxidants alone, particularly during the first 8 weeks of UV exposure.

## GENESTEIN

Recent epidemiologic analysis comparing Asian diets high in soy (average daily intakes of 20–150 mg) (127) with American diets (average daily intake of only 1–3 mg) (127) indicate that soy confers major health benefits by decreasing the incidence of cancer (127–130) and reducing cardiovascular disease (128). Since genistein is the most plentiful isoflavone in soy, this molecule has been the most extensively studied component for its chemopreventive and anticancer effects.

Extensive experimental evidence documents the direct anticarcinogenic action of genistein. Animal studies demonstrate protection against bladder, breast, colon, liver, lung, prostate, and skin cancer (127,131) with oral genistein. Growth of many cancer cell lines is inhibited by genistein (131). Dietary soy inhibits chemically induced skin cancer in mice (132). Exciting research demonstrates that genistein arrests the growth and induces the differentiation of malignant melanoma (133) and inhibits pulmonary metastases of melanoma (134,135).

The mechanism by which genistein inhibits carcinogenesis may be through its proven inhibition of tyrosine protein kinases (TPKs), the enzymes that phosphorylate proteins necessary for regulation of cell division and transformation (136). Of particular importance is phosphorylation of TPK-dependent epidermal growth factor receptors (EGF-R) which are related to tumor promotion, including initiation of transcription factors, release of inflammatory mediators (such as prostaglandins), and stimulation of cell proliferation (137). Genistein was found to down-regulate both UVA- and UVB-induced EGF-R phosphorylation in human epidermoid carcinoma cells (138). By similar enzymic inhibition, genistein retards UV-induced apoptotic changes – including caspase-3 and p21-activated kinase 2 activation of human epidermal carcinoma cells (139) and phosphokinase C $\delta$  in human keratinocytes (140). Recent experiments further show that genestein inhibits carcinogenesis in pancreatic (141) and ovarian cancer cells (142) by down-regulation of oncogenic microRNAs. Hopefully this mechanism will be investigated in UV-exposed skin.

Genistein is also a potent antioxidant. Genistein scavenges peroxy free radicals, thereby protecting against lipid peroxidation (143) and (144). The decreased incidence of cardiovascular disease with high soy diets may be due to genistein's inhibiting the oxidation of low-density lipoprotein (LDL) cholesterol in both aqueous and lipophilic environments. Of direct importance in protection of the skin from UV-induced damage, genistein has been shown to inhibit UV-induced DNA oxidation (145) and cellular DNA oxidation induced by benzopyrene and UVA (146) and by phorbol ester stimulation (147), as well as by psoralen plus UVA (PUVA) therapy (148). The fact that

genistein also reduces erythema and histologic inflammation induced by PUVA in mouse skin (149) may have important implications for PUVA therapy, by reducing possible short- and long-term adverse reactions.

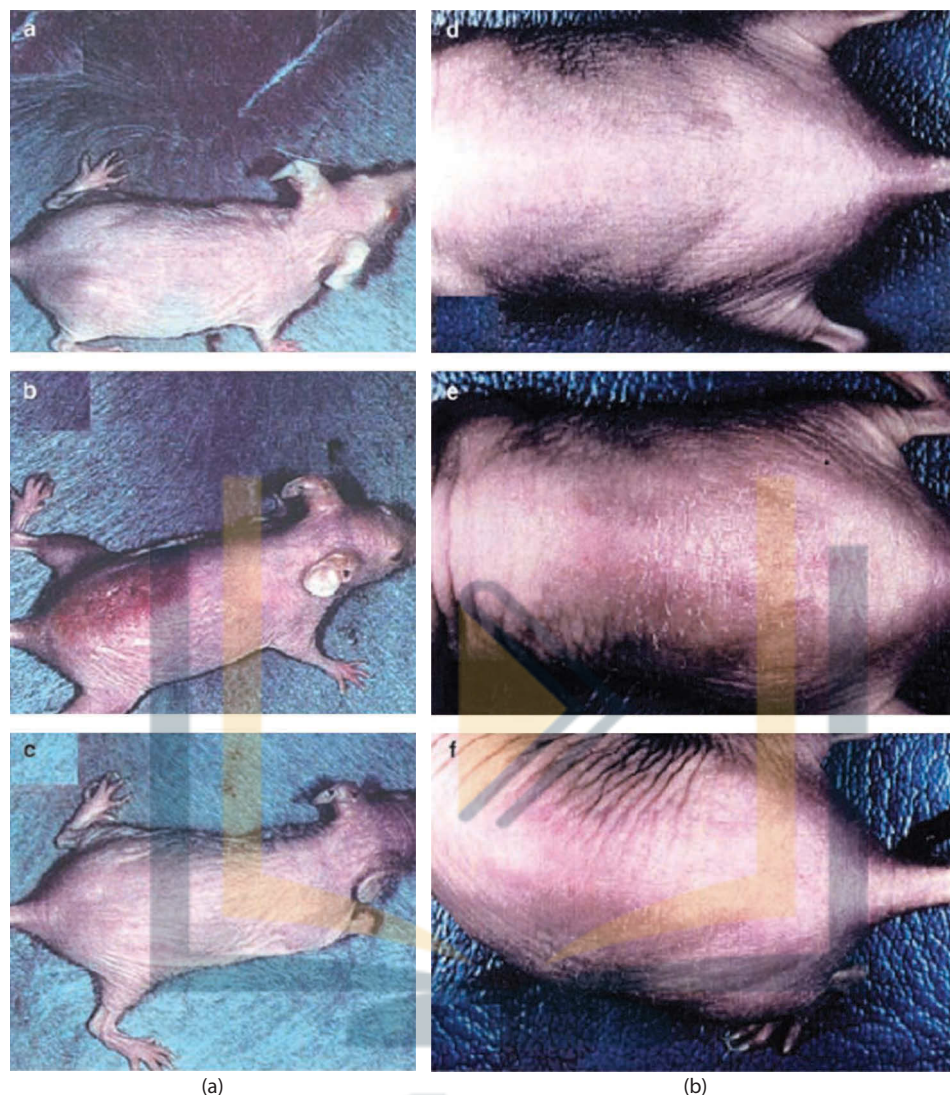
The use of topical genistein to prevent and reverse photoaging has recently been intensively investigated, with promising results. In animal studies, Wei et al. (138) demonstrated that topical genistein (10  $\mu\text{mol}/\text{cm}^2$ ) protects against acute and chronic UV damage to the skin. After exposure of Skh:1 hairless mice to UVB, topical genistein completely blocked acute skin burns and inhibited UVB-induced cutaneous wrinkling (as seen clinically in Figure 24.9) (138). This protection from UV-induced erythema with topical genestein (0.5%) was further confirmed in pig skin by an increase in MED and a decrease in number of sunburn cells after pretreatment with topical genestein (150). Histologic analysis in mice showed that topical genistein substantially blocked the signs of chronic photodamage: epidermal hyperplasia and reactive acanthosis with nuclear atypia (138). At a molecular level, UV-induced damage to DNA as measured by the biomarker 8-hydroxy-deoxyguanosine was markedly reduced (151). Protection was also demonstrated by decreased expression of proliferating cell nuclear antigen (PCNA) (a marker of DNA repair which indirectly indicates degree of UV damage) as well as by decreased cox-2 expression and by increased catalase concentration in mice treated with topical genestein (1–3mg/ml) prior to UVB exposure (152). Further recent experiments in mice corroborated protection from UVB exposure by measuring inhibition of UVB-induced increased levels of nitric oxide (NO), which lead to nitrosative skin injury. Compared to the UV-control group, the group treated with oral genestein (10 mg/kg) showed tissue protection, decreased lipid peroxide and nitrotyrosine formation, low CAT activity (153) and DNA repair. Interestingly, higher doses of genestein (15 mg/kg) showed more histologic damage and less efficient protection against lipid peroxide formation (153).

Inhibition of acute UV-induced erythema with topical genistein was also demonstrated in humans. Topical genistein (5  $\mu\text{mol}/\text{cm}^2$ ) (applied 30 minutes before UVB radiation) inhibited by one MED the UVB-induced erythema (138). Further immunohistologic studies on human reconstructed skin demonstrated that pretreatment with genestein (10–50  $\mu\text{M}$ ) inhibits UV-induced DNA damage, as measured by inhibition of pyrimidine dimer formation and of expression of PCNA (154). The degree of protection is dose dependent, increasing with increasing concentrations of genestein (154). Other experiments using UVB-irradiated human skin cells showed 34%–35% enhancement of DNA damage repair four hours after treatment with the soy isoflavones genestein:daidzein (in a ratio of 1:4 at a concentration of 10  $\mu\text{mole}/\text{L}$ ) (155).

Equally impressive is the fact that topical genistein also inhibits the most serious consequence of chronic UVB damage: skin cancer. Both the incidence and the multiplicity of UVB-induced skin tumors in Skh:2 hairless mice were reduced by about 90% after 25 weeks of UVB exposure (138). Furthermore, after induction of skin tumors in mice by 7,12-dimethyl benzantracene (DMBA) and promotion by 12-O-tetradecanoylphorbol-13-acetate (TPA), topical genistein (10  $\mu\text{M}$ ) inhibited tumor numbers by 60%–75% (151).

In order to investigate the mechanism of inhibition of UV-induced skin cancer, the effect of genistein on the expression of the photo-oncogenes *c-fos* and *c-jun* on mRNA was investigated in mouse skin (156). Expression of these proteins induces matrix metalloproteinases that degrade dermal





**Figure 24.9** (a) Effect of genistein on ultraviolet B (UVB)-induced acute skin burns. Once daily for 10 days, SKH-1 hairless mice were treated with 5  $\mu\text{mol}$  of genistein 60 minutes before UVB irradiation (1.8  $\text{kJ}/\text{m}^2$ ). Photographs were taken 24 hours after final UVB irradiation. (a) Negative (sham) control; (b) vehicle + UVB; (c) 5  $\mu\text{mol}$  of genistein + UVB. (b) Effect of genistein on photodamage in mouse skin chronically exposed to UVB. Twice weekly for 20 weeks, SKH-1 hairless mice were treated topically with 5  $\mu\text{mol}$  of genistein 60 minutes before or 5 minutes after UVB irradiation (0.3  $\text{kJ}/\text{m}^2$ ). (d) Negative (sham) control; (e) vehicle + UVB; (f) UVB + genistein. (From Wei H, Spencer J, Gelfand J et al., *Cosmet Dermatol* 2001; 14:13–19. With permission.)

connective tissue, causing the wrinkles and laxity of photoaging (157). Overexpression of these photo-oncogenes is related to promotion of cell proliferation in oncogenesis. At low-dose UVB, genistein (20 $\mu\text{M}$ ) substantially inhibited both; at high dose, genistein blocked *c-fos* but had little effect on *c-jun* (156). Treatment of mouse skin immediately after UVB irradiation also inhibited the expression of both (156). In human skin, topical genistein (1%) did prevent UV-induced *c-jun*, thereby preventing photoaging and oncogenesis (157).

Another possible dermatologic benefit of genistein is as a phytoestrogen. The skin has both  $\alpha$  and  $\beta$  nuclear estrogen receptors (ER) (158), through which estrogen binding can regulate linked genes of proliferation and differentiation. Genistein has a 30-fold higher affinity for ER $\beta$  than ER $\alpha$  (159) but a greater ER $\alpha$  agonist activity than ER $\beta$  (160). Although estradiol has 700-fold more ER $\alpha$  and 45-fold more ER $\beta$  activity than genistein, the

possible biologic effect of genistein (even through dietary soy isoflavones) may be great. The modulation of estrogen receptors by genistein is being actively investigated in breast cancer in which ER $\beta$  expression indicates more benign tumors and ER $\alpha$ , more malignant tumors (161). Experiments demonstrate that genistein has antiproliferative effects on some breast cancer cells (162,163), especially those with a low ER $\alpha$ /ER $\beta$  ratio (163). The potential benefits and risks of treatment with genistein are dependent on the ER $\alpha$ /ER $\beta$  ratio for each particular breast cancer and on genistein concentration (163,164).

Oral (165,166) and topical (167,168) estrogen increases the collagen content of skin, which diminishes with aging, and especially dramatically in women during and after menopause (169). Genistein may reduce the “crepey” appearance of aging skin by stimulating collagen synthesis. Indeed, genistein does increase collagen gene expression in fibroblasts (170).



**Table 24.1** Correction of Photoaging with Topical Estrogens

	$\beta$ -Estradiol (0.01%)	Genestein (4%)
↑ Epidermal thickness	75%	20%
↑ Number of dermal papillae	135%	0%
↑ Fibroblasts	123%	0%
↑ Blood vessels	77%	36%

*Note:* Biopsies of preauricular skin were taken before and after topical gel treatment once/day for 24 weeks and analyzed by image digitalization (173).

In other studies on normal human fibroblasts exposed to chemical oxidative stress, genestein prevented disturbances in the insulin-like growth factor-1 receptor-mediated collagen signalling pathway (171). Also, treatment of cultured human dermal fibroblasts with soy extract increased synthesis of collagen and hyaluronan (172). Topical application of a soy extract emulsion ameliorated UV-induced flattening of the dermo-epidermal junction and enhanced the number of dermal papillae—thus demonstrating histologically rejuvenation of photoaged human skin (172). Armed with these auspicious studies, a 6-month study of postmenopausal women compared topical  $\beta$ -estradiol (0.01%) with topical genestein (4%) (173). Skin biopsies showed that topical estrogens gave more correction of photodamage than did genestein, as summarized in Table 24.1.

Thus, topical genistein shows great promise not only in protection of the skin against extrinsic acute and chronic UV photodamage, but also in enhancing collagen synthesis, which is diminished with normal intrinsic aging. Because genestein has low water solubility, enhancement of topical delivery to deeper layers of the skin is being developed by formulating with hydrophilic cyclodextrins (174) or within solid lipid nanoparticles (175).

## SUMMARY

There are two great advantages to applying an active formulation of topical antioxidant(s) to the skin. First, the skin attains far higher levels of each antioxidant than can be achieved by only taking these supplements orally (5). For example, the level of vitamin C attained in the skin by topical application is 20–40 times the level achievable with oral vitamin C (5); with topical application, the concentration of vitamin E increases by a factor of 11 (38), and selenium by a factor of 2–8 (62,119). Second, topical application arms the skin with a reservoir of antioxidant(s) that cannot be washed or rubbed off, a protection which stays in the skin for several days after application (5).

Because topical antioxidants work by affecting cellular mechanisms of photoprotection, they supplement sunscreens in preventing photoaging and skin cancer. Indeed, scientific research confirms that topical application of antioxidant(s) offers exciting new possibilities not only in protecting the skin from UV damage (to reduce extrinsic photoaging and to retard intrinsic aging) but also in reversing previously incurred photodamage.

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# Actinic Keratosis

Brigitte Dréno

Actinic keratoses (AK), which are also referred to as solar keratoses, are atypical areas of keratinocyte proliferation and differentiation that occur on sun-damaged areas (Figure 25.1). In places such as northwest England. They affect 15% of 40-year-old men and 34% of 70-year-old men (1). In women the prevalence is lower, with 6% of 40-year-old women and 18% of 70-year-old women being affected (1). In the southern hemisphere, where photoexposure is high, 40% to 60% of elderly populations with a light skin phototype develop AK (1–5).

Risk factors for the development of AK include chronic sun exposure, light skin phototype (I/II), phototherapy, age, exposure to skin carcinogens, history of cutaneous squamous cell carcinoma (SCC), organ transplantation, and long-term immunosuppressive treatments such as chemotherapy or biotherapy (6,7). Recently, cutaneous infection by betapapilloma virus has also been identified as a potential risk factor when combined with other risk factors such as sun exposure, age, and light skin phototype (8,9).

## DIAGNOSIS

AK are diagnosed based on their clinical appearance with no additional investigations being required. Lesions are keratotic, rough on palpation, of variable thickness, and poorly delimited. They are characterized by a diameter of 1 cm or less and a variable degree of erythema. In some cases, they can be pigmented or have a cutaneous horn shape. As AK rarely occur alone, multiple AK are often found in surrounding photoexposed areas.

AK are considered to be a risk marker for cutaneous malignancies, and by some an early stage of SCC (10). As a result, each lesion needs to be evaluated individually. If the skin is thickened, the diameter is >1 cm. If infiltration, inflammation, ulceration, bleeding, or pain on palpation are present, or if there is rapid expansion, the AK should be biopsied (11). In addition, a biopsy should be performed if the lesion recurs within 2–3 months, if it persists after standard treatment, or if it is located on a risk zone such as the lip, the back of the hand, or the ear.

## PROGRESSION TO SQUAMOUS CELL CARCINOMA/SPINOCELLULAR CARCINOMA

Although no clinical, histological, or biological characteristics have been able to reliably predict the progression of an AK lesion (Figure 25.2) to invasive SCC, a clinicopathological continuum of transformation from AK to invasive SCC is believed to exist (10). The relationship between any given AK lesion and the development of SCC, however, is not necessarily linear. If left untreated, AK can either spontaneously disappear, persist without progressing to invasive SCC, or progress to invasive

SCC (12). In fact, the overall rate of progression from AK to SCC is low, with the rate of transformation to SCC over the course of a year ranging from 0.01% to 0.24%, and over 10 to 25 years from 5% to 20% (13–16). Spontaneous regression occurs at a rate of 15% to 25% (15). Fifteen percent of lesions that have regressed recur within a year (17).

## THE FIELD OF CANCERIZATION

The complicated relationship between spontaneous regression, recurrence, and progression is likely to be due to the existence of a field of cancerization, which describes the area of skin surrounding a group of AK and which has been shown to contain genetically altered cells that can provide a reservoir for clonal expansion (17). In any given field of cancerization (Figures 25.3 and 25.4), the ratio of subclinical foci to AK would be expected to be around 10:1 (17). As a result, it is hard to determine if an AK that spontaneously regresses but then recurs is an actual recurrence or the manifestation of another foci within the same field of cancerization.

In most cases the field of cancerization contains subclinical changes in the periphery of the visible AK lesions that can be highlighted using noninvasive reflectance confocal microscopy or photodiagnosis with protoporphyrin IX-emitted fluorescence (17). More invasive analyses that require tissue samples such histology or molecular biology can also be used (18,19).

The concept of field cancerization suggests that the apparently normal skin circling areas of AK sustains the base for the clonal expansion of genetically altered neoplastic cell. The presence of a great number of mutated cells in a field is considered the determinant event to carcinogenesis (17). Moreover, the association of multiple mutations of the keratinocytes and a decrease in cutaneous innate immunity appear to be risk factors. These two factors worsen over time lead to the final formation of AK.

## CHRONICITY AND SECONDARY PREVENTION

The fact that lesions that have spontaneously regressed can recur suggests that there is a chronic nature to the management of AK (17). Whether these are the same lesions or a new lesion from another cell from the same field of cancerization is hard to determine. The clinical implications, however, are the same: at-risk patients should be monitored at least once a year.

Viral infection may be associated with the chronicity of AK. In one study in which 37% of AK lesions were positive for human papilloma virus (HPV) (20), AK lesions recurred within 45–60 days of removal by laser in all HPV positive patients, whereas they did not recur in the HPV negative patients.

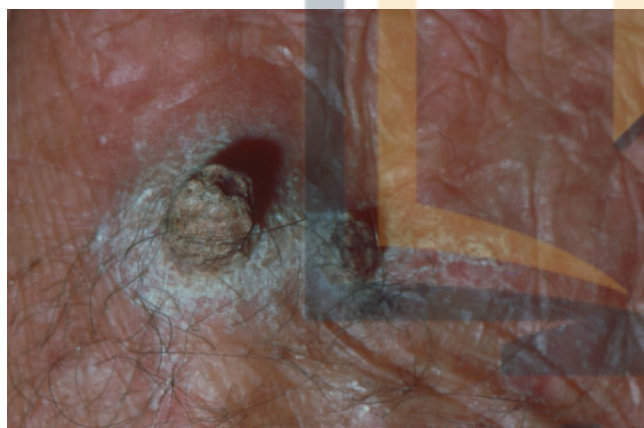




**Figure 25.1** Isolated actinic keratosis.



**Figure 25.3** Field of cancerization.



**Figure 25.2** Hyperkeratotic actinic keratosis.



**Figure 25.4** Field of cancerization.

These data suggest that HPV infection may be an indicator for recurrence.

Organ transplant patients also have a particularly high rate of recurrence (21). Depending on the degree and duration of immunosuppression and the type of transplant, recurrence, as well as the development of new AK, resistance to treatment, and progression to SCC have all been shown to be increased (21,22). These patients are thus high risk and require close monitoring.

### TREATMENT ALGORITHM

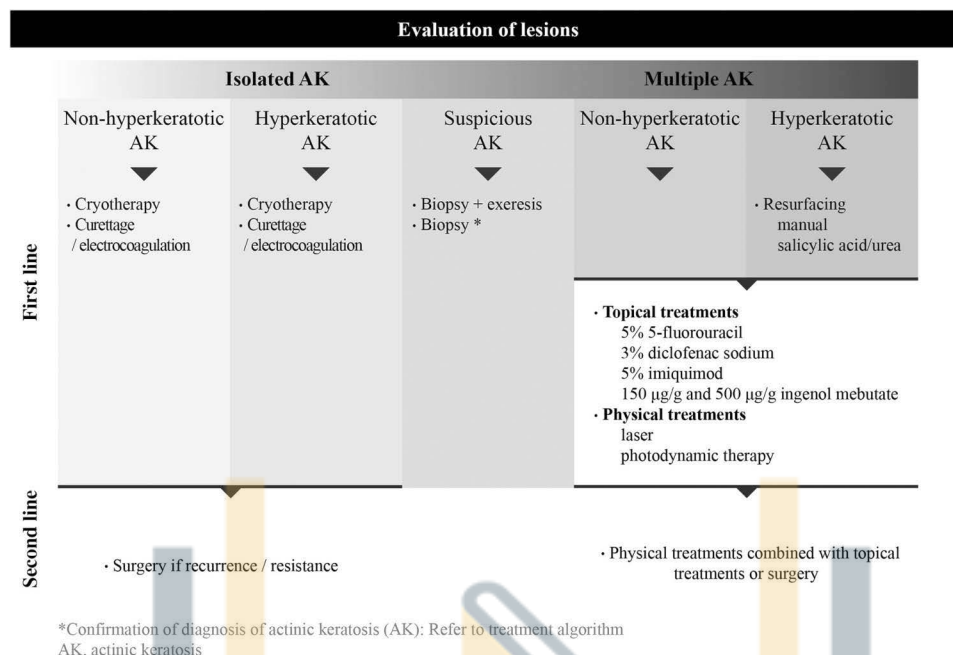
There is no evidence that shows that all AK need to be treated systematically. Rather, the decision to treat should be based on the clinical characteristics of the AK lesion, the patient's cutaneous history, the impact of the lesion on the patient's quality of life, and the patient's preference.

The initial choice of treatment should be based on the number of lesions, their hyperkeratotic status, and their clinical appearance. The algorithm shown in Figure 25.5 was recently published by Dréno et al. (7).

### ISOLATED HYPERKERATOTIC AND NONHYPERKERATOTIC ACTINIC KERATOSIS

For isolated hyperkeratotic and nonhyperkeratotic AK, cryotherapy and curettage followed by electrocoagulation are first-line therapies (23–29). Cryotherapy is a simple, rapid, and inexpensive technique. Rates of complete clearance after one and two applications are between 68% and 75% at 3 months and between 82.5% and 88% at 24 weeks after two applications. Cryotherapy is very much dependent on the protocol used, with more aggressive protocols resulting in fewer recurrences (30,31). Pain during and immediately after treatment is the most frequently reported adverse event (25–29). Hyper- or hypopigmented scars can also occur. Surgery can be used as a second-line therapy if the isolated AK is resistant or if it recurs.

For isolated, suspicious AK with clinical signs evoking SCCs, a complete excision with histology is suitable.



**Figure 25.5** Treatment algorithm for the management of actinic keratosis. The initial choice of treatment is based on the number of lesions, their hyperkeratotic status, their clinical appearance (suspicious or not). (From Dréno B et al., *J Eur Acad Dermatol Venereol*, 28:1141–9, 2014.)

## MULTIPLE, NON-HYPERKERATOTIC ACTINIC KERATOSES

First-line treatments for multiple, non-hyperkeratotic AK include topical treatments and physical treatments such as laser or photodynamic therapy (PDT). Second-line therapies include physical treatments combined with topical treatments or surgery. All treatments should encompass the entire field of cancerization in order to reduce the likelihood of recurrence of a treated lesion and/or the development of new lesions within the same field.

### Topical Treatment Options

#### 5-Fluorouracil 5%

5-fluorouracil is a cytotoxic antimetabolite that interferes with DNA synthesis and to a lesser extent inhibits RNA transcription (32). After 3 to 4 weeks of treatment, the rate of complete clearance varies from 43% to 96% depending on the study (25,33,34). In approximately 65% of patients, lesions recur within 12 months of treatment (25). The main adverse events are pain, pruritus, burning sensation, and hyperpigmentation at the site of application. Application on healthy skin causes can cause an erythematous inflammatory reaction (32).

#### Imiquimod 5%

Imiquimod, which is an imidazoquinoline-derivative, mediates cytokine synthesis and release, stimulates the innate cutaneous immunity, and has an indirect antineoplastic action. It can be used to treat the field of cancerization as well as the AK lesions (35). In studies of the 5% cream, rates of complete clearance were 27% and 55% after one and two cycles, respectively (36–41). The recurrence rate 1 year after treatment ranges from 17% and 39% depending on the study (25,37). Local reactions such as pruritus, burning, erythema, pain,

oedema, dryness, crusting, erosions, and ulcerations have been reported. Systemic reactions, such as myalgia, fatigue, and nausea have also been reported, although more rarely. Few reactions have been shown to occur during the second cycle of treatment. In the special population of immunosuppressed transplanted patients, imiquimod should be used with caution, as safety needs to be evaluated (30,31).

#### Diclofenac 3%, Hyaluronic Acid 2.5%

Diclofenac 3% is a nonsteroidal anti-inflammatory drug that inhibits the cyclooxygenase pathway and decreases prostaglandin E2 synthesis. The rate of complete clearance is 31% after 2 months of treatment and 47% after 3 months of treatment (42,43). After 1 year, the rate of recurrence for treated lesions that had disappeared is 21%. Local reactions occur frequently. These include contact eczema, cutaneous dryness, edema, pruritus, scaly rash, ulcerations, and vesiculobullous rash (42). Altogether, at a dosage of two applications per day for 2–3 months, diclofenac gel appears to be well tolerated, but clinical data suggest that the efficacy profile may be less favorable compared to that of other topical treatments (30,31).

#### Ingenol Mebutate 150 µg/g and 500 µg/g

Ingenol mebutate is a biological compound that has a cytotoxic effect that causes mitochondrial edema and dissolution of cytoplasmic membranes. It also has an immunomodulatory effect that causes an increased production of inflammatory cytokines and the recruitment of neutrophils. In pooled analyses, the rate of complete clearance observed after 2 months of ingenol mebutate treatment was 42% for AK on the face and scalp and 34% for AK on the trunk and extremities (44). Twelve months after the end of treatment, the rate of recurrence was 54% on the face and scalp and 56% on the trunk

and extremities. Local cutaneous reactions, which resolved within 2 to 4 weeks, included erythema, scaling, crusting, edema, vesicles/pustules, and erosions/ulcerations (45).

## Physical Treatments

### *Ablative Ultrapulse Laser Therapy*

Therapy with an ablative ultrapulse laser creates a thermal effect, which causes nonselective tissue vaporization, loss of substance, and coagulation necrosis of the margins. Although this technique is included in recommendations, clearance rates have not been evaluated in double-blind, randomized, controlled clinical trials (24,31). The healing phase is often accompanied by erythema, pain, irritation, pruritus, oedema, and sometimes by secondary infection.

### *Photodynamic Therapy*

Photodynamic therapy selectively destroys tumor cells by targeting them with light after topical application of a photosensitizer (46). Photodynamic therapy can also be used to identify the limits of a field of cancerization (47). The rate of complete clearance of treated lesions after two PDT sessions ranges from 59% to 91% at 3 months (26,27,48,49). After a 12-month follow-up period, the rate of recurrence of lesions was 17% (50). The best clearing results are obtained in studies that use two PDT sessions spaced 1 week apart. The pain associated with application of the light to the photosensitized areas is a major limitation of this technique. In addition, the rate of transient local reactions is high, with 60% to 80% of patients reporting burning sensations, cutaneous pain, crusting, and erythema. In many countries, such as France, the use of this technique is also limited by the fact that dermatologists in private practice rarely own red/blue lamps. European guidelines were published in 2013 (51).

Daylight-mediated PDTs, which require shorter photosensitizer application times and daylight-mediated PDT, have been developed and tested in three randomized controlled trials (51,52). This treatment appears to be associated with good efficacy and lower levels of discomfort than conventional PDT.

## MULTIPLE HYPERKERATOTIC ACTINIC KERATOSES

For multiple hyperkeratotic AK, first-line treatments should include resurfacing prior to the use of the topical treatments and physical treatments described for non-hyperkeratotic AK. Resurfacing can be done manually or using topical keratolytics containing urea or salicylic acid. Cleavage of the corneodesmosomes leads to exfoliation of the superficial layers of the epidermis. Second-line therapies include physical treatments combined with topical treatments or surgery.

## THERAPEUTIC PERSPECTIVES

Due to the chronicity of AK, long-term strategies need to be developed in order to reduce risk.

## Comprehensive Approaches

A multifaceted approach that combines yearly checkups and patient education is likely to reduce risk further. Educated patients are more likely to use protective clothing and sunscreen and to adopt protective behavior. They are also best positioned to notice lesions that are changing rapidly or that have recurred after treatment.

## Repeated Treatments

Repetition of treatment can be used to increase efficacy rates. With PDT, for example, the best clearing results are obtained in studies that use two sessions spaced 1 week apart (51). For imiquimod, the initial cycle of three applications per week for 4 weeks can be repeated once in order to increase efficacy (35). Multiple cryotherapies have also been shown to be more effective than single cryotherapy as the rates of complete clearance at 3 months were 68% after one application and 78% after two applications (26,28).

## Sequential Combination Treatments

The sequential combination of physical and topical treatments is also likely to increase efficacy. Cryotherapy alone, for example, is associated with high rates of recurrence (25). This is likely to be due to the fact that only the AK lesion is targeted and the field of cancerization remains. The combination of a targeted physical approach with a broader topical approach that encompasses the field of cancerization may thus be more effective. After treatment with a combination of cryosurgery and topical ingenol mebutate, for example, the rate of development of new lesions 12 months after treatment was significantly reduced compared to treatment with cryosurgery alone (52% vs 39%, respectively;  $p = 0.02$ ) (53).

In addition, nonablative fractional lasers (Er:Yag and CO<sub>2</sub>), which are not particularly effective for AK in monotherapy (54), can be used in combination strategies. As they create microtunnels in the dermis, they increase the penetration of topical treatments and chromophores. In clinical trials, the efficacy of PDT in combination with fractional laser was significantly higher than the efficacy with PDT alone (88% vs 59% for complete lesion resolution, respectively;  $p = 0.02$ ) (55).

## DNA Repair Enzymes

As UV is intrinsic in the development of AK, topical photolyase creams may become an integral part of longterm treatment plans. These creams contain photoreactive DNA repair enzymes that reverse the dimerization of pyrimidines that occurs from UV damage. Once the cream has been topically applied, the enzyme needs to be photoactivated by 300–500 nm light. Data have shown that use of this technique after exposure to UVB radiation decreases the number of pyrimidine dimers and prevents radiation-associated immunosuppression and erythema formation (56).

## CONCLUSIONS

As Western populations age, the incidence of AK in dermatological practices is likely to increase. The combination of prompt management of suspicious AK, patient education, and long-term follow-up is likely to be the most effective approach to reducing the risk of SCC.

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# Safety of UV Nail Lamps as used in Professional Nail Salons

Douglas Schoon

Questions about the safety of UV nail lamps were raised in 2009 and have since been addressed by four published scientific studies, which are outlined in this chapter. An understanding of how these lamps are designed and used is also important to understanding their safety.

## UV NAIL LAMP DESIGN AND USE

Two styles of UV nail lamps are used in salons, utilizing either fluorescent bulbs or LEDs, as shown in Figure 26.1. Both types emit UV and can quickly harden UV-curable artificial nail coatings. A client's hands are inserted into a covered chamber where UV exposure occurs. LED-style UV nail lamp exposure is typically 30 seconds per hand, while fluorescent-style UV nail lamp exposure times are typically 2 minutes or less. This is repeated three or four times per hand for a total exposure time ranging from 90 seconds to 8 minutes per hand. Salon services are typically semi-monthly exposures for those who keep to the recommended maintenance schedule.

The bulbs used in traditional UV nail lamps are fluorescent tubes, while the equivalent of a bulb used in LED-style UV nail lamps are light emitting diodes (LED). UV nail lamps emit UVA, with very little or no UVB and no UVC. Artificial nail coatings are specially formulated to cure (polymerize) using relatively low levels of near UVA, relying exclusively on wavelengths between 400–350 nm, while the UVA region extends to 315 nm, as shown in Figure 26.2. Fluorescent tubes have peak emissions near 365 nm. The most commonly used LEDs have peak emissions at 405 nm, which is in the visible range, however, wavelengths between 400–380 nm are chiefly responsible for most polymerization. Therefore, it is incorrect to categorize these as visible curing nail coatings. Visible curing is not currently practical or commercially feasible, due almost exclusively to the lack of suitable photoinitiators that are activated by visible light. It is incorrect to refer to UV as “light.” Light is the visible portion of the electromagnetic spectrum. UV is not visible and therefore not considered light, but instead is correctly referred to as UV energy.

Different UV-curable nail product formulations may require differing UV wavelengths for curing. The required wavelengths are determined by the selection of the photosensitive polymerization initiator(s) chosen. Photoinitiators (PI) are activated by specific UV wavelengths, and the intensity of these wavelengths is also important. The degree of polymerization of the nail coating is dependent on exposure to the proper wavelengths at the correct intensity for the necessary duration of time. Therefore, proper polymerization is largely determined by the type of photoinitiator(s) used in the formulations as well as its concentration. The chemical structure of a

typical photoinitiator, ethyl-2,4,6 trimethylbenzoylphenyl phosphinate, is shown in Figure 26.3.

The UV wavelength and intensity (irradiance) needed to properly cure a specific UV nail coating can vary widely, depending on the coating formulation. UV nail lamps that do not properly match the curing requirements of a particular UV nail product can lead to either over- or under-curing the nail coating. Proper curing requires exposing the UV-curable coating to the correct UV nail lamp for the required time.

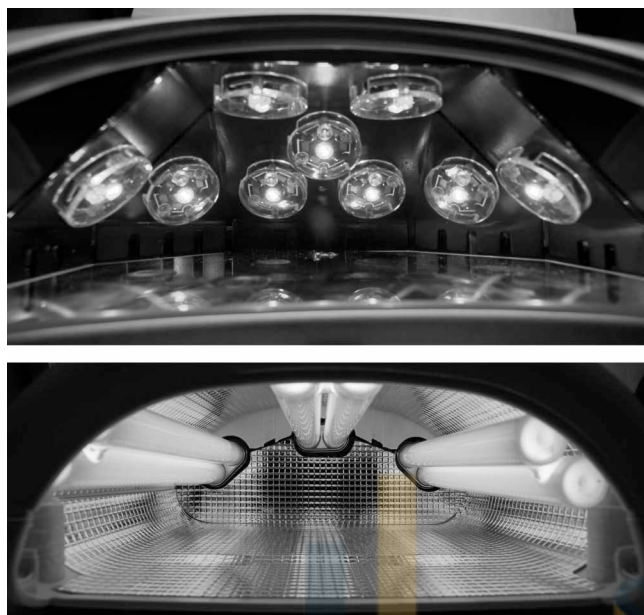
## ACHIEVING PROPER POLYMERIZATION

Nail professionals tend to oversimplify the UV curing process and may not fully appreciate the many factors that influence polymerization and mistakenly believe that wattage of the nail lamp is the critical factor. Wattage is the power consumption, while the far more important property is irradiance (watts/cm/nm). The second mistake that some make is to focus on the range of wavelengths emitted by their UV bulbs, which also ignores the importance of irradiance, which determines the intensity of exposure over an area.

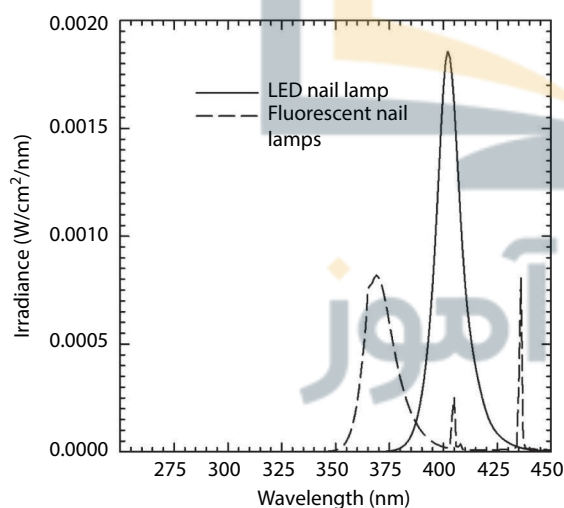
Proper curing of nail coatings is best achieved by consistently applying a thin layer of UV gel, exposing the coating for the proper length of time, and using a UV nail lamp that emits the correct UV wavelengths at the proper intensity for the formulation. When the irradiance is excessive, the nail coating can exothermically release sufficient heat to cause onycholysis. When irradiance is insufficient, unreacted monomer can remain trapped in the nail coating with dust, which can contribute to overexposure and increased risk of adverse skin reactions. When properly cured, these risks are minimized.

Lamp design is also important to ensure proper cure. Physics inverse square law demonstrates that UV intensity is inversely proportional to the square of the distance from the source, which is why the distance between the coated nail plates and the UV source is important, as are the electronic components which power the bulbs or diodes. Different UV nail lamps utilize different electrical components. These various factors help to explain why the identical UV bulbs have differing irradiance when used in two different brands of UV nail lamp, and why it is important to use the UV nail lamp that was designed to cure a specific nail coating. Expired UV bulbs should be replaced with the same type and model of UV bulb, and fluorescent style bulbs should generally be changed two to four times per year, depending on how often the nail lamps are used. LED-style UV nail lamps claim to have usable lives of 10,000 hours, but in practical terms these lamps are expected to last 3–4 years before they must be replaced with a new unit, since the diodes are not replaceable.



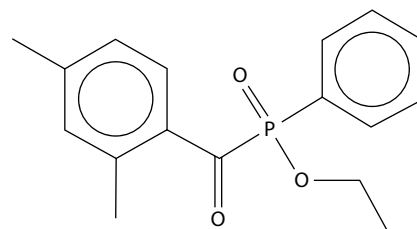


**Figure 26.1** Typical LED (top) and fluorescent-style (bottom) UV nail lamps used to polymerize (cure) artificial nail coatings designed to cure via exposure to relatively low levels of near region of the UV-A wavelengths.



**Figure 26.2** Linear plot of irradiance for LED-style UV nail lamps when compared to fluorescent-style UV nail lamps (1).

Nail technicians may underestimate the importance of using the correct UV nail lamp intended for curing the nail coating product of their choice, and mistakenly believe that “wattage” is the only important factor. UV nail coatings will harden when they are only 50% cured, but to reach the ideal coating durability and minimize the potential for adverse skin reactions, these crosslinked nail coatings should be more fully polymerized. When properly cured, the potential for overexposure to partially cured dust and filings decreases the potential for adverse skin reactions.



**Figure 26.3** 2,4,6-trimethylbenzoyl diphenylphosphine oxide (MW 316), a typical photoinitiator for UV curable nail coatings.

### UV NAIL LAMP SAFETY

Questions were raised in 2009 about the safety of lamps used in nail salons to photopolymerize artificial nail coatings. Texas dermatologists MacFarlane and Alonso claimed to have had two patients who they suspected had developed nonmelanoma skin cancer (NMSC) as a result of using UV nail lamps during regular salon services (2).

MacFarlane and Alonso made no UV measurements—instead, they performed several calculations in an attempt to compare UV nail lamps to tanning beds; however, these calculations were flawed and based on several incorrect assumptions. For instance, they erroneously assumed the spectral output of tanning bed bulbs were the same as those used in nail curing applications; however, the spectral outputs of these two sources are very different, which makes their NMSC risks quite different as well.

MacFarlane and Alonso stated that one of the only two patients described had, “Fitzpatrick skin type III, several actinic keratoses on her face and arms” and was determined to have “moderate recreational UV exposure.” It was claimed this patient’s previous sole exposure to UV nail lamps was approximately eight times over the course of a single year. This indicates that the patient’s exposure was 64 minutes or less, and this was her lifetime exposure to a UV nail lamp. These dermatologists discounted many years of recreational UV exposure of the face and arms to indicate their belief that this instance of NMSC on the hand’s dorsum was attributed to 64 minutes of low-intensity UVA exposure spread over eight nail salon visits during the course of a year. In light of studies that followed, this claim is highly implausible. According to Dowdy and Sayre in *Photobiological Safety Evaluation of UV Nail Lamps*, the most comprehensive paper published concerning the safety of UV nail lamps, MacFarlane and Alonso’s patient would have had more than three times greater NMSC risks from natural sunlight if she walked for 8 minutes under mid-angle sunlight to and from the salon for her appointments, twice per month (3). Dowdy and Sayre’s study is the only study that utilized the proper international standard testing methodology and made risk calculations based on threshold limit values of the American Conference of Government Industrial Hygienists (ACGIH). Other published papers have observations made by utilizing inadequate testing equipment, incorrect calculations, and/or failing to adhere to the internationally-recognized standard testing protocols.

UV energy sources are difficult to measure properly. To overcome these inherent difficulties the ANSI/IESNA RP-27 is the recommended standard for photobiological safety evaluation of lamps and lamp systems. Part 2 of the Standard outlines

ways in which significant errors are introduced when incorrect equipment and testing procedures are utilized:

... special problems related to photobiological hazards measurements. The results of hazard evaluations will be influenced by the instruments' characteristics. The bandwidth of the monochromator will change the weighted results of any spectrum with varying levels. All finite bandwidths instruments report signal at the wrong wavelength, leading to errors in a weighted sum. (4, p. 3)

Relying on improper testing procedures and/or inadequate testing equipment will skew results up to 30%–50% and result in significantly increasing the weighting of the shorter UVA wavelengths. This results in significant overestimation of skin exposure for these lower wavelengths and a large overestimation of the actual NMSC risks. Other than Dowdy and Sayre's study, the spectrometers used in the published studies discussed were not suitable for this type of analysis per this International Standard.

Dowdy and Sayre measured five UV nail lamps—three fluorescent-style and two LED-style—using a dual monochromator spectroradiometer and integrating sphere, calibrated using a tungsten filament spectral irradiance standard traceable to the National Institute of Standards and Technology (NIST) to provide an uncertainty of measurement of < 5% allowing for source positioning uncertainty estimated to be no greater than ~ 2.5%. Secondary calibration to a deuterium spectroradiometric standard lamp was conducted to extend the calibration spectral measurement range down to 200 nm.

Three other studies, Curtis et al., Markova and Weinstock, and Shipp and Wagner, each utilized nonstandardized testing by not following the published International Standard, ANSI/IESNA RP-27, and therefore their results are likely to be overapproximations that do not correctly reflect the true NMSC risks (5–7). UV measurement devices used by these researchers were low-end devices that are especially prone to the errors previously described and which caused a significant overestimation of NMSC risks. Part 3 of the International Standard states,

The performance of the necessary measurements is normally not an easy task without sophisticated instruments... The purpose of this standard [RP-27.3] is to provide guidance for the proper categorization, classification and informational requirements of lamps so that such sources may be properly applied... (8).

For their study, Dowdy and Sayre utilized a dual monochromator spectroradiometer that meets the necessary requirement of the RP-27.2-00 internationally accepted standard to make proper measurements of UV nail lamps, and they then compared these results with the UV output of tanning bed lamps. Dowdy and Sayre performed a risk analysis based upon the ACGIH threshold limit values for exposure to UV and stated,

The results indicate that a person could in their workplace, once every day, put their hand under a UV nail lamp for 25 minutes and remain within the permissible daily occupational exposure limits for workers, according to the applicable international ANSI/IESNA RP-27.1-05 standard. (9,10, pers. comm.).

Dowdy and Sayre concluded that the UV lamp tested for photobiological safety to skin properly belonged within the Risk Group 2-Moderate Risk (RG-2) with  $S(\lambda)$  weighted actinic UV ranging 1.2–1.7  $\mu\text{W}/\text{cm}^2$  and permissible exposure times of 29.8–129.31 minutes. This indicates that these UV nail lamps are permissible for up to approximately 30 minutes of

continuous exposure on a daily basis. Actual usage is 8 minutes or less, twice per month.\*

For all nail lamps tested, exposures were in the Exempt classification for both skin and eyes when UV was measured at the specified distance and angle, 20 cm (7.8 inches) and a 45° angle from the nail lamp. Radiant output was below maximum levels allowed for Exempt classification for UV risk to skin and eyes.  $S(\lambda)$  weighted actinic UV ranged 0.009–0.078  $\mu\text{W}/\text{cm}^2$  and unweighted near UV (320–400 nm) ranged 0.091–0.483  $\text{mW}/\text{cm}^2$ . The retinal photochemical blue light hazard was also within the Exempt range. Meter readings detected negligible Infrared (IR); consequently retinal thermal and cornea/lens IR were also Exempt. For one device, however, the aphakic eye hazard (individuals implanted with non-UV blocking intraocular lenses) slightly exceeded this limit, rising into Class 1 (low risk). These specific devices were all found to be classified into Risk Group Class 2 (moderate risk) for actinic UV to skin exposed inside the chamber with no other risks to normal individuals.

Although they did not adhere to the RP-27 standard as did Dowdy and Sayre, Markova and Weinstock reached a similar conclusion. They tested three commonly used UV nail lamps—two fluorescent-style and one LED style—and used the SCUP-h action spectrum to determine a UV nail lamp session's carcinogenic-effective irradiance in terms of narrow band ultraviolet B (NB-UVB) phototherapy courses, assuming 10 minutes per UV nail lamp session for each device's carcinogenic effective irradiance. They then compared the measured UV dose with that of a single course of NB-UVB, assuming a cumulative UV dose of 25  $\text{J}/\text{cm}^2$  was received per patient per NB-UVB course. The carcinogenic equivalence was calculated in terms of NB-UVB courses to determine that depending on the UV lamp tested, 13,000–40,000+ sessions lasting for 10 minutes would be required to expose the nail plate and surrounding skin to equal the UV dose received during one NB-UVB course. They concluded that given the tiny fractional exposure when compared to a single NB-UVB course, the UV nail lamps tested and hence do not produce a clinically significant increased risk of developing skin cancer. They stated that “One would need over 250 years of weekly UV nail sessions to experience the same risk exposure.” These authors also commented on the calculations made by MacFarlane and Alonso and stated, “... this case review is anecdotal and the spectral irradiance cannot be calculated by using bulb wattage (bulb's power requirements) and exposed to body surface area, but must be measured spectroradiometrically...” (2, p. 2).

Curtis reported very small MED of 0.06–0.09 per nail service and estimated that after 1 year of exposure, the MED could be 1.1–1.5, which is very low. Yet these authors refer to the nail lamps as “high-intensity” devices that produced “high-dose UVA irradiation,” which is in contradiction to their own measured results. Semi-monthly exposure of 0.06–0.09 MED which they reported and no justification is provided to support the contention that these are “high-intensity lamps” (6, p. 1). This is in contrast to Dowdy and Sayre, who stated, “All of the various UV nail lamps submitted for evaluation were found to be significantly less hazardous than might be anticipated based on the initial concerns raised by MacFarlane and Alonso” (3, p. 3).

Had Curtis utilized the proper test equipment and procedures or been provided with Dowdy and Sayre's results

\* Exposure is approximately 2 minutes or less per applied layer of nail coating, totaling approximately 8 minutes or less. The exposure is not a continuous 8 minutes since the lamps take only one hand at a time. LED lamp exposure is even less since each coat requires only 30 seconds.

prior to their publication, they would have seen that they erred in their cursory evaluations and conclusions. Curtis made a misleading statement by claiming these lamps produce 4.2 times the energy of the sun between 355 and 385 nm, but failed to recognize this is the least risky portion of the spectrum and sunlight contains a much greater concentration of more harmful shorter UVA wavelengths not emitted by UV nail lamps. This helps explain why exposure to UV nail lamps is much safer than exposure to sunlight and not riskier as erroneously suggested by Curtis. UV nail lamps utilize the safest region of the UVA spectrum. When the spectral risk weighting functions are properly applied as required by the RP-27 Standard, it is clearly not correct to suggest UV nail lamps have greater NMSC risk than natural sunlight, when in fact UV nail lamps create less exposure to harmful wavelengths and instead produce safer, low intensity energy, emitting near-UVA and visible light wavelengths.

Since both lamps tested by Curtis were also properly tested by Dowdy and Sayre using the appropriate international standard for testing, it can only be concluded that Curtis erred in the measurement techniques utilized, which negatively affected the conclusion and led to an overestimation of risks.

Shipp and Wagner also failed to utilize the proper test equipment and did not cite the results published by Dowdy and Sayre. Instead, they purchased a \$170 UV detector and developed improper test methodology which produced inconsistent and inaccurate results causing the authors to significantly overestimate the risks of NMCS from UV nail lamps. Their testing of various UV nail lamps produced widely varying results with variances so significant that proper statistical analysis was impossible, "Using the Shapiro-Wilks test however, the data failed normality testing because the local irradiance values of the 5 different positions were not homogeneous" (5, p. 1).

Rather than recognize this failure as a result of inadequate measuring devices and poorly designed test methodology, these authors ignored problems with the data to publish inaccurate conclusions about NMSC risks.

Dowdy and Sayre also compared the International Electrotechnical Commission (IEC) indoor tanning annual NMSC exposure limits to sunlight and to the UV nail lamps studied. Table 26.1 compares fluorescent-style UV nail lamps with fluorescent-style tanning lamps to both overhead and

mid-angle sunlight by comparing their exposure times needed to reach the 25 kJ m<sup>2</sup> NMSC effective dose. The lower the reported number, the greater the NMSC risk. The table demonstrates what Dowdy and Sayre's paper states, i.e. "Using spectral weighting relative to overhead and mid-angle sunlight the UV nail lamps were 11–46 times less NMSC effective-irradiance than at overhead 1 atmosphere solar spectrum and 3–12 times less than mid angle 1.5 atmosphere sun" (3, p. 5) Also shown is a comparison of FDA sunlamp calculations for determining maximum exposure times (Te) for tanning applied to UV nail lamps as an objective index of comparison (11). This table demonstrates that UV nail lamps would not function well as miniature hand tanners, as erroneously suggested by MacFarlane and Alonso.

Mid-angle sunlight is safer for skin exposure than overhead sunlight because of the association between UV intensity and the ratio of shadow length to the object's height; longer shadow lengths equal lower UV intensity of sunlight. Mid-angle sun produces erythema after 20–30 minutes; erythema is produced after only several minutes when the sun is overhead. The UVA nail lamps measure clearly present many times lower risk than equal time exposure to mid-angle sunlight.

Table 26.1 shows the International Electrotechnical Commission (IEC) indoor tanning annual NMSC exposure limits to sunlight compared to fluorescent-style tube UV nail lamps, tanning lamps which utilize use fluorescent tubes and to both overhead and mid-angle sunlight. The lower the effective dose; the greater the NMSC risk.

Manufacturers provide users with detailed directions for proper use and warning labels which accompany UV nail lamps that help ensure that users know how to properly use the nail lamp.

Dowdy and Sayre state their discussion demonstrate that nail lamps are "... vastly less hazardous. In terms of NMSC-weighted exposure, the most powerful UV nail lamp was more than an order of magnitude less than the most powerful sunlamp" (3, p. 3).

UV curing of artificial nail enhancements (artificial nails) and other types of UV-curable nail coatings have been widely used as a popular nail services in Europe, Canada, Australia, Japan, and the United States for more than 25 years. UV nail lamps, when correctly tested under appropriate standards, are demonstrated to be acceptable according to the cited standards and therefore should be considered safe as used for curing of artificial nail coatings. Normal exposure to UV nail lamps provides a low bioeffective skin dose from the least risky portion of the UVA spectrum for about 8 minutes or less, at 2–3 week intervals. Identically exposing skin to mid-angle sunlight in this fashion would pose an insignificant NMSC risk. UV nail lamps have at least three times lower NMSC risks when compared to mid-angle sunlight. Most people are likely to not be overly concerned with 8 minutes of exposure to mid-angle sunlight twice per month and neither should they be concerned with 8 minutes of exposure using UV nail lamps, which have a significantly lower NMSC risk than natural mid-angle sunlight.

## CONCLUSION

It can be concluded that total exposure following typical salon exposure times and exposure steps accumulate to only a small fraction of the permissible daily exposure under ANSI/IESNA RP-27. These risks are further mitigated in realistic use

**Table 26.1** IEC Indoor Tanning Annual NMSC Exposure Limits Compared to UV Nail Lamps and Natural Sunlight

UV Source	IEC Annual Tanning Limit (25 kJ NMSC)	FDA Maximum Timer for Tanning Session (Te)
UV Type 5 tanning booth	8 hours	14.47 minutes
<b>Overhead sunlight</b>	<b>10 hours</b>	<b>20.47 minutes</b>
CIE AMIG T7/1		
UV Type 4 body tanning lamp	13 hours	23.47 minutes
UV Type 4 facial tanning lamp	15 hours	29.73 minutes
<b>Mid-angle sunlight</b>	<b>29 hours</b>	<b>62.68 minutes</b>
CIE AM1.5G 77/2		
UV nail lamp F3*	96 hours	145 minutes
UV nail lamp F4*	114 hours	176 minutes
UV nail lamp F2*	154 hours	234 minutes

\*Not a tanning appliance.



scenarios, since they are not a daily occurrence. Also, the natural nail plate is a very efficient blocker of UV, protecting the nail bed making the UV exposure risks to the nail bed comparable to that of skin protected by high SPF topical sunscreen, and the dorsum of the hand is four times more resistant to UV than the forehead or cheek and three-and-a-half times more resistant than a person's back, making the dorsum of the hand the most UV acclimatized, photo adapted, and UV-resistant body site (12,13,14).

To put things into the proper perspective, it is very unlikely for anyone to suffer from overexposure to UV through normal use of a UV nail lamp, and it is highly improbable that even the most dedicated nail salon client would approach an unsafe level of exposure.

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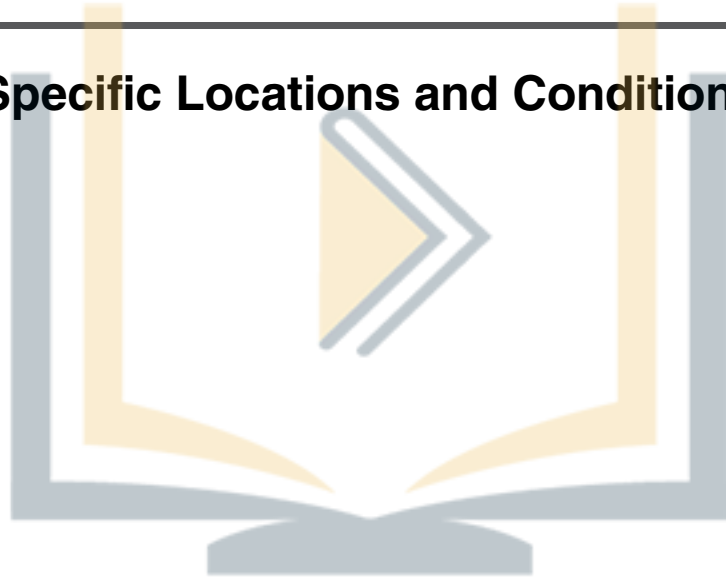
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## Section IV

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**Specific Locations and Conditions**



بزشک آموز



پزىشک آموز



# Hair Care

John Gray

## INTRODUCTION

Hair is one of nature's survivors and, although technically "dead," one of its most resilient creations. The visible hair shaft may appear very simple but the mechanism to create it, the hair follicle deep in the skin, is a highly complex biological system.

Hair first arrived on the evolutionary scene some 310 million years ago on reptilian-like animals. It preceded and survived the rise and extinction of the dinosaurs and emerged as the dominant skin appendage of mammals (1). As part of this class, modern humans have "inherited" skin which, although bearing several million hair follicles, has largely confined itself to growing hair in any profusion on the scalp in women and scalp and face in men.

The modern obsession for the complex grooming of hair is entirely consistent with mammalian habits which enhances appearance, and subconsciously imparts status, health, physical attractiveness, and perhaps less romantically, helps remove parasites. Hair care is an (almost) ubiquitous human habit in the 21st century and is driven by conscious as well as subconscious social and evolutionary pressures.

The varied appearance of human hair types can be explained to some extent by inherited regional phenotype based on founder effects and partly by both adaptive consequences to the environment and sexual selection that occurred after the modern human diaspora out of its origins in Africa some 70–90,000 years ago. These differences now play an important role in determining grooming habits and the type of hair care products people use as part of their grooming ritual.

## HAIR

Some mammals such as Polar bears invest an estimated 30% of their dietary protein in the manufacture of hair (2). Despite their reputation as naked apes, humans still invest extraordinary amounts of protein, particularly at a young age, in growing scalp hair to a greater length than any other mammal (Figure 27.1).

The length of an individual's hair depends on the duration of continuous growth (anagen) from the follicle (3). Only the merino sheep has a comparable duration of hair growth, although this is artificially induced by selective breeding.

It is open to debate quite why *homo sapiens* are so blessed with profuse head hair when young, and why subsequently so many lose it. Human hair's function may now primarily be that of a critical signaling device, conveying age, health, and even social status to others of the species. In prime condition it can act as a powerful beacon of sexual attraction; in a damaged or dishevelled state, quite the reverse (5).

## HUMAN HAIR AND THE FOLLICLE

This chapter focuses primarily on the hair shaft. The origin of the visible hair and what influence the follicle has on hair health is also addressed.

### The Hair Follicle

By definition, a follicle is a mammalian skin organ that produces hair. It contains many of the complex biological systems found throughout the body and is a reservoir of stem cells for regeneration of both hair and skin. These attributes infer the fundamental importance of such tiny organs to the body as a whole and reflect our mammalian heritage where fur and pelts are essential to survival.

Of the 2–5 million hair follicles on the human body, those on the scalp are the most fascinating and important. Possibly as a mark of its importance, the hair follicle has, like the eye, brain, and human reproductive organs, been granted "immune privilege." It is postulated that the demise of this privilege is an initiating phase of alopecia areata (3).

### Types of Follicle

In infancy, hair follicles are generally small other than those on the scalp which produce significant hairs. After puberty and under androgenic influence, selected follicles enlarge and develop sebaceous glands and terminal hairs which are produced on other body areas such as the trunk, although there are large inter- and extra-regional differences. These large and heavily pigmented hairs are described as terminal hairs (3).

Of the 100,000–150,000 follicles on the scalp, 75%–90% produce terminal hairs. The associated sebaceous glands deliver sebum—a natural mixture of triglycerides, wax esters, and squalene which helps to maintain the integrity of the scalp and has both protective and thermoregulatory properties (3). The remaining 10%–25% of scalp follicles are small and produce fine vellus hairs.

## COSMETIC ASPECTS OF HAIR

The result of this hair growth activity is the production of a terminal hair shaft. This may be described, like the carapace of a crustacean, as a filamentous biomaterial since it contains no living matter and possesses no biofeedback mechanisms. From this point on, the fate of the hair shaft may be regarded as cosmetic, which includes beauty, aesthetics, or appearance. In reality, modern proteomic research has identified that behind these cosmetic changes are structural and measurable changes to the core of the hair.



**Figure 27.1** Female, age 7. Massive amounts of protein are used to manufacture a signaling device of youth and health.

### The Terminal Hair Shaft

A single human hair is merely a bundle of compressed protein with a small quantity of fats and traces of minerals and vitamins. It contains no living cells and receives no support other than mechanical from the follicle. The visible hair shaft has no active metabolism, yet in combination with its neighbors can exude an almost mystical vitality and strength (Figure 27.2).

There are three essential types of hair in humans which are age related and dependent to some extent on the size of the follicle.

- **Lanugo hair** is fine and non-medullated hair which appears on the fetus, and with rare exceptions is shed prior to or immediately after birth.
- **Vellus hair** is fine (less than 40 microns in diameter), short, and non- or lightly pigmented and is the most numerous of human hairs. It can be seen from the neonatal period onward, covering all surfaces except the palms and soles of the feet. At puberty, some vellus hairs enlarge to become terminal hairs and develop sebaceous glands. Vellus hairs occur on the scalp but are far less numerous than terminal hairs.
- **Terminal hair** is thick (50–150 microns), long, and pigmented. Terminal hairs are the dominant hairs on the scalp, eyebrows, lashes, axillae, and genital areas. In men, terminal hairs are variably found on the trunk and legs. There is great regional difference in terminal body hairs. Terminal body hair is relatively uncommon in Asians and more common in Indo-Europeans.

The cross section of the hair terminal shaft reveals three major components: the cuticle, the cortex, and (rarely, other than in



**Figure 27.2** Scanning electron microscope image of hair shaft.

grey hairs) the medulla. The main constituents of these structures are sulphur-rich proteins, lipids, water, melanin, and trace elements.

### The Cuticle

The cuticle is composed of specialized keratins and consists of six to eight layers of flattened overlapping cells with their free edges directed upward to the tip of the hair shaft. There are several layers to each cell. The innermost endocuticle is covered by the exocuticle which lies closer to the external surface and is comprised of three parts: the b-layer, the a-layer, and the epicuticle. The b-layer and the a-layer are largely protein. The epicuticle is a hydrophobic (water resistant) lipid layer of 18-methyleicosanoic acid attached by a covalent chemical bond to the surface of the fiber. This is commonly known as the f-layer. The f-layer is of critical importance to hair “health” since it affords considerable protection to the shaft (Figure 27.3).

The cuticle’s complex structure allows it to slide as the hair swells, and the f-layer imbues a considerable degree of water resistance (hydrophobicity). It is critical in protecting the hair and rendering it resistant to the influx and outflow of moisture.

The normal cuticle has a smooth appearance, allowing light reflection and limiting friction between the hair shafts. It is primarily responsible for the luster and texture of the hair (Figure 27.4). The cuticle may be damaged by four different “insult” sources: environmental, mechanical, chemical, and heat.

Chemical removal of the f-layer (Figure 27.5)—particularly by oxidation during bleaching or perming—eliminates the first hydrophobic defense and leaves the hair more porous and vulnerable. If the cuticle is damaged there is little change in the tensile properties of hair; however, its protective function is diminished (3).

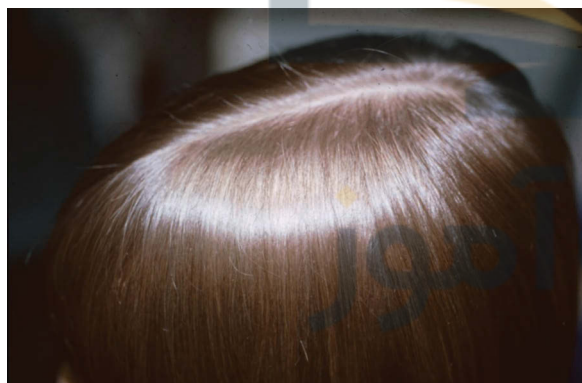
### The Cortex

The cortex forms the main bulk of a fully formed (keratinized) hair shaft and contributes almost all the mechanical properties of the hair, particularly strength and elasticity.

The cortex consists of closely packed spindle-shaped cells rich in keratin filaments comprising 400–500 amino acid residues paired together to form protofilaments which



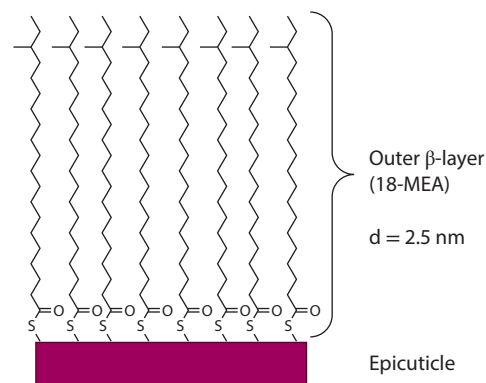
**Figure 27.3** Overlapping scales of the cuticle.



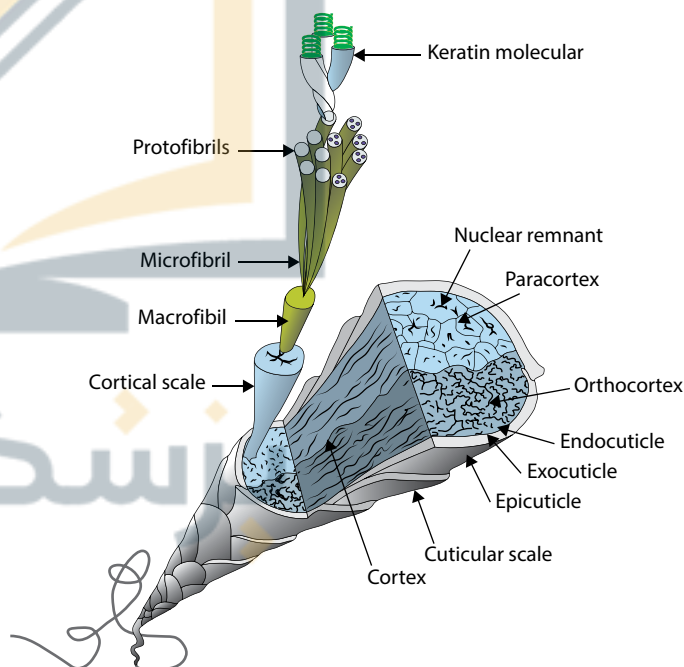
**Figure 27.4** Healthy hair. Reflection from the intact cuticles of well aligned hair is largely responsible for hair shine.

make up a keratin chain. These are orientated parallel to the long axis of the hair shaft and embedded in an amorphous matrix of high sulphur proteins. The keratin chains have a large number of sulphur-containing cystine bonds which create a strong cross-link between adjacent chains. These so-called disulphide bonds are critical in conferring shape, stability, and resilience to the hair shaft and can only be broken by external oxidative chemical agents such as occurs with perming or relaxing (Figure 27.6).

Weak hydrogen bonds and salt link the keratin polypeptide chains together. These weaker bonds are easily overcome



**Figure 27.5** Preservation of the outer f-layer is of critical importance in maintaining “hair health” or homeostasis. Removal renders the hair shaft potentially vulnerable to further damage. See further ref. 10. (Courtesy of JR Smith, University of Portsmouth SPM Dept and P&G Beauty.)



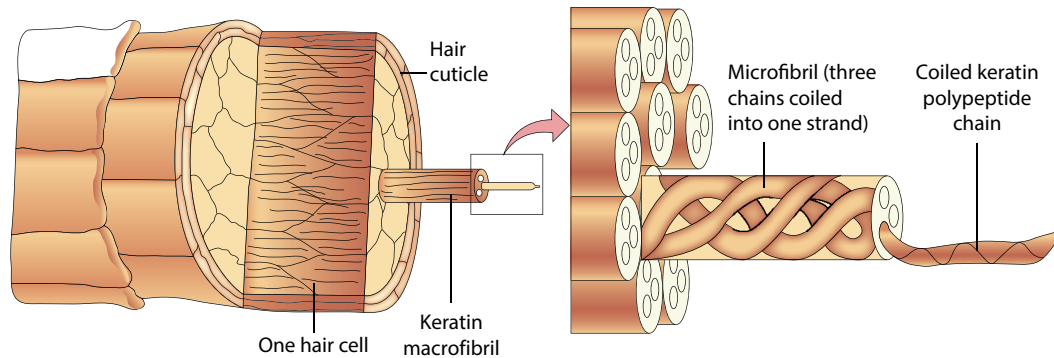
**Figure 27.6** The outer composition of the cortex.

by water rendering curly hair—temporarily—straight. The powerful disulphide bonds and weaker hydrogen bonds are crucial to hair health. The cortex contains melanin granules which color the fiber based on the number, distribution, and types of melanin granules (Figures 27.7 and 27.8).

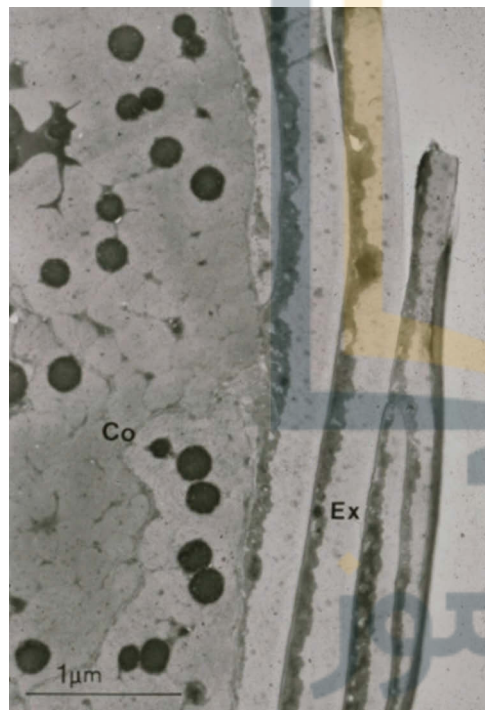
### The Medulla

The medulla is a soft, proteinaceous core present in thicker and white hair. It has no known function in humans.





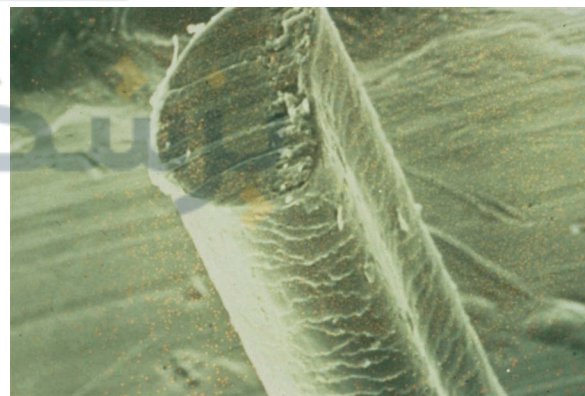
**Figure 27.7** Composition of the cortex: macrofibrils and microfibrils.



**Figure 27.8** Melanin granules in the cortex with overlapping scales of the cuticle.



(a)



(b)

**Figure 27.9** (a) Uncombable hair due to (b) abnormality of the hair shafts.

### Abnormal Hairs

Not all hairs are "normal." Genetically inherited hair diseases may produce abnormal hairs which result in weakness and/or major problems in grooming. One such example is cheveux incoiffables (uncombable hair syndrome; Figure 27.9).

### Hair Diameter and Texture

The diameter of human hair varies from 17 to 180 micrometers (0.00067 to 0.0071 in). Hair less than 40 microns is regarded as vellus hair.

Hair texture is a less precise term but is usually classified as follows:

- Fine: less than 60 microns
- Medium: 60–80 microns
- Thick: 80–150 microns

Many women, on questioning, believe their hair to be finer than accurate measuring would confirm. This misunderstanding may affect their less than ideal choice of hair care products.

## Hair Types

Not all terminal hairs have the same structural phenotype. Terminal hairs have traditionally been described as round, oval, or flat. Further, these shapes have been assigned genetically redundant racial acronyms. In reality there is a broad spectrum of hair shapes and although individuals tend to bear essentially one type on their scalps, it has been observed that all three can be present.

While peoples from subequatorial Africa and their dispersed descendants have hair follicles which are curved, the final shape of the hair emerging from the follicle is more determined by the activity of the matrix cells deep in the follicle and the manner in which the keratin proteins are laid down in the cortex.

The hypothesis that hair shaft shape is principally due to the shape of the follicle is further confounded by the ability of terminal hair follicles to produce different phenotypes under altered circumstances. Patients after chemotherapy routinely report dramatic change in hair shaft phenotypes, presumably due to a change in arrangements of keratins in the cortex from a “reprogrammed” matrix.

## The Concept of Aging of Hair

The tissues of the scalp and hair follicle inevitably age and their activity declines. The proliferative tissues in the hair matrix are subject to the intrinsic factors associated with constant metabolic activity and natural (chronological) aging. External (extrinsic) factors may also impact on the quality of hair produced. These factors inevitably occur in conjunction and are cumulative over time.

Intrinsic factors are overwhelmingly inherited and result in, for instance, male pattern balding and premature greying. Extrinsic factors include the effects of ultraviolet radiation (UVR), smoking, and possibly nutrition.

As a result of continuous metabolic activity, highly reactive oxygen molecules (ROS) are generated in the cells, leading to oxidative stress which is now believed to play a major role in the aging process. The damaging effects of these ROS are induced from the mitochondria in the matrix cells and melanocytes and externally from the environment. The follicle possesses enzymes and vitamins (E and C) which help to quench the ROS. Eventually, the ROS overwhelm the defenses leading to damage to the active cells. The effects of this are seen as greying and an imperceptible but steady decrease in hair production both in terms of quantity and quality.

Allied to this concept is that of hair shaft “aging.” Hair as it emerges from the follicular opening is in its most perfect state. As this growth progresses, the hair shaft quality deteriorates or “ages” due to external factors such as environmental, chemical, and physical insults. The process is known as weathering and is the most common hair disorder encountered. The combined effect of the intrinsic and extrinsic factors at a follicular level and extrinsic aging of the shaft is to render the hair thinner, weaker, and less “healthy.” This concept of hair aging has become important in the development of hair care products targeted to meet both intrinsic and extrinsic aging.

## Hair Thinning: Reducing Hair Density, Diameter, and Hair Mass

A common complaint of women as they age is that of losing hair density, or in common parlance, thinning. This may be manifest as a tangible loss of bulk, a visible scalp, or reduction in the volume of a ponytail. It is often difficult for an observer, stylist, or dermatologist to interpret early thinning, particularly if there is no evidence of greater than normal daily hair loss. By the time this reduction in total hair mass is apparent, some 50% loss of hair may have occurred (Figure 27.10) (5).

## Hair Density

At all ages, there is a wide and normally distributed scatter of hairs over the scalp. Possibly as a result of the impact of ROS described above, density in women gradually declines from 293 cm<sup>-2</sup> at age 35 to 211 cm<sup>-2</sup> at age 70.

## Hair Diameter

Mean hair diameter increases from age 22 to a peak in the mid-30s but gradually declines with advancing age (Table 27.1). In addition, some follicles cease functioning altogether.

As an individual ages, the keratin production slows and the hair shaft becomes thinner with a mean diameter of terminal hairs. Finally, the interval between cessation of growth of one hair and the commencement of a new (kenogen) extends (4).

All these factors contribute to a gradual reduction in hair mass caused by reduced number of hairs, reduced diameters, and extended intervals of growth. This perception or reality of reduced hair density can have a major impact on the self-perception of healthy hair and self-esteem (3).



**Figure 27.10** Visible scalp indicates up to 50% hair loss.



**Table 27.1** Mean Hair Diameter Increases from Age 22 to a Peak in The Mid-30s but Gradually Declines with Advancing Age (4)

Age (Indo-European Women)	Average Hair Density (hairs/cm2)	Delta (%) in Density vs Age 30
30	290	
40	270	−6.8
50	263	−9.3
60	235	−18.9
70	211	−27.2
80	185	−36.2

**Phenotype and Hair Health**

The shape of the shaft(s) has significant implications for long-term hair health. Increased susceptibility of the cuticle to physical damage is more common in flatter hair. The greater the diameter of the cortex, the greater the the resistance of the hair shaft to environmental and self-inflicted damage.

**HAIR DAMAGE: NATURAL AND ACCELERATED WEATHERING**

For most adults, there is both a natural and progressive deterioration in the internal and external condition of the hair shaft over time. This is the result of environmental and self-inflicted, repeated damage. This process, known as weathering, can vary from minimal to extreme (Figure 27.11).

In young women who do not use chemical processes or excessive heat on their hair, this weathering process is almost impossible to detect with the naked eye. In women with repeated chemical applications, the damage (Figure 27.12), particularly at the tip, is evident.

Modern research techniques (P&G data on file) now allow hair scientists and researchers to examine the breakdown of the structures of the hair at a nano scale (i.e. the breakdown of chemical bonds). It is possible to extrapolate not only how these breakdowns result in changes in a single fiber marker (such as loss of tensile strength) but how these single fiber changes impact bulk properties, such as noticeable shine. The extent of the structural changes and their manifestations will vary depending on the morphology of hair—i.e. diameter and curl—and on the prevailing hair style (long vs short).

**The Process of “Weathering”**

Hair is an exceptionally resilient structure able to withstand many differing traumas—environmental, mechanical, physical, and chemical. In the 21st century, in spite of this resilience, badly weathered hair is epidemic, particularly in the developed world (Figure 27.13).

When hair first emerges from the scalp, the cuticle consists of up to ten layers of long “scales.” However, the cuticular layers are only 3 or 4 μm thick and may have to last for 6 years or more.

**Natural weathering** involves a wearing away of the cuticle of the hair shaft, primarily from physical acts of grooming.

**Accelerated weathering** occurs as a result of additional and excessive physical and most importantly, repeated chemical injury. This accelerated weathering, which is generally regarded as “damage,” involves destruction not only of the f-layer and damage to the cuticle but ultimate exposure and



(a)



(b)

**Figure 27.11** (a) Minimal and (b) severe weathering.

degradation of the proteins in the cortex. The latter becomes increasingly unable to maintain the structural and homeostatic integrity of the hair and at its most extreme, the hair proteins may unravel, causing split ends or breakage in mid-shaft (Figure 27.14).

**Sources of Damage**

Major damaging sources include wetting, friction, sunlight, heat from drying and styling appliances, chemicals, and heavy metals in swimming pools and in the home. Most devastating





**Figure 27.12** Difference in integrity from root (a) to tip (b).

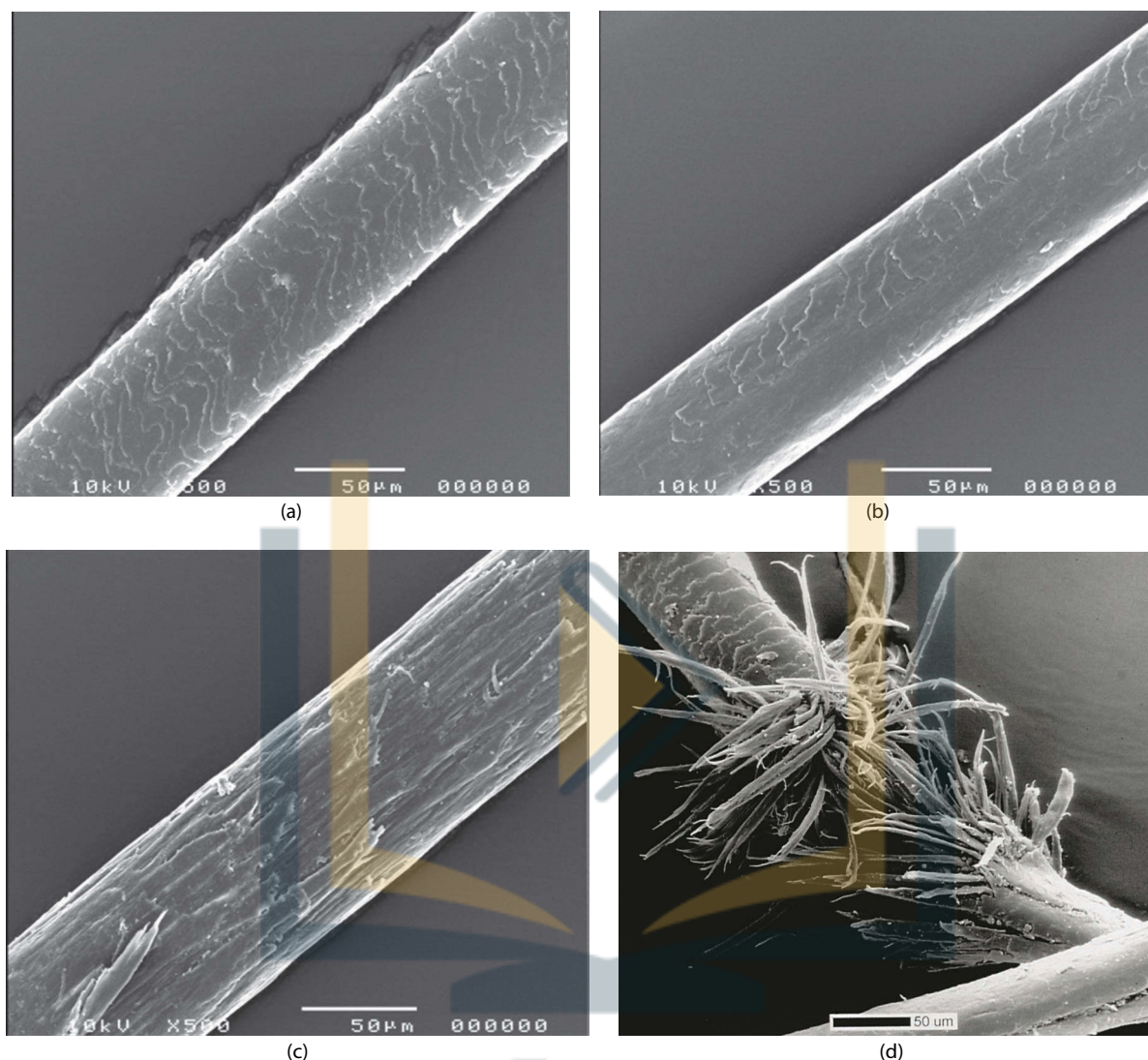


**Figure 27.13** In the 21st century, badly weathered hair is epidemic. (a) Copenhagen, (b) New York, (c) South Africa, (d) Singapore.

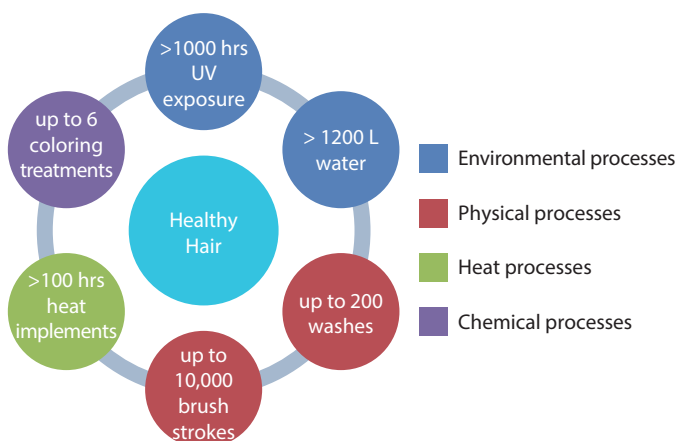
are chemical procedures, notably bleaching, perming, relaxing, and straightening. Other insults are due to poor habits and practices and include physical damage from brushing and combing and the use of excessive heat from implements such as blow dryers and flat irons. It is now recognized that poor brushing and combing are more detrimental than previously thought.

### Causes of Hair Damage

Figure 27.15 shows an average exposure to these various insults over 1 year, which equates to approximately 12–15 cm of growth. In Figure 27.15 the types of damage have been color-coded to four main processes—environmental, physical, heat, and chemical. These insults do not occur in isolation. The final damage to hair is due to a combination of all these factors, their



**Figure 27.14** EMGs of progressive severe weathering to a hair shaft. (a) Root, (b) mid-shaft and (c) tips, and (d) trichorhexis nodosa from a woman with 15-cm length hair.



**Figure 27.15** Causes of hair damage. (Data courtesy of P&G.)

frequency and intensity. If a woman possesses hair of 40 or 50 cm length, there may be 4 or 5 years of these cumulative insults on the hair ends.

Most of these insults impact at the nanostructural level by causing changes to protein and lipid structures. The insults themselves cannot be detected but can be measured by techniques such as roteomics and lipidomics that identify the exact structural changes. As a result there are micro-structural and single-fiber changes that will eventually manifest as macrostructural or bulk hair changes. As an increasing number of fibers lose cuticles, the cortex will eventually be exposed, which further reduces shine and will increase the propensity of hair to form split ends. As more protein damage occurs, the tensile strength of hair will decrease and eventually lead to breakage, which women will then notice as additional hairs in the brush or as a lack of smoothness from the broken ends (Figure 27.16).





**Figure 27.16** Disruption of the normal structure and physiology of the hair shafts results in dry (a), weak (b), and unmanageable (c) heads of hair lacking shine.

## COSMETIC HAIR CARE PRODUCTS

Selection of the correct products from the plethora available is not necessarily intuitive. The desire for constant change, which is a hallmark of the age, leads to constantly changing to different product ranges. Research demonstrates that this offers no advantage and indeed is potentially deleterious. A clear understanding of the state of the hair, selection of the correct regimen type to address this, and the regular use of these products is the key to prolonged hair health.

To restore and maintain healthy hair an appropriate regimen is necessary. This is a combination of products selected for an individual's hair type, desired end benefit, and which fits with their lifestyle and economic circumstance.

### Hair Care Regimens

A basic haircare regimen includes a shampoo, a range of conditioning variants, and ancillary products such as serums, heat protectors, smoothing agents, and styling products.

## HAIR CARE PRODUCTS FOR DIFFERENT HAIR NEEDS

The cosmetic industry takes certain factors into account when designing a regimen for different needs and which to a large measure are independent of regional stereotypes, although in areas such as the Americas and increasingly in Europe, all phenotypes are present in the marketplace.

Product ranges are now principally designed to manage, fine, thick, curly, and chemically damaged hair wherever they occur and whatever the hair phenotype.

### SHAMPOOS

Early products were relatively inefficient and many were harsh to the cuticular surface, the skin, and the eyes. Today such products are highly efficient, aesthetically pleasing, and mild to the surfaces they touch. In addition to removing sebum from the hair and the detritus it inevitably collects, many now contain ingredients designed to enhance the natural properties of hair and mitigate environmental and self-inflicted damage. Products carry ingredient labels in compliance with worldwide and company regulations (Figure 27.17). Hair-care





**Figure 27.17** Products carry ingredient labels in compliance with worldwide and company regulations.

products, in comparison to skin care, are inexpensive and widely distributed.

#### General Attributes of Shampoos

Shampoo formulations seek to maximize the following qualities:

- Effective cleaning
- Easy rinsing
- Good finish after washing hair
- Pleasant in-use aesthetics (lather, product thickness, perfume, etc.)
- Minimal skin/eye irritation
- No damage to hair
- Outstanding safety profile
- Good biodegradability

#### Modern Shampoo Formulations

Shampoos are invariably an aqueous (water-based) product and consist of three major components:

1. Primary surfactants for removing dirt and foaming power
2. Secondary surfactants to improve and condition the hair
3. Additives which complete the formulation and add special aesthetic effects and endow secondary benefits such as volume and shine

**Surfactant Mode of Action** Both soaps and shampoos contain surfactants—compounds that lower the surface tension between a liquid and a solid. They may act as detergents (a mixture of surfactants with cleaning properties in dilute solutions), wetting agents, emulsifiers, foaming agents, or dispersants.

Soaps can bind to oils with such affinity that they remove too much if used on hair. Shampoos use certain surfactants balanced to provide the level of surface cleaning suited to hair fibers. These surfactant systems are called *syndets*—synthetic/detergents which are manufactured with a range of properties (6).

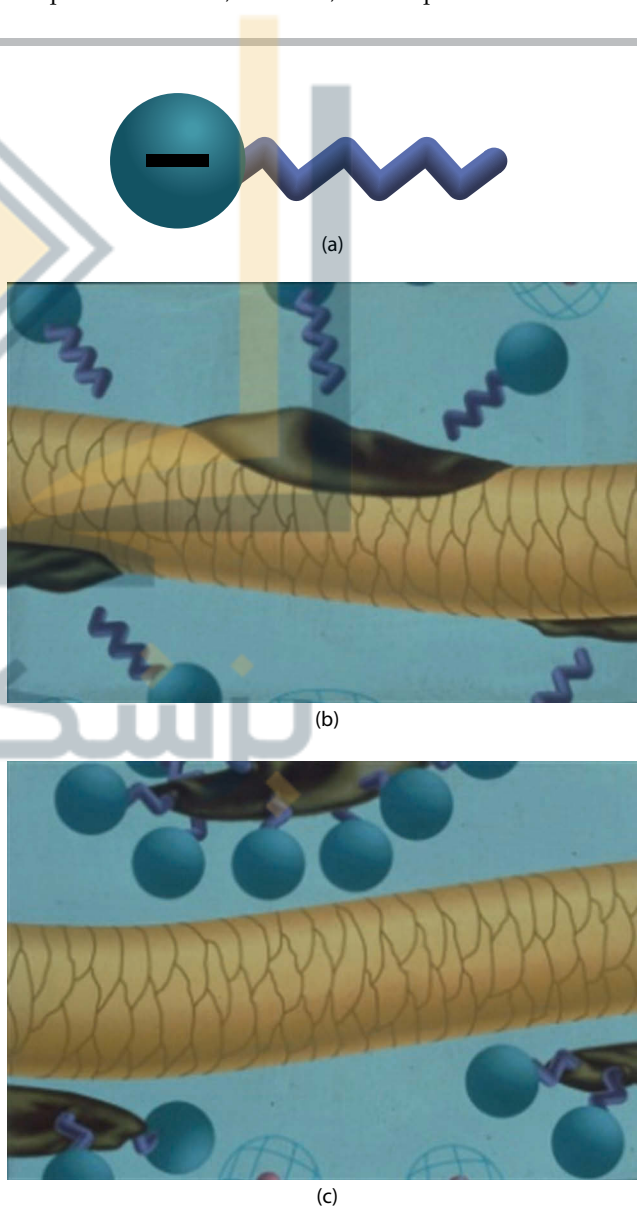
**Mode of Action** Undamaged hair has a negatively-charged hydrophobic (water-resistant) surface to which lipids (fats) adhere but from which water is initially repelled. Sebum and

detritus are not easily removed when the hair is rinsed with plain water. Shampoo applied to wet hair is absorbed into the oil/hair interfaces.

A surfactant inherently moves toward “surfaces” and cleans hair by removing dirt and oils (sebum) that are not water soluble. It also forms a lather, which is a signal desired by many that the shampoo is working.

A surfactant has a head group that is hydrophilic (water loving/oil hating) and a tail group that is hydrophobic (water hating/oil loving). Surfactants will either migrate to the air/water interface or to an oil material, with their hydrophilic head group toward the water and the hydrophobic tail group toward the oil or air. The surfactant will try to surround the oil droplet and in doing so will lift it off the surface, where it will be rinsed off down the drain (Figure 27.18).

The three types of surfactants or detergents found in shampoos are anionic, nonionic, and amphoteric.



**Figure 27.18** (a) Surfactant molecules with hydrophilic heads act by (b) removing the dirt from the hair with a lipophilic (fat-loving) component and (c) transferring it to the rinse water with hydrophilic (water-loving) component.

- **Anionic:** These have a negatively charged hydrophilic component and are mainly used as primary surfactants. Laureth sulfates and lauryl sulfates are often used to gently cleanse. They are highly effective and possess the foaming properties desired by the consumer. Products which do not foam are often regarded as “ineffective” and may reduce compliance.
- **Nonionic:** These surfactants condition the hair and perform gentle cleansing. They can increase the quality of lather in a shampoo as well as its viscosity and solubility. They can be added to for mildness or improve the anti-static qualities of a shampoo, e.g., ethoxylated fatty alcohols.
- **Amphoteric:** These surfactants contain a balance of positive and negative charges. They are very mild cleansers. They condition the hair but are generally less effective cleaners than anionic surfactants. They are also non-irritating to the eyes, which is why they are commonly used in baby shampoos.

### Key Ingredients of Shampoos

The key ingredients of shampoos are described and the chemical names found on products are included in Table 27.2.

### Specialized Shampoos

**Anti-Dandruff** In addition to regular shampoos, the most popular scalp products sold worldwide are targeted at preventing dandruff, a condition which affects over half the adult population at some time in their lives. Actives such as zinc pyrithione, climbazole, and salicylic acid are used in both shampoos and conditioners to improve scalp health by the elimination of the putative fungus *malassezia globosa* and reduce surface sebum levels. If used regularly they can prevent signs and symptoms of dandruff.

**Conditioning (2-in-1) Shampoos** Combination, or 2-in-1, products were developed first by Procter and Gamble in the late 1980s and delivered for the first time cleansing and conditioning benefits from a single product source. Since this innovation, it has become possible and commonplace to incorporate conditioning ingredients into shampoos—primarily to prevent tangling but also to facilitate styling.

The challenge was to deliver conditioning actives from a product that has to both clean hair and deposit conditioning ingredients onto the hair surface. The main mechanism of achieving this is via a coacervate formation wherein an anionic polymer is formulated with a surfactant and silicone to deposit the silicone on hair as the shampoo is diluted during rinsing.

There exists a range of polymers that can be used for this including natural polymers, celluloses, and synthetic polymers. These play a role in creating the coacervate (a miniscule

spherical droplet of assorted molecules which is held together by hydrophobic forces from a surrounding liquid). This enables deposition of silicone on hair but in some cases can imbue wet feel benefits (Figure 27.19). Consequently, hair can feel smooth during shampooing and with decreased friction forces.

**Shampoos for Children** Shampoo for infants is formulated so that it is the same pH level as the eye, making it less irritating if it were to get into the eyes. Most contain sodium laureth sulfate and/or sodium lauryl sulfate. Alternatively, infant shampoos may be formulated using other classes of surfactants, most notably non-ionics, which are much milder than any charged anionics used.

**Moisturizing Shampoos** Moisturizing products are a huge need globally and across all hair types. Moisturization is not the same as adding moisture to hair and is more about delivering a soft and conditioned feel. It has been shown that hair with higher moisture actually feels drier than hair with low moisture levels. The benefit can be delivered at different levels depending on the product. There are very intense formulations for women with very damaged and dry hair and lightweight versions for women who want conditioned feel but are concerned about a tradeoff of weigh down.

**Shampoos for Color-Treated Hair** In recent years products have become more advanced in terms of efficiency of deposition of conditioning actives and also adjustment to different needs. Of particular interest have been shampoos and conditioners designed for women who color and chemically treat their hair. Not only will these women potentially have more physical damage in terms of split ends, etc., but the efficiency of deposition of silicones such as dimethicones on colored hair is significantly lower than on non-colored hair. This is driven by surface energy changes that occur during coloring and specifically the increase in surface hydrophilicity caused by loss of the surface bound f-layer lipid.

Polymers are also being used in shampoo formulations to enhance silicone deposition on previously colored hair. As an example, a high charge density polymer, poly(diallyldimethyl) ammonium chloride, on dilution forms a hydrophobic layer on hair, enhancing silicone deposition from both the shampoo itself and from the subsequent conditioner.

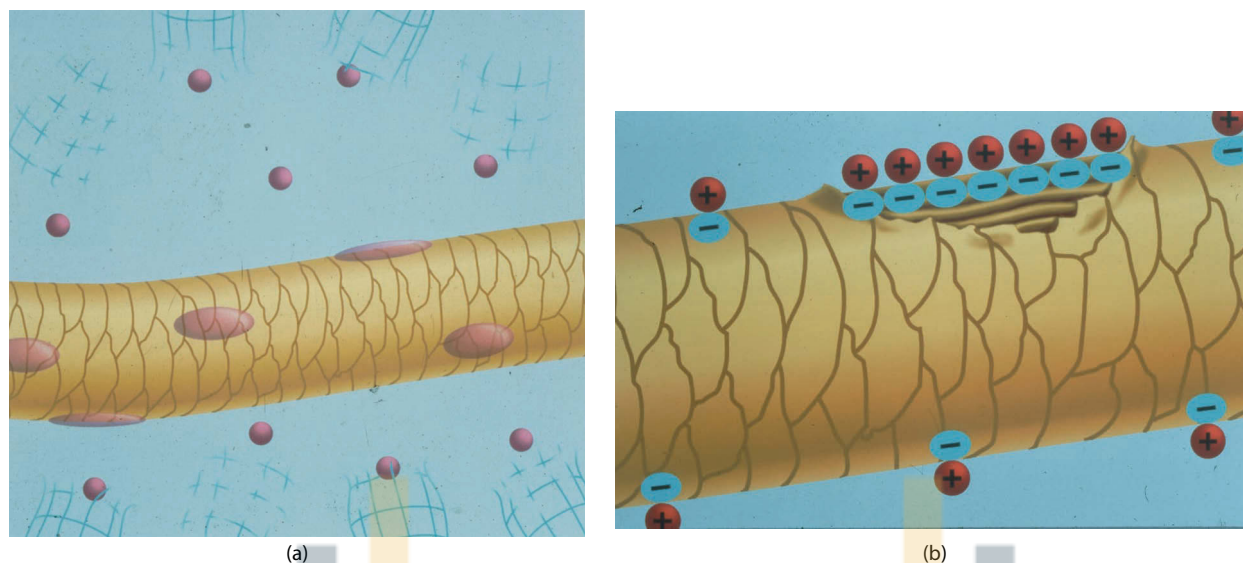
## CONDITIONERS

The concept of conditioning hair is not new. Essential oils (tea tree, jojoba) were used historically and continue to this day. The Victorians were keen on Macassar oil and invented the antimacassar cover for chair backs to prevent greasy residue. Brilliantine is largely regarded as the first of

**Table 27.2** Key Ingredients of Shampoos

Material class	Name to look for on products	Function
Surfactant	Sodium lauryl sulfate	Cleans sebum, dust, and dirt from hair as well as previously applied product (e.g. gels, hairsprays)
	Sodium laureth sulfate Cocamidopropyl betaine	Provides lather
Silicone	Dimethicone	Makes hair easier to comb, softer and smoother Increases shine by increasing fiber-to-fiber alignment
Polymer	Polyquaternium-76	Provides wet feel
	Guar hydroxypropyltrimonium chloride Polyquaternium-6	Aids in depositing silicone conditioning materials on hair
Additional ingredients	Hydroxypropyl methylcellulose	
	Panthenol	Targeted benefits, e.g. moisturization, antioxidant,
	Vitamin E Trisodium ethylenediamine disuccinate	chelant for UV protection





**Figure 27.19** Deposition of silicones onto negatively-charged surface of the damaged hair from a 2-in-1 product.

modern conditioners but was largely employed for softening moustaches.

The practice of regular conditioning after shampooing is relatively recent and still not part of a global culture. In light of worldwide coloring and bleaching in addition to daily weathering, conditioning hair is critical to its sustained health as it inevitably weathers in an accelerated manner.

Early “conditioners,” which were equivalent to greasy pomades, offered protection against the harsher effects of relaxers, permanents, straighteners, and colorants. Modern intensive conditioners can be formulated in a range from light to heavy and have much greater aesthetic properties. If used regularly they can obviate the effects of chemical and physical processes.

### Conditioners' Mode of Action

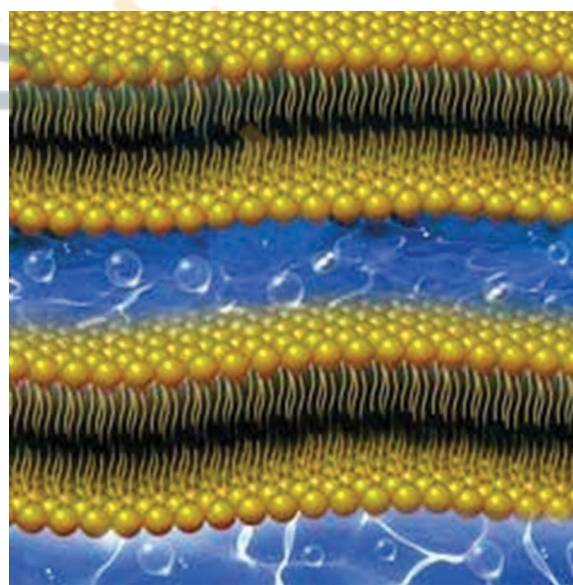
A conditioning product deposits actives onto the surface of the hair shaft. It typically does this by creating a lamellar gel network structure composed of fatty alcohol and cationic surfactants with silicone suspended in the hydrophobic part of the gel network.

During product application the gel network spreads on hair, giving a very smooth feel, reducing knots and tangles. As the product then dries the silicone spreads evenly over the hair surface forming a thin layer and increasing surface hydrophobicity. This hydrophobic layer will change hair feel, especially when hair is dry, giving it a smooth, soft feel, and it will reduce hair friction and combing forces (Figure 27.20). Importantly, this will also reduce knot and tangle formation and reduce combing breakage.

Early silicone products were limited by the ability of formulators to include this type of technology into aqueous solutions. In the last 20 years there has been an explosion in silicone technology such that one recent innovation has been the introduction of functionalized silicones such as terminal amino silicones (TAS) which add amine groups to the end of the silicone chains. By more closely matching the interfacial tension of the silicone to the surface energy of hair, the TAS silicone materials will deposit significantly better onto colored and damaged hair than uncharged silicones like dimethicone.



(a)



(b)

**Figure 27.20** Deposition of conditioning actives by creation of a lamellar gel network.

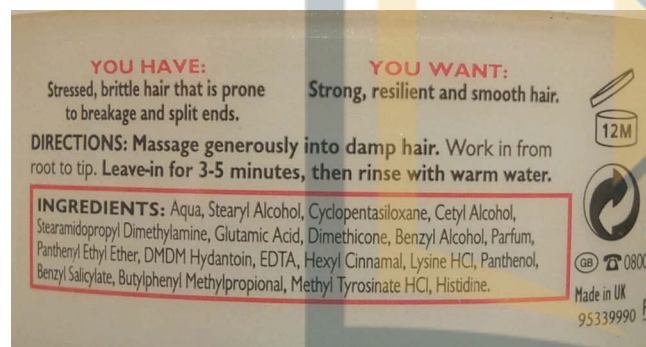


These materials also have an additional benefit of improved ability to deposit on severely damaged hair tips than previous dimethicone silicones.

### Different Conditioning Variants

The level of conditioning benefit can be increased by varying the levels and types of silicones and cationic surfactants used (in general longer chain surfactants deliver higher conditioning).

- **“Intensive” conditioners** are heavy and creamy in consistency since they contain high proportions of fatty alcohols, and are well suited to hair that is significantly damaged or coarse. Such conditioners can be left in the hair for a long time, and can temporarily hold split ends together (Figure 27.21).
- **“Leave-in” conditioners** tend to contain lighter-weight conditioning materials, which add little weight to the hair (Figure 27.22).



**Figure 27.21** Intensive conditioner. High levels of fatty alcohols (see ingredient listing) designed for significantly damaged hair.



**Figure 27.22** Rinse-out conditioner; part of the regimen for thinning hair.

There are also “hold” conditioners, which are combination products that provide the benefits of conditioning while also holding the hair in place like a mousse. This effect is achieved using cationic polymers.

### Conditioning Products in Ethnic Usage

In Africa many women who retain their natural phenotype employ light conditioning agents as their primary hair care product. As the essential need is for moisturization rather than cleansing, this by experience is often their preferred method of hair care.

### Oil Inclusion in Conditioning Products

Oils have a long history of use in hair care and are invariably culturally based. This history has driven the introduction of natural oils into modern hair care products to deliver dry and wet feel benefits. In some cases these ingredients supplement silicones. Examples of these oils include argan oil, Moroccan oil, and coconut oil.

Other ingredients in modern conditioners and hair care preparations similarly work to smooth the outer layers of the cuticle. These may include protein extracts (collagen, and the amino acids obtained from silk) and panthenol and similar compounds, which are related to vitamin B5. Some of these are known to penetrate into the cortex and to help to increase its moisture content. Keratin or hydrolyzed keratin is another common ingredient added to products. Keratin proteins will typically not readily penetrate inside hair due to their large size. Hydrolyzed keratins are more likely to penetrate but neither active will replace lost proteins.

### Key Ingredients of Conditioners

The key ingredients of conditioners are described and the chemical names found on products are included in Table 27.3.

#### Silicones

Silicones are an example of polymers and are now widely used as ingredients in hair conditioners, shampoos, and hair gel products. They are synthetic and chemically inert. Some silicones, notably the amine functionalized amodimethicones, are excellent conditioners, providing improved compatibility, feel, and softness, and lessening frizz. The phenyltrimethicones is another silicone family and these are used in reflection-enhancing and color-correcting hair products, where they increase shine and glossiness.

### Ethnic and Cultural Differences in Conditioning Preferences

The level of conditioning required to achieve optimum desired performance will depend on the hair phenotype and morphology (curl, diameter, etc.), hairstyle, and the level of hair damage. In general women with European phenotype hair tend to prefer lower-conditioning products more suitable for their fine hair (Figure 27.23). Women African descent tend to prefer high-conditioning products that give high protection against knots and tangles (Figure 27.24). Indian and Asian women will also tend to prefer high-conditioning benefits to drive a high shine, smooth look (Figure 27.25).

### Special Hair Needs

Dry, woolly hair generally requires heavier deposits of conditioners than other hair types. The use of leave-in and “intensive” conditioners is growing. The use of moisture-retaining

**Table 27.3**    Key Ingredients of Conditioners

Material class	Name to look for on products	Function
Silicone	Dimethicone	Makes hair easier to comb, softer and smoother Increases shine by increasing fiber to fiber alignment
	Amodimethicone	
	Bis-aminopropyldimethicone	
Cationic surfactant	Stearamidopropyldimethylamine	Positively charged molecules preferentially attracted to areas of damage Makes hair easier to comb, softer and smoother and less static
	Behentrimonium methosulfate	
	Behentrimonium chloride	
	Dicetyldimonium Chloride	
Fatty alcohol	Cetyl alcohol	Gives hair a smooth fee when dry and improves wet combing Give products a thick, creamy appearance
	Stearyl alcohol	
	Cetearyl alcohol (mix of cetyl and stearyl alcohol)	
Oil/emollient	Hydrogenated coconut oil	Moisturizes hair to improve softness
	Mineral oil	
	Argan oil	
	Moroccan oil	
Additional ingredients	Panthenol	Targeted benefits, e.g. moisturization, antioxidant
	Vitamin E	



**Figure 27.23**    In general women with European phenotype hair tend to prefer lower conditioning products more suitable for their fine hair.

ingredients (humectants) such as panthenol can be augmented by cationic ingredients (e.g., polyquarternium derivatives), which leave hair more manageable.

**How to Select the Correct Hair Care Regimen**  
One of the major concerns women have for hair care products is choosing the correct one for their hair type and style. Too low a conditioning level will not deliver the feel, shine,



(a)



(b)

**Figure 27.24**    Women of African heritage tend to prefer high-conditioning products that give high protection against knots and tangles.





**Figure 27.25** Indian and East Asian women tend to prefer high-conditioning benefits to give a high-shine, smooth look.

and frizz benefits they desire but too high a conditioning level may create weighed down hair and a greasy, sticky feel and look. These negatives can be an issue when a high-conditioning product is used on fine and/or straight hair. To help women avoid these issues, version names are being chosen to indicate a desired benefit and the level of conditioning is commensurate with this and the assumed state of the hair.

Products may be labeled as a collection (regimen) under the following main benefit categories (from lowest to highest moisture capability):

- Volume
- Clean
- Defined curls
- Smooth
- Moisture
- Strength/anti-breakage
- Color
- Damage repair

Within these broad categories will be a range of shampoos (including clarifying), conditioners of varying intensity, and styling products including mousses, gels, heat defense, hair-sprays, and serums.

#### *Volume*

Women in this category tend to have finer, thinner hair texture or shorter length, desire more cleaning, and wash more frequently. They tend to use blow dryer, curling iron, and styling products to create and keep desired look.

Women state that they want to achieve healthy volume, but find their flat, lifeless hair is also prone to overstyling and overconditioning. These products tend to have low conditioning levels and contain polymers which add lift at the root. They

are suitable for women with fine hair/low volume/undamaged hair (Figure 27.26).

*Technical Solution:* The technical solution for women requiring volume is to increase cleaning and lather cushion for detangling without depositing undue levels of conditioning agents on the hair. Consequently, products incorporate:

1. Higher surfactant levels, to increase cleaning
2. Cationic polymer, to improve lather cushion and detangling but rinse clean to prevent deposition onto the hair
3. Low or no silicone, to minimize deposition on the hair shaft in order not to weigh down the hair mass and allow hair's own natural conditioning to provide volume

#### *Moisture or Smooth*

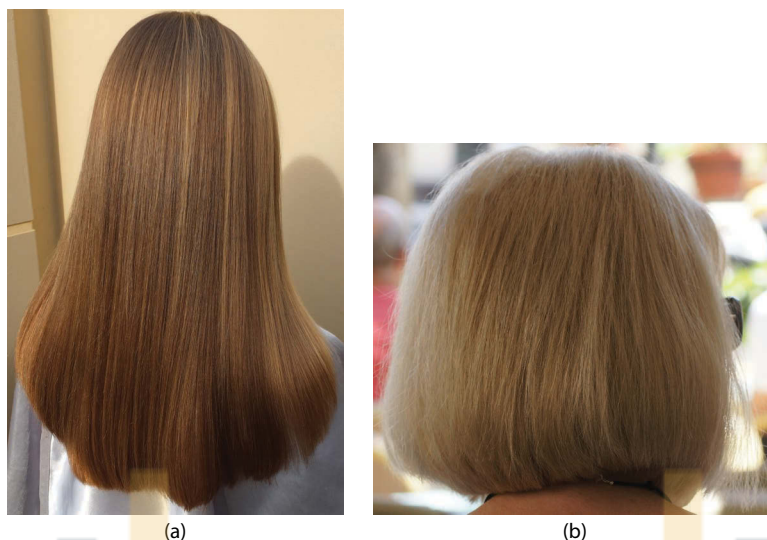
Women seeking this benefit tend to have hair texture ranging from fine to coarse and would probably include hair which is dry with slight damage from heat and coloring. Such hair is susceptible to changes in humidity. Women desire an improvement in hair feel through balancing conditioning with cleaning. Shine is a requirement.

Products for these benefits are likely to have higher conditioning levels and are suitable for women with a degree of damage and a tendency to develop frizz and flyaways. Women with unruly hair prone to dryness or frizz would normally find a variant in this category (Figures 27.27 and 27.28).

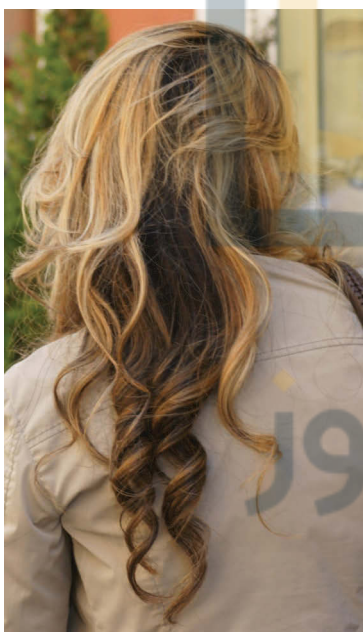
#### *Technical Solution:*

1. Higher molecular weight cationic polymer to provide more hair lubricity, detangling, and silicone deposition.
2. Silicone, which provides increased wet detangling and excellent dry conditioning with less weight on the hair
3. Oils are often added to minimize frizz and flyaway





**Figure 27.26** Women with finer, thinner hair texture (a) and with shorter length (b) tend to wash more frequently.



**Figure 27.27** Products for these benefits are likely to have higher conditioning levels and are suitable for women with a degree of damage and a tendency to develop frizz and fly-away. Women with moderately unruly hair prone to dryness or frizz would normally find a variant in this category.



**Figure 27.28** Products for these benefits are likely to have higher conditioning levels and are suitable for women with a degree of damage and a tendency to develop frizz and fly-away.

of silicone is low. These products will have the highest conditioning levels and are almost mandatory for women with repeated bleaching where the hair is fragile, porous, and prone to tangling and breakage.

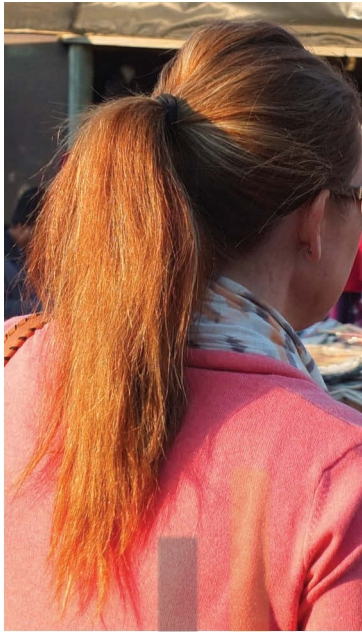
This category would include intense conditioners including “masques,” heat defense serums, and potentially specialized products to manage severely damaged tips. The different conditioning materials in Table 27.3 are generally specific to the product used to deliver the benefit.

*Technical Solution:*

1. Higher level of cationic polymers to protect hair during washing and wet combing (Figure 27.30)
2. Silicones to give high levels of dry conditioning and a moisturized, smooth feel.

*Damage Repair*

Women in this category tend to have hair with high levels of damage from overprocessing and overstyling (Figure 27.29). They seek high conditioning levels from products but also need sufficient cleaning, as they are likely to be using multiple steps. A technical challenge for this level of damaged hair is the strong negative charge it carries, so the deposition efficacy



**Figure 27.29** Women in this category tend to have hair with high levels of damage from overprocessing and overstyling.



**Figure 27.30** Products contain polymers which help define even the most challenging of natural curls.

Other categories as listed below will have carefully balanced cleansing and conditioning products aimed at addressing the prime concern of the woman.

#### *Strength*

These products are designed for the woman with a significant breakage problem.

#### *Curls*

Products contain polymers which help define natural curls.

## Summary

Conditioning materials are formulated as emulsions and are traditionally applied after shampooing to increase hair “quality” before grooming. They reduce negative charge, prevent flyaways, increase manageability, and hence reduce tangling. Other ingredients are classed as humectants—essentially, they have the ability to increase moisture content and minimize moisture movement in and out of the hair.

Modern, high-quality conditioners increase the manageability, shine, and moisture content of each hair shaft and are designed to provide one or more of the following functions:

- Increase the ease of wet and dry combing
- Smooth, seal and realign damaged areas of the hair shaft; minimize porosity;
- Impart sheen and a silken feel to hair
- Provide some protection against thermal and mechanical damage
- Moisturize
- Add volume and body
- Eliminate static electricity

## STYLING AND HAIR HEALTH

For most women, a “good” cut, utilizing the intrinsic nature of the hair and its patterns, is the first essential step for good style. Prior to this is the necessity for cleansing and regular conditioning, particularly if the hair is subjected to repeated insults.

Each day, millions of women strive to achieve their desired style for work and recreation. In order to do this they may employ nothing other than natural drying or alternatively, a wide range of implements and products.

The application of physical forces on the hair (combing, brushing, teasing, backcombing) and the use of heat (natural or forced drying, direct heat) have potentially serious consequences for the hair shaft, especially since these actions will most likely be repeated thousands of times. The judicious use of moisturizing detanglers and heat protection sprays can improve the quality and integrity of the hair shafts and help in the preservation of hair health.

The technologies behind these products is described here along with a practical guide to their use.

## Styling Problems

Of all the various issues with hair styling, time and the intrinsic nature of each woman’s hair are the most problematic. Hair phenotype dictates what is easily achieved. Whatever the issue, modern hair care products applied correctly and regularly can contribute significantly to the improvement of these situations.

Certain hair phenotypes have inherent problems—Northern European hair is very fine and tends to lankness and lack of body. Very curly, dry hair suffers from physical grooming problems. Indian and Asian women typically desire a smooth and sleek look but many have a considerable hair mass and even significant wave which requires excessive amounts of time to manage. Wavy or very curly Caucasian hair suffers from frizz and flyaways and needs products to control it.

## Volume Control

Control of “volume” is the most common driver for “styling.” Volume in the broadest sense implies either too little or too



much. It may be the desire to increase volume for those with fine or thinning hair, or it may be the control of wayward curls. The desire to control volume and keep it in a day-long style is the ultimate end benefit for millions of women (Figure 27.31).



(a)



(b)



(c)

**Figure 27.31** Three volume problems: (a) not enough, (b) too much, and (c) managing curls.

## Factors Influencing Hair Volume

The key factors affecting volume are:

- Diameter
- Density
- Stiffness
- Friction
- Cohesion

For women with fine to medium hair who desire to increase or control volume there are a number of strategies which in combination with styling and heat protection products can produce the desired end benefit.

### Blowdrying

With the introduction of precision cutting techniques in the 1970s, blowdrying became universally popular in the salon and at home. The blowdryer works by using a heated air flow (up to  $\sim 80^{\circ}\text{C}$ ) to first rapidly remove water between hair strands. It then evaporates water from inside each hair strand, at which point the hair can be shaped into the desired style, forming a “wet set.” The wet set is improved by evaporating as much water as possible, and inadequate drying is one reason style does not hold. How long the wet set will keep its style during the day depends to some extent on the temperature and humidity. At high relative humidity, moisture in the air will penetrate hair, break temporary hydrogen bonds holding the style in place, and the hair will revert back to its natural shape.

### Flat/Curling Irons

Flat irons or curling irons are also used to achieve the desired volume. These implements can reach temperatures up to  $220^{\circ}\text{C}$  and are effective at removing water and creating a very effective wet set. However, these implements can cause significant damage, especially if used at a high temperature setting or used for an excessive amount of time (Figure 27.32). Care should be taken to keep the heat setting below  $190^{\circ}\text{C}$  and limit the number of passes.

Heat protection products can help reduce damage by smoothing the hair surface, making it easier to pass the flat iron through hair and reduce localized heating.

### Temporarily Increasing Hair Friction for Volume

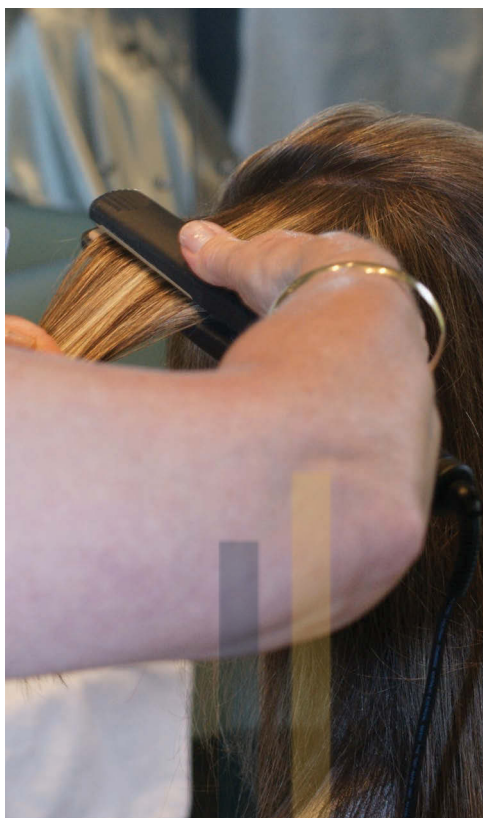
There are three ways of temporarily increasing friction, which also helps increase volume and fullness:

1. **Cleaning:** Hair's natural oils or sebum can build up over time, making hair more slippery and reduce friction and make hair limp. Cleaning these oils off with a volume-building shampoo will restore hair's natural tendency to volume.
2. **Teasing or backcombing:** Teasing or combing hair backward from tip to root will roughen the cuticle, drastically increasing friction. This temporarily creates volume but is potentially very damaging over time. Teasing causes the cuticle layers to roll and peel up (Figure 27.33).
3. **Styling products:** These products can create stiffness at the root to create volume. Spacer particles can also be added to provide volume by separating fibers.

## Straightening

There are several ways to straighten hair depending on the starting level of curl and how permanent the treatment is.





**Figure 27.32** The temperatures generated by some implements, hair dryers, and hot combs, can exceed 200°C and may cause significant damage.



**Figure 27.33** Teasing or combing hair backward from tip to root will roughen the cuticle, drastically increasing friction.

As permanency increases there are also tradeoffs with hair health, as these treatments involve reactive chemistry that can impact fiber integrity.

The least permanent method is blow drying and/or flat ironing, as discussed previously. This method uses heat to create a wet set which will only last until the next wash. Using

excessive heat (>190°C) can be detrimental to hair health, as is excessive use of such implements.

A recent popular straightening treatment is keratin treatments such as the Brazilian keratin treatment. These products use formaldehyde to crosslink hair into a straight shape. The active is applied and then crosslinking is activated by heat during the flat-ironing final step. The formaldehyde-containing products have become less common in the last few years as regulations have restricted its use globally due to safety concerns. Alternative products are emerging including those containing glyoxylic acid and glyoxyloyl carbocysteine, which work via a similar crosslinking mechanism. There have been reports of these products causing hair breakage which could either be due to the high flat iron temperatures used to create the original style or repeated treatments where multiple crosslinks eventually make hair very brittle and easy to break. Crosslink products are more effective for women with wavy hair versus very curly hair and typically last 2–3 months depending on wash frequency.

An alternative for women with curlier hair are Japanese straightening treatments. The products, which have been in the market from many years, employ thioglycolate technology to straighten hair prone to frizz. Care must be taken when applying the thioglycolate chemistry as it can irreversibly damage hair by breaking down the disulphide bonds which give hair its strength if left too long on hair. Typically this chemistry is best applied in the salon by experts who can accurately judge processing time and get the optimum result. These products will work well on women with wavy and curly hair and will last 3–6 months. However, they generally will not be able to fully straighten high curly hair.

For women with highly curled hair, e.g., women of African descent, straightening is achieved with relaxer treatments. Most relaxers are high-pH products in the form of heavy creams consisting of very high oil-in-water emulsions which are combed through the hair, where they slowly break down the structural bonds. The aggressiveness of the caustic is controlled by the incorporation of suitable emollient oils. The two most common types of relaxers are sodium hydroxide (lye) and guanidine hydroxide (no-lye). Also on the market are potassium and lithium hydroxide relaxers and ammonium bisulfite relaxers. However, sodium hydroxide and guanidine hydroxide have proven to be the most effective. Guanidine hydroxide relaxers are considered less irritating to the scalp than lye-based relaxers; therefore, some women prefer them. No-lye products, although considered less harsh, can still burn the scalp, eyes, and ears.

Relaxers are the most damaging to hair structure of all the straightening products but they are also the most permanent and effective. Relaxed hair thus requires considerable aftercare (Figure 27.34). Modern hair care products have been developed for this market and the conditioners include fatty alcohols and light mineral oils to maintain the critical moisture content.

## STYLING PRODUCTS

A range of styling products to create long-lasting styles have emerged to complement the new generation of cleansing and conditioning products. These can enhance or alter most common aesthetic styling problems. Foremost among these is the control of volume—either too little or too much. Managing frizzy hair is also important, and products for so-called “ethnic” hair are emerging.



**Figure 27.34** Most relaxers are formulated in the form of heavy creams consisting of very high oil-in-water emulsions which are combed through the hair, where they slowly break down the structural bonds. Relaxed hair allows for easier grooming.

Styling products help keep long-lasting volume by creating reinforcing bonds between hair shafts at critical locations to the style. These bonds come in two types:

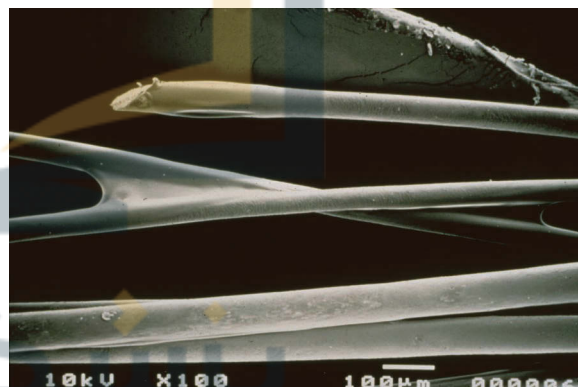
- **Seam welds:** Bonds created that hold two hair shafts together in in side-by-side alignment.
- **Spot welds:** Found where hairs cross each other to create a support structure. The styling polymer glues the shafts together at this critical structural point (Figure 27.35).

### Hairspray

Hairspray (also called hair lacquer or spritz) is sprayed onto dry hair to keep it stiff or in a certain style. The spray can be dispensed from a pump or aerosol spray nozzle. Modern hairsprays were developed around the time the aerosol can was introduced in the 1940s.

Hairspray is the most common styler and is a solution of polymer in a mixture of solvents and propellants that is sprayed on the hair in droplets. The droplets are formed when the liquid is forced through a pinhole in the nozzle of the can. In aerosol hairsprays, the force is supplied by propellant. In non-aerosols, the force is supplied via mechanical action of pumping the nozzle. Typically, non-aerosol's propellants provide more force than mechanical pumping resulting in smaller droplet sizes. Smaller droplets dry faster giving aerosol hairspray a "drier" feeling than non-aerosol hairspray.

Ingredients are a blend of polymers that provide structural support to hair and include copolymers of polyvinylpyrrolidone (PVP) and polyvinyl acetate (PV). This copolymer mixture is usually modified to achieve the desired physical properties (adhesive strength, foaming, etc.). As the product dries the polymer forms spot welds to hold the desired style in place



**Figure 27.35** Spot welds are found where hairs cross each other to create a support structure. The styling polymer glues the shafts together at this critical structural point.

### Mousse

Mousses are typically added to wet hair before styling. They use a propellant and a surfactant in addition to water-soluble styling polymers to create a smooth, creamy foam which is easy to spread through hair. The hair is blow-dried and the polymers form spot and seam welds to hold style.

### Hair Gels/Waxes/Pomades

These products are typically added to dry hair and are popular with men and women with short hair styles. They can hold hair in different shapes and create, for example a "wet look" or a "texturized" look. They contain polymers and high levels of waxes which hold the hair in place

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# Dandruff and Seborrheic Dermatitis

James R. Schwartz and Thomas L. Dawson, Jr.

## INTRODUCTION

Modern society considers the formation of large, individually distinguishable flakes of scalp skin an abnormal condition (1). These flakes are dislodged by mechanical action and are visible either within the hair or on horizontal surfaces immediately below the hair such as shoulders and top of the back. This condition is known by a number of equivalent names, each having slightly different connotations. The most common, yet not very descriptive, is “dandruff.” “Seborrheic dermatitis” describes essentially the same condition with an emphasis on excessive oiliness (seborrhea) and irritation/redness (dermatitis) (2). Names reflecting the fungal causality (though none of them correctly termed based on today’s knowledge; see below) are captured in “pityriasis simplex” and “pityriasis capitis” (referring to *Pityrosporum*) and “furfuracea” (referring to *Malassezia furfur*). As all of these terms are in common use today; the practitioner and diagnostician simply needs to understand they represent the same symptomology based on the same etiology, and prescribe treatments as summarized below (2).

## CLINICAL FEATURES

Normal scalp has few flaking areas (Figure 28.1a) and healthy appearing, smooth skin. Dandruff is characterized by patches of loosely adherent, oily flakes, often accompanied by pruritus. Dandruff has the clinical feature of small white or gray flakes that accumulate diffusely on the scalp surface or in localized patches (Figure 28.1b). Dandruff does not exhibit the overt inflammation seen in seborrheic dermatitis, and is confined to the scalp.

In seborrheic dermatitis the scales are greasy and yellowish in color (Figure 28.1c). Seborrheic dermatitis flakes accumulate in adherent mounds on the scalp, and underlying inflammatory changes (seen as surface erythema) are evident. The lesions of seborrheic dermatitis vary in appearance, with the characteristic presentation being patches of red, flaking, greasy skin, particularly on the scalp, nasolabial folds, ears, eyebrows, and chest. However, patients often vary with respect to the degree of erythema, amount of flaking, and the extent to which the affected areas have a greasy appearance. It is also important to note that, while patients with seborrheic dermatitis may have oily skin, this is not necessarily the cause (3–5). As the terms dandruff and seborrheic dermatitis are widely accepted to refer to differing severities of the same etiology, herein we will refer to them as dandruff/seborrheic dermatitis (D/SD).

Recently, it has been demonstrated that the unhealthy skin state associated with D/SD has negative consequences to the quality of hair (6). Hair from D/SD scalp is thinner, has a more brittle surface, and is less shiny than hair from a normal scalp. These observations are very similar to those associated with scalp psoriasis.

## PREVALENCE

Dandruff and seborrheic dermatitis are the most common scalp afflictions of adolescence and adult life and are rare and mild in children (1,2,4). Historically, it was thought that about 50% of the world’s population is afflicted to some degree with onset at puberty and peak incidence and severity reached at the age of about 20 years. Dandruff becomes less frequent after the age of 50 years (1,2). A broad, well-defined survey consisted of 1408 Caucasians, African Americans, and Chinese subjects from Minnesota and Georgia, the United States and Beijing, Shanghai, and Guangzhou, China, and suggests that severity and prevalence of noticeable dandruff and seborrheic dermatitis is much higher in the adult population than first thought, at 81%–95% in African Americans, 66%–82% in Caucasians, and 30%–42% in Chinese subjects (Table 28.1, Figure 28.2a) (5). Additionally, the prevalence of dandruff was as high in U.S. teens as their adult counterparts, with prevalence at 75%–95% in Caucasian and African-American teens (Table 28.1, Figure 28.2b) (7,8). Based on this survey, dandruff occurs in 60%–90% and seborrheic dermatitis in 3%–5% of immunocompetent adults. In AIDS patients, the prevalence of seborrheic dermatitis increases to 30%–33% (9).

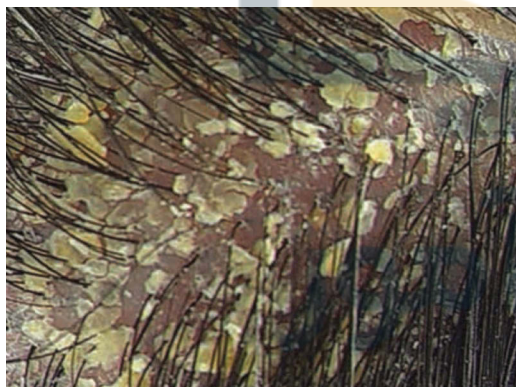
Dandruff appears to have little variation due to climate, as incidence and severity are similar in north to south of United States and China during the winter (Figure 28.3)(7,8). Higher shampooing frequency appears to result in lowered dandruff severity in all populations (Figure 28.4) (8). However, when examining population statistics, the frequency of antidandruff product use needs careful consideration. Despite higher shampoo frequencies and the ready availability of highly effective over-the-counter and prescription antidandruff shampoos in the United States, the most recent prevalence study in both adults and teens suggests dandruff is occurring at a much higher rate and severity than initially thought in the United States versus China (8). The higher prevalence of dandruff in the United States is most likely associated with the lower use of antidandruff shampoo in routine hair care regimens (10%–20%) versus China (40%–52%) (Figure 28.5). While the decreased incidence and lower severity of dandruff in China may lead to the assumption that there is a different etiology in Chinese subjects, recent clinical data indicates that in all geographies treatment remains similar in effectiveness (9,10). Further, while current DNA-based microbial identification can detect subtle differences distribution of genetic subtypes (11–13), it is yet to be evidenced that these differences are relevant to treatment paradigms (14). In addition, the majority of clinical studies demonstrated that scalp itch, a key symptom of dandruff and seborrheic dermatitis, was found to be correlated with the severity of dandruff flaking across all ethnic groups. Dandruff has recently received much attention as its presence can lead to loss of self-esteem and a negative social image (5,8,16).



(a)



(b)

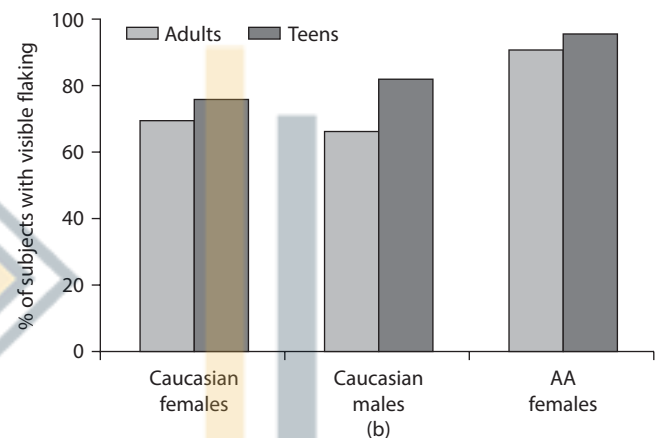
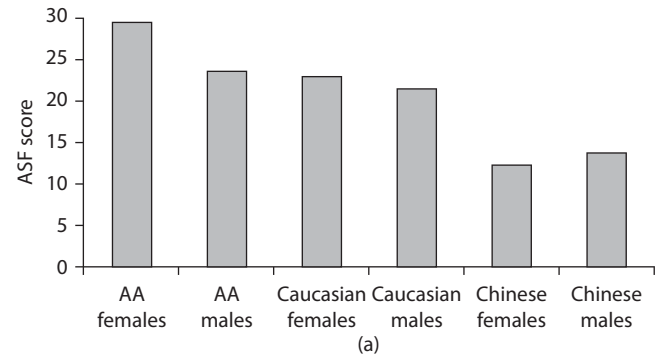


(c)

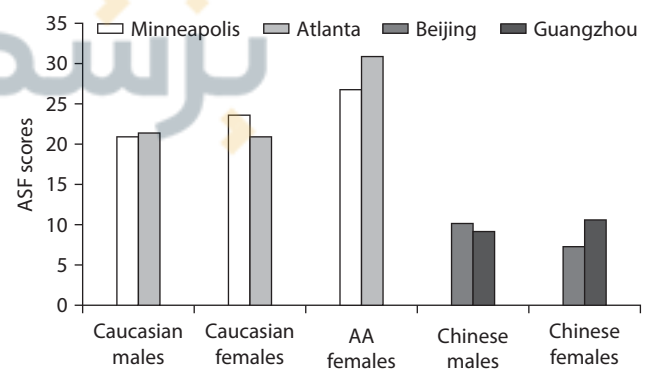
**Figure 28.1** Clinical features of normal (a), dandruff (b), and seborrheic dermatitis (c) scalp.

**Table 28.1** Adherent Scalp Flaking Severity Scores in Adult and Teens in the United States and China

	Adults	Teens
African-American females	29.3	27.1
African-American males	23.4	26
Caucasian females	22.7	22.8
Caucasian males	21.3	23.7
Chinese females	12.1	12.4
Chinese males	13.6	11.2



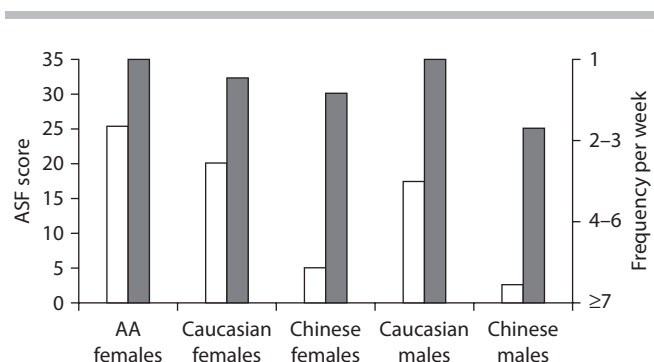
**Figure 28.2** Dandruff incidence among ethnic groups and teens. (a) Adherent scalp flaking (ASF) scores in African Americans (AA), Caucasians, and Chinese. (b) Dandruff incidence as high in teens as in adults.



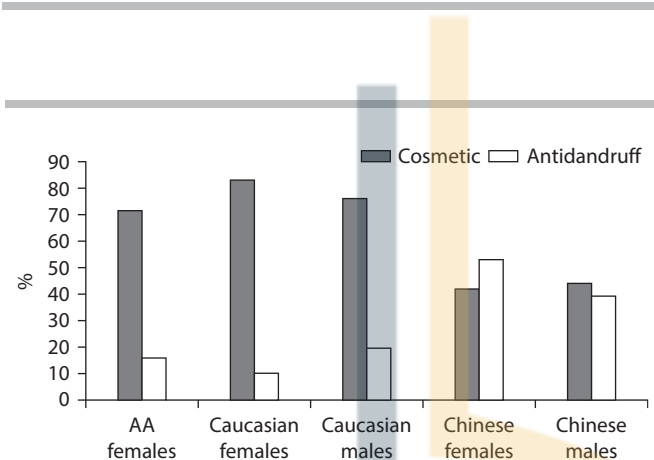
**Figure 28.3** Dandruff severity across climatic conditions. ASF, adherent scalp flaking; AA, African American.

## PATHOLOGY

The superficial flaking and redness that are the outward symptoms of dandruff and seborrheic dermatitis are manifestations of an abnormal epidermal structure and function (17). Flakes are generally believed to occur in “patches” on the scalp and these eruptions randomly “move” about on the surface over time. However, the underlying stratum corneum irregularities



**Figure 28.4** Adherent scalp flaking (ASF) score (white bars) versus shampoo frequency (shaded bars). AA, African American.



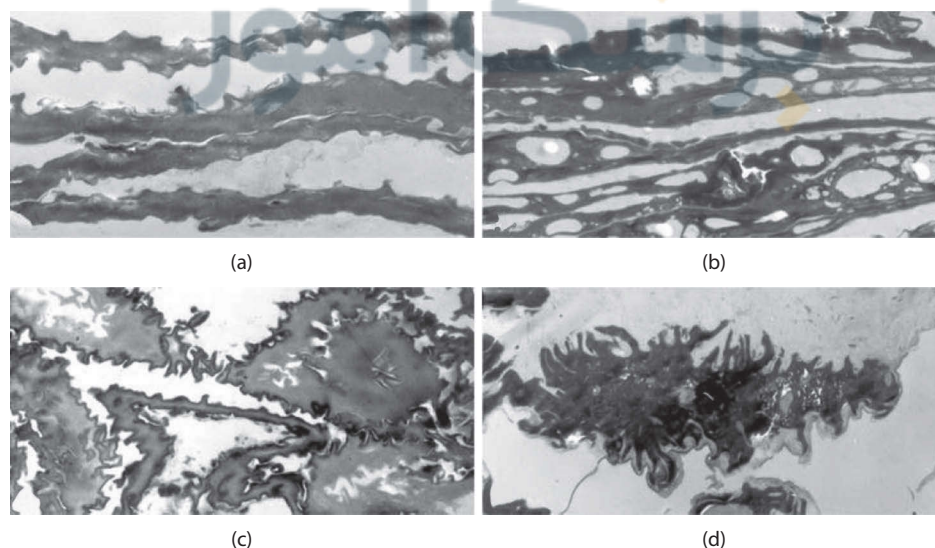
**Figure 28.5** Cosmetic versus antidandruff shampoo users. AA, African American.

occur throughout the scalp of affected individuals (17), suggesting the actual flakes are the end result of a cycle of skin distress that may or may not be visible to the unaided eye.

The stratum corneum of affected individuals shows striking features consistent with a hyperproliferative state, as is indicated by functional studies that measure accelerated epidermal maturation times (2). The physical features accompanying this state are dramatic (4,5). An electron microscopic study of stratum corneum using new fixation methods (17) reveals that dandruff stratum corneum exhibits parakeratotic nuclei, lipid droplets within corneocytes, a decreased number of desmosomes, irregular corneocyte envelope structure, intercellular *Malassezia* yeasts, and massive quantities of unstructured intercellular lipid (Figure 28.6). All of these features are consistent with a state in which the feedback between epidermal synthesis and maturation rate is lost and uncontrolled growth leads to corneocytes reaching the surface that are immature and not ready to be shed as individual cells.

The intercellular lipid abnormalities are quite striking in their size as well as lack of tightly ordered lamellae. As expected, there is a lack of true intercellular lipids (ceramides) with most of the lipids being sebaceous in origin (18). Again, this is indicative of the lack of an ordered temporal chain of events, resulting in low epidermal lipid secretion into the intercellular space. Simply topically applying such lipids is unlikely to be meaningful as they cannot displace the sebaceous lipids present, and they not initiate the formation of missing features such as a tight lamellar structure or desmosomes and the other characteristics required for orderly desquamation.

Treatment of dandruff and seborrheic dermatitis are discussed below, but it is appropriate to mention here that as the outward symptom of flakes is improved, the underlying skin condition is also being restored (17). There is a direct correlation between clinical flaking and the severity of the stratum corneum abnormalities, suggestive of the cause and effect relationship between the superficial (flaking) and subsurface (morphology) symptoms.



**Figure 28.6** Stratum corneum features in dandruff. (a) Normal scalp. Note the closely apposed cells, flat, solid cell morphology, and close junctions between cells. (b–d) Dandruff scalp. Note the lipid droplets between cells (b), interdigitated cell membranes (c,d), parakeratotic nuclei (d), and vast amount of extracellular lipid (d).



## ETIOLOGY

The relationship between dandruff and seborrheic dermatitis has at times been controversial. While most investigators regard seborrheic dermatitis of the scalp as severe dandruff, others believe that dandruff should be used to describe any flaking of the scalp (19–22). The microbial origin of dandruff centers on the causal role of yeasts of the genus *Malassezia* (23,24). Originally named *Malassezia* by Malassez in 1898 (25,26), this genus was renamed and referred to as *Pityrosporum* during the second half of the 20th century (27,28). In the mid 1990s, studies of the morphological, ultrastructural, physiological, and genomic differences of the genus *Malassezia* led to the identification of the following seven species: *M. furfur* (*Pityrosporum ovale*), *M. Pachydermatis*, *M. sympodialis*, *M. globosa* (*P. orbiculare*), *M. obtusa*, *M. restricta*, and *M. slooffiae* (29,30). Recently, there have been seven additional *Malassezia* species accepted: *M. dermatis*, *M. equi*, *M. nana*, *M. yamatoensis*, *M. japonica*, *M. cunicula*, and *M. capri* (31–34). As detailed DNA-based identification techniques are more broadly applied to the *Malassezia* genus, there will almost certainly be more species identified. Investigation of the etiologic mechanism(s) associated with *Malassezia*-associated disease has become of significantly increased interest due to the sequencing of multiple *Malassezia* genomes (35,36).

While the pathogenic role of *Malassezia* as the principal causative agent of dandruff and its association with disease severity has been reported (21,23,24,37,38,41), it was a novel molecular biological technique using terminal fragment length polymorphism (tFLP) analysis that identified *M. globosa* and *M. restricta* as the predominant fungal species present on both dandruff (flaking scores of >24) and normal (flaking scores of <10) scalps (Figure 28.7) (39–41). *M. furfur* (*P. ovale*) was absent on human scalps. Though *M. furfur* was the predominant species previously identified using culture techniques (41–43), it is not the species present in vivo. This suggests that *M. furfur* is likely not the causal organism for dandruff, and perhaps is an artifact of isolation or culture techniques, or use of supplanted nomenclature. In the 1950s to 1990s the entire *Malassezia* genus was referred to as *M. furfur* (27,28), and only in the 1990s was the species *M. furfur* split into the genus *Malassezia* with multiple unique species

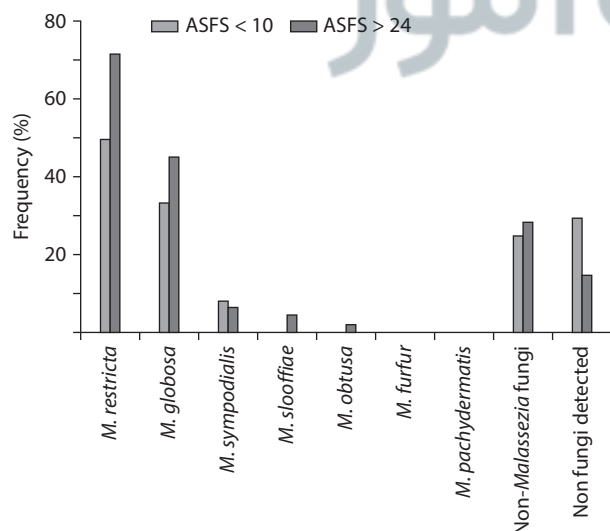
(29,30). The species which retained the name *M. furfur*, while the most robust in culture and likely the most pathogenic, is very rarely isolated from normal human skin (39). More recent molecular genotype analyses the *Malassezia* microflora on skin of atopic dermatitis and seborrheic dermatitis patients support the presence of *M. globosa* and its likely involvement in disease expression (25,44–46). The observation that the human eukaryotic microbiome is far less complex than the prokaryotic microbiome, as well as the observation that *M. globosa* and *M. restricta* are the dominant members associated with the head, has been confirmed by modern techniques and the human microbiome project (47).

Increased interest in the role of *Malassezia* yeasts in the development of dandruff and seborrheic dermatitis has provided additional evidence that in most cases dandruff is indeed a mild form of seborrheic dermatitis. The vast majority of recent data supports a direct causal link between *Malassezia* fungi and dandruff. First, effective treatment of the condition can occur with a wide range of material types, from zinc and selenium salts to highly specific azoles, with the only known functional link between these materials being antifungal activity (24). The second supporting factor is that improvement in dandruff correlates exquisitely with reduction in scalp *Malassezia* level (40,48). While the absolute level of *Malassezia* correlates less well with the dandruff, its reduction among those individuals that express the symptoms strongly supports its role.

Identification of *M. globosa* as a lipophilic *Malassezia* species which is both on the scalp and capable of digesting sebaceous triglycerides (40), thereby releasing free fatty acids (oleic acid), led to the hypothesis that these fatty acids penetrate the stratum corneum and break down skin barrier function (49). This barrier breakdown results in hyperproliferation as well as the secretion of more sebum, which then acts as food to sustain further *Malassezia* proliferation (40). Confirmation of the role of fatty acids in dandruff genesis is proven by the demonstration of oleic acid-induced dandruff-like flaking when applied to scalps of dandruff-susceptible human subjects in the absence of *Malassezia* (40). The resurgence in interest in *Malassezia* biology has also resulted in the sequencing of the *M. globosa* and *M. restricta* genomes (50). Key findings include confirmation of many lipase genes and their expression on scalp, as well as proteomic identification of many other enzymes which would be detrimental to scalp and hair. For example, of 24 proteases identified, more than half were shown to be expressed on human scalp. Also, multiple genes for generation of peroxides were identified, making it likely that *Malassezia* are involved in damage to the hair shaft as well as the scalp. This hypothesis is also supported by recent work indicating that hair sampled from dandruff sufferers was less healthy than that isolated from non-dandruff subjects (51).

Since both *M. globosa* and *M. restricta* are found in dandruff and normal scalps (39), the host immune system may have a role in manifestation of this scalp disorder. Why some individuals get dandruff and others with similar absolute levels of *Malassezia* do not will require further research into individual susceptibility.

Investigation of molecular markers and precursors of skin inflammatory, immunologic, and infectious processes in normal, dandruff, and seborrheic dermatitis scalps indicate that skin cellular immunity is involved in this scalp disease process (52). Significantly higher levels of IL-1 $\alpha$ /TP (total protein) levels ( $p = 0.03$ ) and IL-1 $\alpha$  to IL-1 $\alpha$  ratios were recovered from dandruff ( $p = 0.07$ ) and seborrheic dermatitis ( $p = 0.002$ ) scalps versus the scalps of normal subjects (Figure 28.7). The IL-1 $\alpha$  and the IL-1 $\alpha$  to IL-1 $\alpha$  ratios correlated with scalp flaking severity in the diseased versus the nondiseased scalps.



**Figure 28.7** *Malassezia* sp. on human scalp. ASFS, adherent scalp flaking score.

The TNF $\alpha$ /TP levels recovered from dandruff scalps were significantly higher ( $p = 0.02$ ) than levels recovered from the seborrheic dermatitis and normal scalps. IL-2/TP was significantly increased ( $p = 0.01$ ) and IFN- $\gamma$  and NO levels were significantly decreased ( $p = 0.05$ ) in seborrheic dermatitis versus normal scalps (34). Recent work shows that pyrithione zinc has the ability to abate surfactant-induced IL-1 expression (53).

## ANTIDANDRUFF ACTIVES AND MODES OF ACTION OF ACTIVES

Multiple topical agents identified in the last several decades have proven to be successful therapies for the treatment of dandruff and seborrheic dermatitis. These agents include pyrithione zinc (17,19,54–68), selenium sulfide (19,54–59), salicylic acid (54), sulfur (54), coal tar (54,69), hydrocortisone (54), and ketoconazole (19,58,59,64,66,68,70–85) in the United States. In addition, piroctone olamine and climbazole are accepted materials in other countries. A consistent therapeutic mode of action of all effective actives is their antifungal activity against *Malassezia*. In vitro fungistatic and fungicidal tests of ketoconazole (17,19,58,59,73,74), pyrithione zinc (17, 9,58,59,64), and selenium disulfide (17,19,58,59,64) have demonstrated extremely low minimum inhibitory concentrations of growth (MICs) against *M. furfur* as the marker organism (17). A recent investigation (76) indicates the molecular basis for antifungal activity of ZPT involves transport of copper into cells which then inactivates iron–sulfur active centers of mitochondrial enzymes. Coal tar (72) was also demonstrated to possess activity against 54 *Malassezia* strains isolated from patients with dandruff, seborrheic dermatitis, and pityriasis versicolor, but with a much lower potency. Other antimycotic agents such as itraconazole, terbinafine, bifonazole, climbazole, fluconazole, clotrimazole, dithranol, and liquor carbonis also have the ability to inhibit *P. ovale* (presumed to be *M. furfur*, due to culture conditions) (59,73).

Salicylic acid, sulfur, and liquor carbonis possess exfoliative qualities expected to improve the appearance of scaling, while the antimitotic effect of topical corticosteroids and coal tars might also be involved in reducing the hyperproliferation associated with dandruff scaling.

Traditionally, non-scalp seborrheic dermatitis has been treated with either topical or oral steroids (48). However, the renewed interest in the role of *Malassezia* yeasts and the known side effects of topical steroids have made antifungal medications an increasingly popular choice. Tacrolimus has been shown to have potent antifungal activity against *M. furfur* in vitro (86). It has been suggested that topical tacrolimus and pimecrolimus may be useful alternatives to corticosteroids, as they exhibit anti-inflammatory activity but do not have the side effects associated with long-term corticosteroid use (87). Further, tacrolimus and pimecrolimus may be effective as they possess both anti-inflammatory and antifungal activity.

The fungal etiology of both dandruff and seborrheic dermatitis leads to a refractory condition. *Malassezia* yeasts are commensal microflora, so cessation of antifungal therapy will result in a return of the condition. When considering any topical therapy for long-term prophylaxis, particularly when concerning hair, it must be cosmetically acceptable enough to assist in compliance. This highlights that for dandruff and scalp seborrheic dermatitis the use of cosmetic antidandruff shampoos should be the first choice, with less cosmetically acceptable shampoos, lotions, and foams reserved for use in severe or refractory cases (88,89).

## THERAPIES AND EFFICACY

The objective of treatment of dandruff and seborrheic dermatitis is to control the scalp condition at the lowest possible cost and inconvenience to the patient (1,69,88,89). Since the 1960s, various shampoos, conditioners, and treatment products have been marketed as over-the-counter or prescription products for the treatment of seborrheic dermatitis and dandruff. Many of these products not only treat the scalp, but can also provide hair grooming needs for cleansing and conditioning hair (17). It is critical that the hair care products selected to treat D/SD have no cosmetic tradeoffs compared to purely cosmetic products, to increase the likelihood of long term compliance (17,70).

Therapies considered to be effective include pyrithione zinc (17,19,54–68), selenium sulfide (54–57,61,65,70,71,90), salicylic acid (4,5,54), sulfur (4,5,54), coal tar (4,5,55), hydrocortisone (48), and ketoconazole (19,58,59,64,68,70–85,91) in the United States. In addition, ciclopirox olamine (54,55), piroctone olamine (90), and climbazole (73) are approved for use in other countries. A consistent mode of action of most of the actives is their antifungal activity against *Malassezia* (19,24).

**Pyrithione zinc (ZPT)** shampoo and conditioning rinse-off products have been marketed since the 1960s. This category of antidandruff products has been approved for over-the-counter use in the United States for the treatment of dandruff and seborrheic dermatitis at levels of 0.3%–2% ZPT in shampoo and rinse-off products (54–57), and 0.1%–0.25% ZPT in conditioner and leave-on products (56,57). The efficacy of these products has been demonstrated in many clinical trials (19,27,54–59,70). While pyrithione zinc possesses high intrinsic microbial inhibitory activity against *Malassezia* (27,54–59), its practical efficacy is dependent on a number of formulation parameters (93): mass of ZPT retained after rinsing, the spatial distribution of ZPT particles on the scalp surface and follicular infundibula, and the biomolecular activity of ZPT. The amount of ZPT retained on the scalp surface is controlled by delivery via coacervate, an association complex that phase-separates upon product dilution, trapping and depositing ZPT particles on the scalp. The details of this technology are beyond the scope of this review, but the exact nature of the coacervate can impact spatial distribution of ZPT particles, with large, highly aggregated coacervates impeding efficient spatial distribution.

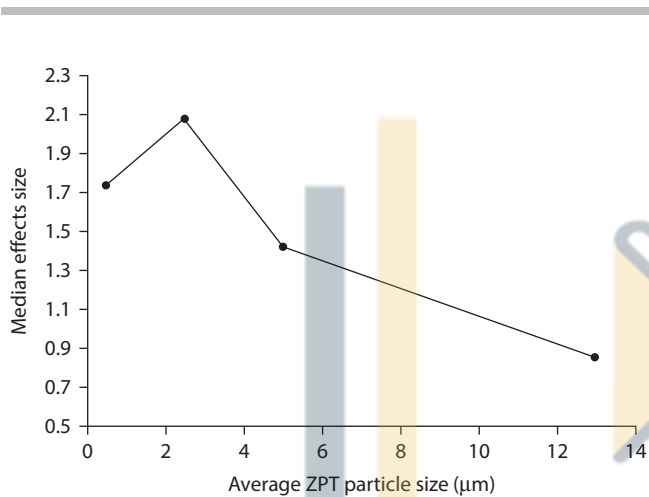
Another factor that can impact efficient spatial distribution is the size/shape of the ZPT particles themselves. For example, platelet ZPT at a particle size of 2.5 micron is optimal for deposition on scalp from shampooing and provides scalp surface coverage (Table 28.2, Figure 28.8) (17). The authors have compiled data from 14 separate clinical studies involving antidandruff shampoos with 1% ZPT of different average particle size. To gauge the impact of particle size, the median effect size was evaluated relative to placebo as measured by the standardized mean differences of the reduction in scalp flaking after 6 weeks of product use. (Since some ZPT shampoos appeared together in the same study, the effect sizes were computed accounting for the correlation and for unequal variances.) The results are represented graphically in Figure 28.9. It can be seen that particle size is a significant variable in ZPT-based product efficacy and that not all ZPT-based shampoos can be assumed to work equivalently. In practice, products containing 2.5 micron platelet ZPT appear to be most effective (17, 94,95).

Finally, bioactivity of delivered ZPT can be affected by other formulation components. For example, a unique formulation technology incorporates an additional source of zinc in

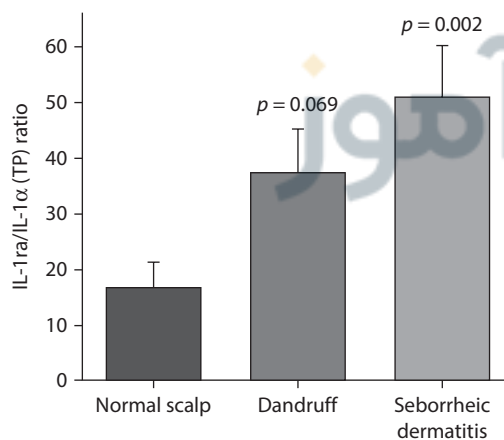
**Table 28.2** Optimization of Active Particle Size Increases Dandruff Efficacy of Marketed 1% Pyrithione Zinc Shampoos

1% Pyrithione Zinc Shampoo	Avg. Particle Size ( $\mu\text{m}$ )	Median Effect Size	Range of Effect Sizes
Shampoo A	13	0.85	1 study only
Shampoo B	5	1.41	[1.36, 2.93]
Shampoo C	2.5	2.08	[0.55, 2.14]
Shampoo D	0.5	1.74	1 study only

Note: Individual effect sizes are standardized mean differences between active and placebo shampoos of the reduction in scalp flaking after 6 weeks of use. The results were taken from 14 separate studies. Since some pyrithione zinc shampoos appeared together in the same study, the effect sizes were computed accounting for the correlation and for unequal variances.



**Figure 28.8** Effect of particle size is a significant variable in PTZbased product efficacy. Note that, in practice, products containing 2.5 mm platelet PTZ appears to be most effective.



**Figure 28.9** Ratio of interleukin (IL)-1ra/IL-1 $\alpha$  normalized to total protein (TP) in normal scalps and scalps of dandruff and seborrheic dermatitis sufferers. Values are mean  $\pm$  SE.

the formula to maximize the activity of ZPT, this is referred to as a potentiated ZPT formula. The mechanism apparently relies on the additional zinc to alter an equilibrium between active and inactive forms of ZPT, enhancing the presence of the former.

**Selenium sulfide** has been approved for over-the-counter use for the treatment of dandruff and seborrheic dermatitis at levels of 0.6% (micronized form) and 1% (54–57). Shampoos containing selenium sulfide have proven efficacy (21,37,38). Since selenium sulfide is a particulate, efficacy is dependent on the particle size to optimize coverage. Differences in efficacy may be related to the particle size of the selenium sulfide in the shampoo. Further, selenium sulfide is a complex mixture of multiple isoforms and the relative constitution of formulations affect efficacy.

**Ketoconazole**, an imidazole antimycotic agent, has been used orally for the treatment of mycoses. Several large anti-dandruff efficacy studies have demonstrated efficacy against pityriasis capitis and seborrheic dermatitis (21,24,37,38,54–57). Ketoconazole has been approved for topical over-the-counter use at 1% in shampoos and prescription use at 2%. Twice-weekly treatments are currently recommended for ketoconazole-containing shampoos. To achieve efficacy, these products are recommended to be left on the scalp for 5–10 minutes before rinsing, thereby requiring a change in shampooing habits and practices.

**Coal tar**, approved for over-the-counter treatment of dandruff, seborrheic dermatitis, and psoriasis at levels of 0.5%–5% (tar equivalent) (54–57) reduces the number and size of epidermal cells and decreases epidermal proliferation and dermal infiltrates. Coal tar has also been reported to have slight antifungal activity, which could explain its limited anti-dandruff efficacy (54–57,69). Coal tar-containing shampoo and treatment products have been marketed for decades, mainly for psoriasis. This shampoo has been anecdotally reported to negatively impact hair color.

**Salicylic acid**, approved for over-the-counter treatment of dandruff, seborrheic dermatitis, and psoriasis at concentrations of 1.8%–3% (54–57) is an exfoliative agent that loosens the scales, enabling them to be washed away. Sulfur is approved for over-the-counter treatment of dandruff at levels of 2%–5%. Combinations of salicylic acid and sulfur have not been approved for over-the-counter use in the United States.

**Climbazole**, an antimycotic agent present in antidandruff shampoos in the European continent with high in vitro and in vivo efficacy against *Malassezia*, was evaluated for efficacy and safety (97). This shampoo is not marketed in the United States.

### COMPARATIVE EFFICACY OF ANTIDANDRUFF THERAPIES Potentiated ZPT Formulas versus Standard ZPT Formulas

A recent clinical evaluation (102) compared a potentiated 1% ZPT shampoo to a standard 1% ZPT shampoo (which additionally contained 0.5% climbazole). Even with the secondary



climbazole, the potentiated ZPT formula was found to be significantly more effective at reducing the appearance of flakes. This conclusion was substantiated by quantitation of the objective biomarker histamine as well (103).

### Ketoconazole versus ZPT

Comparison of optimized ZPT and ketoconazole products has demonstrated equivalent performance. As discussed above, it is important to consider the particle size of the ZPT contained in each product, as optimized ZPT particles have been shown to have increased performance. The relative antidandruff efficacy of a 1% size-optimized ZPT particle-containing shampoo has been compared to 1%–2% ketoconazole shampoos (17,64,68). In a 364 patient, 6-week, randomized, double blind, parallel group study (64), three groups of 112 patients were assigned a 1% ZPT shampoo, or a 1% ZPT shampoo with a different use regimen, or a 2% ketoconazole shampoo; a fourth group of 28 patients was assigned to a placebo shampoo. The antidandruff efficacy of the two 1% ZPT shampoos, regardless of the use regimen, was found to be comparable to the 2% ketoconazole shampoo. All three active treatments were significantly more efficacious than the placebo shampoo. In a small 60-patient, 8-week, randomized, double blind, parallel group study (65), a 1% ketoconazole shampoo was found to be more efficacious than a 1% non-optimized large particle ZPT-containing shampoo after 6 and 8 weeks of therapy in a subpopulation of patients with severe dandruff. The differences observed in this study are attributed to a shampoo with a larger non-optimum particle size ZPT which was therefore less efficacious than some of the currently marketed ZPT shampoos.

In a recent clinical study (66), the efficacy of 2% ketoconazole shampoo was compared to a 1% non-optimized ZPT shampoo in severe dandruff and seborrheic dermatitis sufferers. This randomized parallel group study in a total of 341 sufferers consisted of a 2-week run period with a neutral non-antidandruff shampoo followed by 4 weeks of active treatment with either 2% ketoconazole twice weekly or 1% ZPT shampoo at least twice weekly, and a subsequent 4-week follow-up phase on non-active shampoo. While significant benefits were observed for both shampoos in comparison to non-dandruff shampoos, the 2% ketoconazole shampoo achieved a 73% improvement in total dandruff severity scores compared to 67% improvement for 1% ZPT shampoo at week 4 of active treatment. These differences were significant at  $p = 0.02$ . Side effects were minimal. Efficacy differences observed in this study were attributed to a shampoo with a larger particle size ZPT, therefore a less efficacious product than currently marketed ZPT shampoos.

In a postmarketing study examining the efficacy of a combination shampoo containing 2% ketoconazole and 1% pyrithione zinc (68), greater than 90% reduction in dandruff severity was observed for all areas of the scalp in 236 moderate to severe dandruff sufferers in 4 weeks of treatment. In addition to the significant reduction in dandruff severity, improvements in erythema and pruritis and minimal side effects were observed. This combination shampoo, while offering a safe and effective option for the treatment of dandruff in India and perhaps other geographies, is not approved for use in the United States.

### Ketoconazole versus Selenium Sulfide

In a comparative efficacy study of a 1% selenium sulfide shampoo versus a 2% ketoconazole shampoo (70), a 1% selenium

sulfide shampoo was found to be more effective than the 2% ketoconazole shampoo after 4 weeks of therapy. This was a large, 350-patient, 6-week, double-blinded, randomized parallel group study where two groups of 150 patients with moderate to severe dandruff or seborrheic dermatitis were randomly assigned to either 1% selenium sulfide shampoo or 2% ketoconazole shampoo, and one group of 50 patients was randomly assigned to placebo shampoo. Adherent scalp flaking scores (ASFS) were assessed at baseline, and weeks 2, 4, and 6. Both the 1% selenium sulfide shampoo and the 2% ketoconazole shampoo were significantly more efficacious than the placebo shampoo at all treatment time points. While the efficacy of these shampoos was comparable at week 2, the 1% selenium sulfide shampoo was found to be significantly more effective at reducing adherent scalp flaking in comparison to the 2% ketoconazole shampoo after 4 and 6 weeks of therapy. The superior efficacy associated with the 1% selenium sulfide shampoo may be a function of shampoo frequency. When the hair was shampooed three times weekly, patients using the 1% selenium sulfide shampoo had significantly better improvement than patients using the 2% ketoconazole shampoo. When the shampoo frequency was comparable at two times per week, the efficacy of the two active products was comparable.

In a 4-week study (70), 246 patients with moderate to severe seborrheic dermatitis and dandruff used either a 2% ketoconazole shampoo, a 2.5% selenium sulfide shampoo, or a placebo shampoo twice weekly for 4 weeks. Both active shampoos produced significant improvement in total adherent dandruff scores relative to the placebo shampoo. The 2% ketoconazole shampoo was found to be significantly better than the 2.5% selenium shampoo after 1 week, but not after 2 and 4 weeks of therapy. Assessments of the reduction in yeast cell counts paralleled the adherent flaking results, with the ketoconazole and selenium sulfide shampoos having significantly reduced yeast cell counts relative to placebo treatment. Following the active treatment phase, 103 patients who responded to treatment entered the regression phase, where they used a nonmedicated shampoo for an additional 3 weeks. A progressive increase in adherent and loose dandruff scores and an increase in the percentage of patients with yeast colonization were noted, indicating relapse on cessation of therapy.

In a 4-week, double-blind study (75), 102 patients with moderate to severe dandruff were shampooed at the test facility with either a 2% ketoconazole shampoo, a 2.5% selenium sulfide shampoo, or placebo shampoo twice weekly for 4 weeks. Adherent scaling and yeast organism density were assessed at pre-, 2, and 4 weeks post-treatment. The 2% ketoconazole shampoo was comparable to the 2.5% selenium sulfide shampoo in flaking reduction scores, and both active shampoos were significantly more effective than the placebo shampoo. The mean yeast count reduction scores paralleled the flake reduction efficacy measures, with the 2% ketoconazole and 2.5% selenium sulfide shampoos producing a significantly higher reduction in yeast density than the placebo shampoo.

### Coal Tar, Selenium Sulfide, and ZPT

In a study comparing the activity of a 1% selenium sulfide commercial shampoo with two commercial shampoos containing 1% ZPT and one product containing coal tar (46,61), loose, adherent, and total dandruff flake scores were obtained. The study was conducted among 199 panelists for 4 weeks.

Most clinical evaluations focus on the adherent scalp flakes, thus this data is referred to here. The change versus baseline at 4 weeks was as follows: selenium sulfide, 7.1; ZPT prototype A, 7.0; ZPT prototype B, 5.7; and coal tar, 5.8. Coal tar does not appear to have substantial antidandruff activity and, as has been seen previously, ZPT-based products can vary considerably in activity depending upon pharmacological delivery of the active.

### Other Actives and Combinations

In a recent trial, the efficacy of a 1.5% ciclopirox olamine and 3% salicylic acid shampoo was compared to 2% ketoconazole in a study with 224 (154 dandruff and 70 seborrheic dermatitis) subjects (78). The shampoos were used three times weekly for a period of 4 weeks followed by a 2-week follow-up period. Clinical and self-assessments of efficacy were made. Both treatments produced significant improvement, with lower clinical and self-assessment scores observed at the end of treatment and follow-up periods. Only subjects treated with the 1.5% ciclopirox olamine and 3% salicylic acid shampoo showed a significant reduction in scalp itching in the seborrheic dermatitis subjects.

A recent randomized double-blind study was conducted in two groups of 30 moderate to marked dandruff sufferers with a non-tar (2% salicylic acid, 0.75% piroctone olamine, and 0.5% elubiol) or 0.5% coal tar shampoo (98). The study consisted of a 3-week run-in washout period, followed by a 4-week treatment and a 4-week post-treatment regression phase. The non-tar shampoo was found to reduce *Malassezia* spp. counts and squamometry values versus the 0.5% tar shampoo. However, the non-tar shampoo contained two antifungal agents, namely piroctone olamine and elubiol, and a keratolytic agent (99).

The effectiveness of 2% sulfur and 2% salicylic acid either alone or in combination in a shampoo were assessed in a double-blind, parallel controlled study (100) using scaling and corneocyte counts as the endpoints for efficacy. A total of 48 patients with moderate to severe dandruff were shampooed twice weekly at the study site for 5 weeks. At weekly intervals, scalp flaking and corneocyte counts were assessed. Significantly greater and earlier reductions in the degree of scaling and corneocyte counts were observed in patients treated with the formula containing 2% sulfur and 2% salicylic acid versus the individual ingredients. This combination active treatment is not approved for marketing in the United States.

In a small (eight patients on selenium sulfide and fifteen patients on miconazole nitrate treatment), parallel group study (64), the antidandruff efficacy of a 2.5% selenium disulfide shampoo was compared to a 2% miconazole nitrate shampoo. Miconazole nitrate was found to possess antidandruff activity equivalent to selenium disulfide. The endpoint for efficacy determination was clinical assessment of disease severity supplemented by cytodiagnosis of exfoliated scalp epidermal cells by smear examination.

A small, 4-week, unblinded, open study (67) reported marked decreases in scaling, seborrhea, erythema, and the burning and itching of the scalp of seborrheic dermatitis patients treated with either a 1% ZPT or a 1% econazole shampoo. The 1% econazole shampoo was assessed to be slightly better than the 1% ZPT shampoo. However, this 1% econazole shampoo is not marketed.

### EFFICACY MEASURES (DERMATOLOGIC AND MICROBIOLOGIC)

The primary efficacy measure of antidandruff activity in clinical trials is the severity of ASFS (15). This assessment is generally based on an 11-point flaking scale ranging from 0–1 (very light scaling) to 8–10 (severe scaling) (54, 65, 66, 68, 75–81) or from 0 (no scaling) to 10 (very heavy scaling) (54, 60, 62). The scalp is divided into six (54, 65–68, 75–80, 83, 84, 91) or eight (54, 60, 62, 64, 70) anatomic sites, and the adherent flaking density is scored after parting the hair at each anatomical site multiple times. The ASFS from each site is then summed across sites (total of 60 or 80) for the primary efficacy measure. An alternative method, the colorimetric method (Chroma C\*), called squamometry, is used to assess the amount of flakes obtained on D-squame tapes collected from the most severely affected area at pretreatment and the same area post-treatment to assess flaking density changes resulting from treatment. Corneocyte counting is another alternative method for assessing flaking changes. These two latter methods are not used in the conduction of current clinical studies. In addition to the ASFS, assessment of loose dandruff, global involvement in the disease process, and subjective assessment of itch and dandruff severity serve as secondary efficacy measures. Other secondary efficacy endpoints include assessment of *Malassezia* density (71, 75, 82). Alternative approaches include molecular genetic techniques (39, 40, 44–46, 101) in species identification and quantification.

### DANDRUFF TREATMENT CONSIDERATIONS

While intrinsic antifungal potency of antidandruff products is important, these therapeutic results can only be achieved if antidandruff shampoos are incorporated into a patient's routine hair care regimen. Despite higher shampoo frequencies and the ready availability of highly effective over-the-counter and prescription antidandruff shampoos in the United States, the most recent prevalence study (17, 54–57) in both adults and teens suggests dandruff is occurring at a much higher rate and severity than initially thought in the United States versus China (7). The higher prevalence of dandruff in the United States may be associated with the lower use of antidandruff shampoo in routine hair care regimen versus China. This suggests that shampooing more often with antidandruff shampoos that have excellent efficacy, aesthetics, and hair conditioning properties similar to cosmetic shampoo could lead to a lower prevalence and severity of dandruff. While most antidandruff shampoos have tradeoffs in usage regimen and hair end benefits such as negative odor attributes, unusual color or hair staining properties, or altered use regimen instructions such as a 5–10 minute residence time versus 30 seconds, the norm for shampooing (80), some cosmetic ZPT-containing shampoos have been uniquely formulated to have no significant tradeoffs, leaving the hair clean and conditioned. Recommendations for more frequent use of antidandruff shampoos with superior cleansing and conditioning properties as a patient's regular hair care regimen may lead to more successful management of dandruff.

Once D/SD is brought under control, effective preventative treatment is required to decrease the high probability of reoccurrence (due to the commensal nature of *Malassezia* and hence their certain repopulation of the scalp). This requires long-term use of an effective antidandruff product that is cosmetically desirable and affordable. ZPT-based shampoos have been shown (51) to be effective over long periods of time without the risk of any physiological accommodation (tachyphylaxis) that could gradually reduce the benefits.

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# The Periorbital Wrinkle

Martin R. Green

## THE HUMAN PERIORBITAL WRINKLE

Wrinkles are the most obvious and perhaps the most disliked aspect of facial aging. Considering this, it is surprising that the biology of wrinkle formation remains unclear, as is the physical structure of wrinkles and why is it so hard to erase a wrinkle once it is formed.

Many people, scientists and laymen alike, have considered wrinkles to be cosmetic and therefore not worthy of research. There was a common belief that as the skin aged, collagen stiffened, elastic networks collapsed, and the mechanical properties of the skin degraded. Wrinkles were just seen as the "points of weakness" where excess, lax skin formed creases.

Nothing has made a larger impact on this simplistic model than the success of botulinum toxin in reducing facial wrinkles. Botulinum toxin is injected into the muscles underneath the skin and causes a long-lasting paralysis and relaxation of those muscles. The fact that this has such a dramatic effect on the appearance of the wrinkle proves that deep wrinkles are not simply a phenomenon of changed properties of the skin but rather are an effect of the entire integumentary system including not only the dermis but also the subcutaneous fat and muscle layers.

Wrinkles also contribute to "perceived age," the average facial age of a person estimated by independent assessors (1,2). Over the past 10 years perceived age has gained credence as a highly useful biomarker of aging and a method by which to study the facial attributes of attractiveness. Perceived age has been shown to be a robust indicator of health (3) and to be influenced by facial wrinkles (2). In a 70+ age group, perceived age predicted human survival and correlated inversely with physical and cognitive functioning (1). As well as facial wrinkles, hair graying and lip height are also major contributors to perceived age. Twin studies have also shown that facial wrinkles are more or less equally influenced by genetic and environmental factors (2) while hair graying and lip height largely influenced by genetic factors (2). More recently factors such as serum glucose (4) and cortisol (5) have been shown to be correlated with perceived age, implying at least an indirect causal link to aging features.

This chapter briefly reviews what is known about the wrinkle and sets out studies which cast new light on how deep periorbital wrinkles ("crow's feet") form and are maintained, pointing to new therapeutic approaches to treat periorbital wrinkles. It should be noted that the complex biological processes leading to wrinkle formation are certain to vary between wrinkle types (6) and that the conclusions drawn here relate only to periorbital wrinkles.

## WRINKLES AS AN ASPECT OF AGED SKIN

There is a considerable literature describing the differences between youthful and aged skin. In many cases, no attempt is made to differentiate between the area of the

wrinkle and the unwrinkled skin around it. In large part this is because it is sometimes difficult to locate a wrinkle in a histological section—a surprising factor to which I will return later.

For a review of the wrinkle literature the reader is guided to the articles by Kligman (7), Contet-Audonneau (8), Lavker (9), Tsuji (10), and Humbert (6). The key findings are as follows:

1. The epidermis thins with age and the properties of the stratum corneum change in a multitude of ways including reduced barrier repair properties and decreased elasticity. These changes are particularly important in causing "fine lines" and are the changes that are countered by the vast majority of effective topical moisturizing products.
2. The intricate, interconnected, elastic fiber network in the dermis degrades and is replaced (especially in the case of sun exposed skin) by large deposits of poorly organized nonfunctional elastin deep in the dermis. This leads directly to loss of resiliency—the ability of the skin to quickly return to its original shape after distortion. In extreme cases the large elastin deposits (so called elastosis) can cause the skin to look sallow and yellowish in color. Aspartic acid residues in elastin are also prone to racemization and the protein is known to be turned over very slowly if at all (11). Accordingly, functional elastin networks in skin appear easy to destroy and nonfunctional elastotic deposits very hard to remove.
3. Collagen fibers become less well organized and the collagen itself undergoes chemical changes including crosslinking that reduce its mechanical flexibility. Repeated imperfect collagen repair can lead to "scar-like" patches of stiff, aligned collagen. The dermal thickness under major wrinkles decreases (12) and there may also be direct-dermal UV-mediated damage of proteins (13).
4. The skin glycosaminoglycan composition changes greatly. In sun-exposed skin with chronic (i.e. years of) photodamage there is a marked increase in types of sulphated glycosaminoglycans (versican) at the expense of others (decorin). Since these molecules can hold as much as 1000 times their own weight of water in an elastic gel these changes hugely affect the water content of the dermis.
5. The subcutaneous fat layer, especially in women, decreases markedly in thickness. Major wrinkles also form dermal invaginations into the subcutaneous fat layer (12).



Skin which has undergone deleterious changes is obviously prone to wrinkles. However photodamage is not sufficient on its own to cause some types of wrinkling to occur. The extra factor that seems to be necessary is repeated movement of the skin causing folding of the skin. Over time, and combined with the overall aging changes set out above, wrinkles form and progressively get worse. Thus wrinkling can be seen on the face where “expression lines” form but not in other sun-exposed areas even though the histological degradation of the skin may be worse. The toxins introduced by cigarette smoking also make a marked, adverse contribution to wrinkling (14), particularly around the mouth.

### LOCAL DIFFERENCES IN THE WRINKLE COMPARED TO SURROUNDING SKIN

Considering what an obvious feature a wrinkle represents, there are limited published data contrasting the wrinkle with the surrounding skin. Such studies that have been done suggest that the classic features of solar elastosis are less in the base of the wrinkle than in the surrounding skin (8,10).

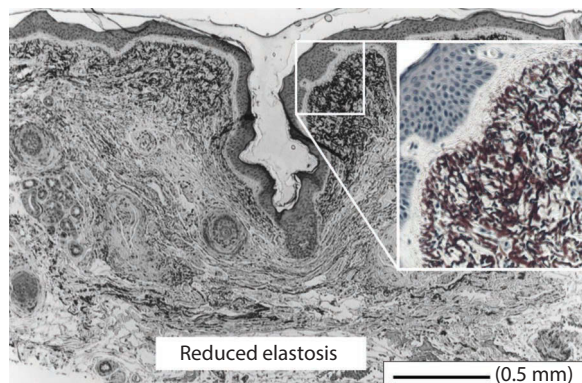
One reason for the limited number of these studies is that it is difficult to identify wrinkles—even deep ones—in histological sections. When the skin is excised, the wrinkle opens and partially disappears—obvious evidence that major wrinkles are under compression and in part maintained by the properties of the surrounding skin. To overcome this problem a novel technique was used whereby a line of cyanoacrylate glue, visible in Figure 29.1, was introduced into the wrinkle and allowed to set prior to taking a biopsy of the wrinkle. With this precaution, the shape of the wrinkle was preserved, allowing a better correlation of histological changes to the architecture of the wrinkle.

Sections of these biopsies, which were taken from the periorbital “crow’s feet” areas, were stained by a variety of techniques using both conventional and immunostaining techniques. We confirmed the finding (8,10) that the elastic fiber network was far less damaged at the base of a deep wrinkle than on the sides of the wrinkle or adjacent skin (Figure 29.1). This is thought to be a consequence of the base of the wrinkle being less exposed to ultraviolet (UV) light during and after formation than the surrounding skin. It suggests that the damage to the elastic fiber network in unlikely to be a primary cause of the wrinkle.

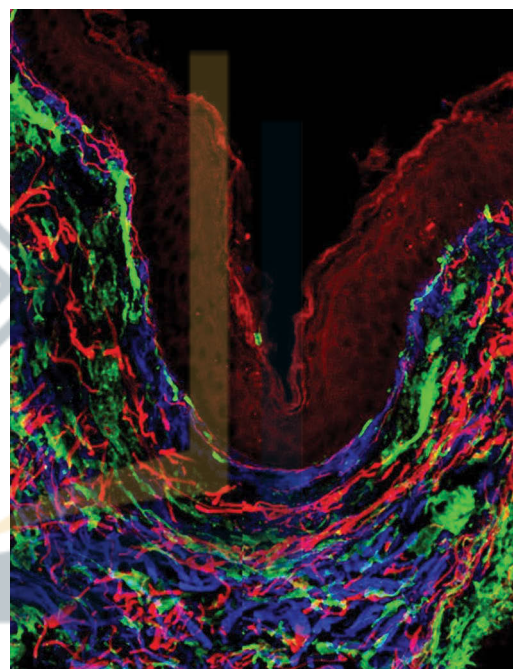
In contrast to the normal-appearing elastin at the wrinkle base, the “elastin” at the sides of the wrinkle is heavily disorganized (elastotic), a phenomenon that is worse on the lower, more

sun-exposed side, as viewed when a wrinkle would have been positioned in situ on the face. The collagen fiber orientations were also quite abnormal. In stark contrast to the sides of the wrinkles, where the collagen fibers tended to run parallel to the long edge of the wrinkle and were in places relatively normal, at the wrinkle base there was a dense band of highly aligned collagen fibers running perpendicular to and underneath the wrinkle (Figure 29.2, also clearly visible in Figure 29.1).

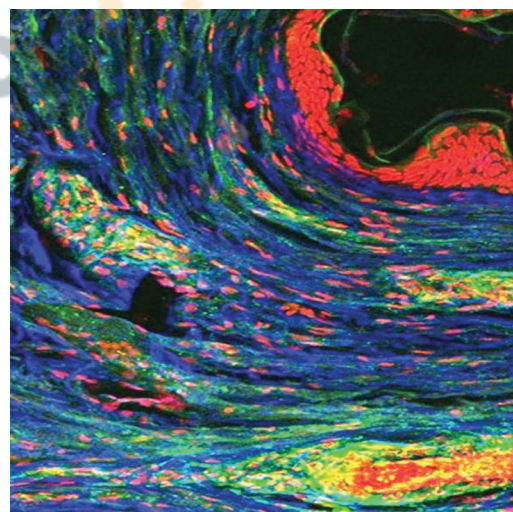
This band of highly aligned collagen was one of the few features of the permanent periorbital wrinkle that correlated clearly with the wrinkle location and gave a clue to what dictated the shape of the wrinkle. Recently Pessa et al. have reported that lymphatic vessels with perilymphatic fat lie very close to or



**Figure 29.1** Elastic fibers in the periorbital wrinkle (orcein stain).



(a)



(b)

**Figure 29.2** Highly aligned collagen at the base of the wrinkle. Red, tropoelastin; green, fibroblasts; blue, mature collagen (a). Red, nuclei; green, fibroblasts; blue, mature collagen (b). (Photographs courtesy of J. Wares).

directly underneath major facial wrinkles (15). Regrettably, lymphatic features were not evaluated in this study.

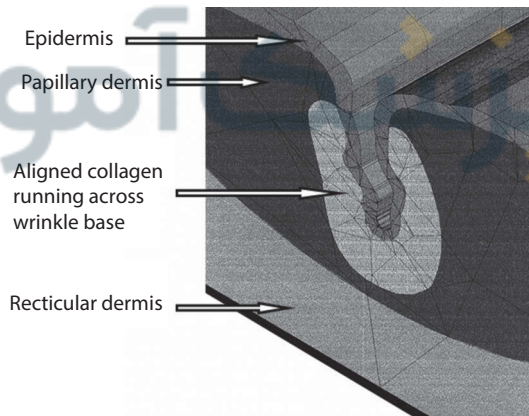
To investigate how attached collagen fiber alignment may occur, a computer model of the skin was developed (M. Eastwood and M.R. Green, unpublished data) incorporating the structural information derived from the histology and building in physical material constants for the different parts of the skin. Other simulated models for immediate wrinkle formation after skin compression have been prepared (16,17) but these do not take account of the effect of changing tension on the long-term biological responses of the skin, and local changes in wrinkle architecture. A novel method using digital image speckle correlation has also been developed to study the mechanical properties of skin, showing that lines of increased tension in skin coincide with the position of prominent wrinkles (18). Using a new method, Yasui et al (19) have employed polarization-resolved second-harmonic-generation microscopy to study collagen fiber alignment in UV-irradiated mouse skin. The results show striking collagen fiber patterns developing around major wrinkles over 16 weeks of UV treatment, with fiber alignments developing consistent with those reported here.

### COMPUTER MODEL OF THE PERIORBITAL WRINKLE

Figure 29.3 shows the basic structure of the computer model. It incorporates four different layers: the epidermis, the papillary dermis, the reticular dermis, and the highly aligned zone of collagen. Material properties of these zones were estimated from literature values, as shown in Table 29.1. The model was based on finite element analysis using Pro Mechanica® software. The base of the skin in the model was anchored to an inflexible substrate. No attempt was made to simulate the effects of muscle or the attached subcutaneous fat layer.

The first and critical finding in preparation of the model was that the simulated wrinkle could only maintain its shape with compressive forces pushing the wrinkle closed. Three possible sources of this compressive force exist:

1. Overall pressure from the surrounding skin. If older skin has a larger natural area than it is constrained to by its attachment to the underlying tissue, then the whole area of skin will be under compression. This is probably a significant factor where there has been significant loss of the subcutaneous fat layer leading to shrinkage of the attachment area for the skin. Conventional "facelift" procedures act on this compression effect by reducing the natural area of the skin so that wrinkle compression is replaced by a tension. Skin is usually maintained under tension as is evident from its behavior when cut (it will gape) or from instrumental measurements (20–22).
2. Action of subcutaneous muscles. The integumentary system is extensively attached to muscle groups that can actively create tensions in the skin. This is particularly so on areas such as the forehead and around the mouth. Where muscle groups act to compress areas of skin, the compression can generate and sustain wrinkles. Botulinum toxin injections act on these muscle groups causing them to relax for as long as 6 months. The fact that in certain types of wrinkle such injections virtually eliminate the visible wrinkle proves that for some types of wrinkles the muscle generated compressive forces are a primary cause.
3. Tension carried within the aligned collagen at the wrinkle base. Figure 29.4 shows the computer wrinkle model when the wrinkle has been allowed to open up, as if it were excised. The color scale shows the amount of stress in the different elements of the model. A major stress is found in a narrow zone at the base of the wrinkle, in the aligned collagen domain. This stress arises because of the

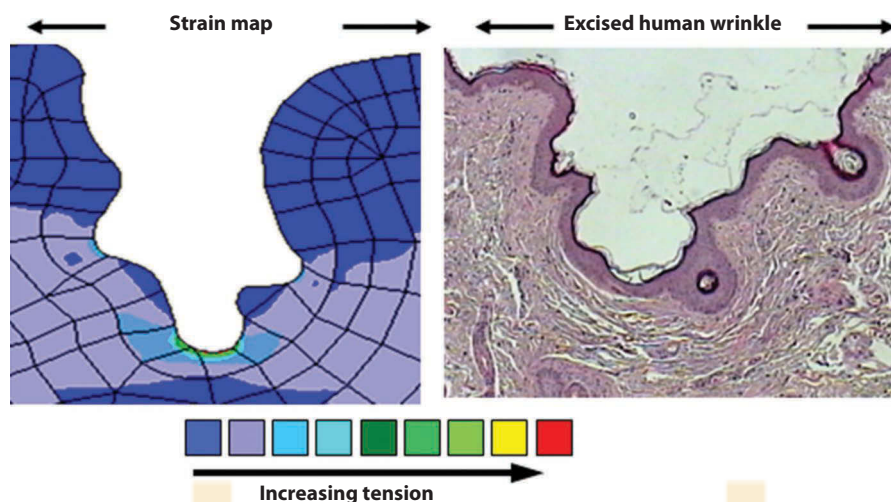


**Figure 29.3** Visualization of the wrinkle computer model. (Picture courtesy of M. Eastwood).

**Table 29.1** Physical Constants Used in The Computer-Generated Wrinkle Model

	Epidermis	Papillary dermis	Reticular dermis	Aligned collagen
Young's modulus (MPa)	0.06	0.05	0.048	0.065
Poisson's ratio	0.45	0.49	0.49	0.49





**Figure 29.4** Strains calculated in the computer-wrinkle model of a “relaxed” wrinkle (left image) compared to the histology of an excised, “relaxed” human periorbital wrinkle where the cyanoacrylate glue failed to hold the wrinkle sides together (right image).

removal of the compressive force on the wrinkle sides, and gives an indication of what might happen during day-to-day flexing of the wrinkle on the face. Put simply, the aligned collagen band is stiff but under little stress when the wrinkle is closed. As the wrinkle opens, the stiff collagen band deforms and resists the opening of the wrinkle. This strongly implies that the aligned collagen band is a major factor in maintaining the shape of the periorbital wrinkle.

### ORIGIN OF THE ALIGNED COLLAGEN LAYER

Why does the wrinkle possess this deep zone of highly aligned “scar-like” collagen? While it is clearly important to the maintenance of the wrinkle once formed, it is unlikely to have a role in the initial formation of the wrinkle. Indeed if such a zone were present in an area of unwrinkled skin it might cause the wrinkle to form nearby rather than in an inflexible zone. To understand how the zone could form it is necessary to consider the interplay of forces within the tissue and the biological behavior of fibroblasts within the tissue and response to mechanical force.

Fibroblasts are richly endowed with surface molecules, particularly integrins, that will bind to components of the connective tissue—collagen, fibronectin, etc. When these surface molecules bind to the connective tissue matrix, they transduce mechanical forces (23,24) acting through the binding sites into biological changes in the cell. One outcome of this is that fibroblasts within a connective tissue matrix that is under tension increase their synthesis of collagen and deposit it preferentially in the direction of the tension (25–27).

What does this mean for the skin? In unwrinkled skin under uniform “360°” tension, individual collagen fibers have a random direction of alignment. When unwrinkled skin is flexed so as to form a temporary “expression” fold, the greatest tension is experienced by the tissue at the base of the fold and causes collagen fibers to align perpendicular to the furrow or fold line. In response to that tension, fibroblasts deposit collagen in the direction of the tension, perpendicular to the fold, adding to the aligned collagen. The next time the skin is flexed and the fold forms, the tissue at the base of the fold is just a little

stiffer due to the deposited collagen. That makes the tension at the wrinkle base just a little higher and induces the deposition of still more aligned collagen. Over time this vicious circle continues until the aligned collagen comes to dominate the stress field in the skin to the point where it maintains a permanent wrinkle. Sunlight, particularly UVB radiation, exaggerates this effect by activation of proteases and the triggering of further collagen realignment (28) and loss of elastic fibers (29).

### IMPLICATIONS FOR TREATMENT OF WRINKLES

It is clear from the discussion above that there are multiple distinct factors such as acute and chronic sun exposure, natural skin tension, skin flexing, intrinsic aging changes including the thinning of the dermis and loss of subcutaneous fat, causing and sustaining wrinkles. In all body and facial sites it is likely that all the factors contribute to the overall wrinkling effect, but the relative contribution of the different factors will differ, giving rise to different wrinkle structures, suggesting different optimum treatment regimes for wrinkle types may be needed.

On the forehead, the dominant factor sustaining wrinkles appears to be the action of the subcutaneous muscles. Botulinum toxin injections are known to be highly effective.

On the cheeks and around parts of the mouth, loss of subcutaneous fat and elastic fibers leads to a general loss of tension of the skin. In these cases, botulinum toxin is less effective and surgical removal of flaps of skin or generalized contraction of the skin using laser treatment is necessary to have a substantial effect.

The crow’s feet area is characterized by wrinkles maintained by the stiffened aligned collagen zone at the wrinkle base. Botulinum toxin has relatively little effect on these wrinkles and while surgical “facelift” procedures may have temporary benefits, the analysis above suggests that stretching the wrinkles out does not change the underlying histology, probably ensuring that the wrinkles will return.

The analysis above suggests that a promising route for treatment of such periorbital wrinkles would involve stimulation of deep dermal collagen remodeling, to put into reverse



the vicious cycle that caused the aligned collagen zone to form. If collagen turnover could be stimulated while the skin was exposed to tension parallel to the long edge of the wrinkle, then the existing perpendicular collagen fibers would be reduced and remodeled while new fibers remodeled parallel to the wrinkle would no longer sustain the wrinkle shape. Retinoic acid might be effective used in this way, as it has been shown both to increase collagenase expression in skin and to increase the deposition of newly synthesized collagen. To our knowledge, such a combined topical tension and topical treatment experiment has yet to be attempted. Recently, promising clinical results for facial wrinkle reduction have been achieved after long-term consumption of a supplemented drink containing isoflavones, lycopene, vitamin C, vitamin E and fish oils (30). Such a dietary approach may be better able to deliver benefit agents to deep wrinkle sites which topical agents may not be able to affect.

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## Cosmetology for Normal Nails

Robert Baran and Douglas Schoon

### INTRODUCTION

Evolving from the primeval claw, the nail's working uses became less important, while over time their aesthetic value has grown in importance. The application of cosmetics to the nail can be done to enhance length, beautify or address specific needs. The nail is a convex, hard, horny plate covering the dorsal aspect of the tips of the fingers and toes (Figure 30.1). Its appearance is determined by the integrity of the terminal bony phalanx and the paronychium, and i.e. matrix, nail bed and hyponychium, and nail folds. The nail plate, produced by the matrix, grows from a pocket-like invagination of the epidermis and adheres firmly to the nail bed. Tissue from the undersurface of the proximal nail fold also tightly adheres to the surface of the nail and as the nail grows, emerges from underneath the eponychium to create the cuticle. This cuticle tissue creates a resistant gasket-like seal which helps protect the nail pocket from infection, irritants, etc. The most distal part of the matrix, the whitish semicircular lunula, is visible wherever it extends beyond the proximal nail fold. Juxtaposed to the lunula, the pink nail bed epithelium is made from parallel longitudinal rete ridges and subepithelial capillaries running longitudinally at different levels. Adjacent to the nail bed, the hyponychium, an extension of the epidermis under the nail plate, marks the point at which the nail separates from the underlying bed tissue. There is small area between the nail and the distal bony phalanx that is occupied by non-keratinizing nail epithelium, as well as highly vascular mesenchyme containing glomus organs.

### The Following Should Be Borne in Mind

- The proximal matrix forms the surface of the nail plate, and the distal matrix forms its inferior part. It is therefore possible to locate initiating pathology by a thorough examination of the nail.
- Fingernails grow at a rate of 0.1 mm a day; toenails grow much more slowly. It can take 12–18 months to replace a large toenail as opposed to 5–6 months for the fingernail. The nail's functional role is to protect the nail bed and help prevent breakage or fracture of the bony tips at the end of each finger and toe. The underside provides counter pressure on the pulp; essential for good tactile sensation involving the fingers. Nail beauty depends on several main factors: the shape and length of the nail, its color (or decoration), and its texture.

### CARE AND ADORNMENT OF THE NORMAL NAIL The Shape of the Nail

The shape of the nail depends on proportion and contour. The ratio of length to breadth of the nail is critical to its aesthetic appeal, and the two dimensions should be

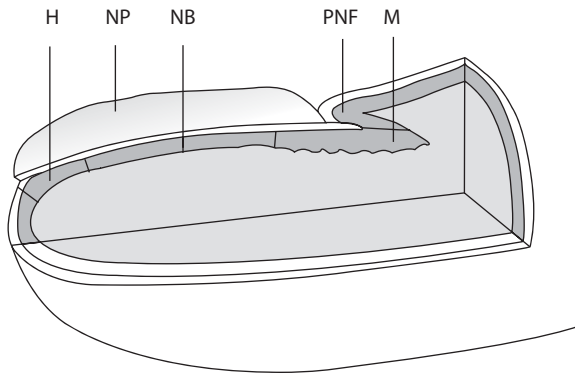
approximately equal (1), at least on the thumb. When the “magic” ratio differs from the ideal, the nail is less attractive (Figure 30.2).

Until the early 1990s, oval-shaped nails were most popular, but the current trend is to cut the tip more or less squarely at the free edge (Figure 30.3) to create an illusion of thinner, more tapered and graceful fingers. When too long, however, nails may not only become unsightly, the length may interfere with hand performance and increase the risk of injury. A long nail is more likely to become caught and, acting like a lever, can lead to catastrophic separation of the nail plate–nail bed attachment, a condition called onycholysis. The form or style of the nail can vary geographically. In some locales, shorter nail lengths are preferred, usually with rounded or almond-shaped free edges which can create a very natural-looking nail, if skilfully applied, while in other areas the preference is for longer nails with higher apex arches. Artificial nails are often used to seal the surface of the nail to help prevent mild peeling or splitting at the free edge as well as to enhance their look for cosmetic purposes, i.e. reshape flat nails with more attractive natural looking arches or to create more dramatic curvature at the free edge. They can also be used to correct the coloration of the nail or underlying tissue bed or to create the illusion of a longer nail bed. It is a misperception that artificial nails are mostly worn to lengthen the nail plate.

### Decoration of the Nail

For nails of equal length and corresponding contour, a colored or painted nail is usually considered more attractive (Figure 30.4). Interest continues to grow for the many types of sculptured artificial nails and the current trend is to decorate them with everything from a painted snowflake to a three-dimensional flower or flag to a portrait of a grandchild. Colorant powders or colored UV gels or glitters are used to create artificial nails with permanent designs that can be very intricate and beautiful. In some places this is considered a new art form, highly sought after and collected by nail art enthusiasts. Diamonds or emeralds have even been fixed into the elongated nails, while some wear intricate jewelry attachments to the free edge (Figure 30.5). Like jeweler, some practitioners utilize preformed nails of gold or goldplate, some studded with jewels.

Modern nail art was invented in the late 1960s when American teens began to paint colorful designs on their nails. The fad evolved during the 1970s when Japanese nail technicians began mixing colored pigments into their artificial nail enhancement products. Airbrushing with paint became popular for creating artistic designs on the nails and this service is still performed in salons to a lesser degree. The newer



**Figure 30.1** Anatomy of the nail apparatus. M, matrix; NB, nail bed; H, hyponychium; NP, nail plate; PNF, proximal nail fold. (After Dr E Haneke.)



**Figure 30.2** The racket nail of the middle finger is less attractive than the nail exhibiting the ideal ratio.

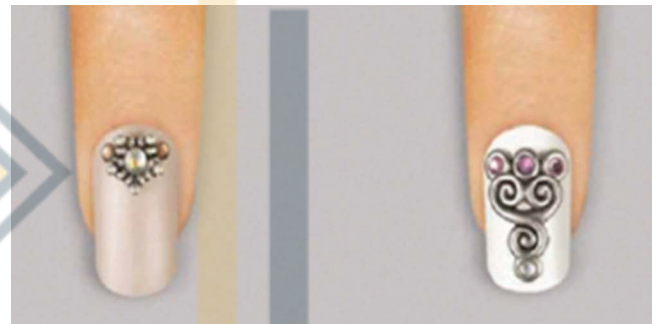


**Figure 30.3** There is a tendency to cut the tips of the nails more squarely.

technique is to use decorated films (coated on one side with a heat-sensitive adhesive backing) to decorate either natural or artificial nails. There are many hundreds of colors available



**Figure 30.4** Painted nails are more attractive than plain nails.



(a)



(b)



(c)



(d)

**Figure 30.5** Intricate jewelry can be attached to the free top extremity of the nail.

for UV gels and acrylic nail powder users and hundreds of preprinted designs and decorations available on adhesive backed film. The newest technique involves UV gels designed



to closely mimic traditional UV gel coatings, but formulated more like a nail varnish to be easily brushed on the nail and more easily removed (Figure 30.6).

### Texture of the Nail

The appearance and condition of the nail can determine its aesthetic appeal. The nail may be exposed to external agents or conditions which may render it soft, brittle, or peeling. The brittle nail is vulnerable to single or multiple longitudinal splitting and horizontal splitting into layers (onychoschizia) (Figure 30.7), or less often to transverse breaking (Figure 30.8).

Frail nails can benefit from external treatments with oils or waxes which may absorb to soften or may seal the surface of the nail to prevent excessive moisture loss or absorption. Such treatments are popular and may provide value, especially when used in conjunction with avoidance of "wet work." In addition, the age-dependent decrease in cholesterol sulfate levels might explain the previously observed higher incidence of brittle nails in women (2).

### Items for Nail Care

The following are examples of tools used for nail care (3).

#### *Nippers and Cutters*

Nippers are used to chip away or loosen artificial nail placed over the lunula area in preparation for reapplication in the area where the nail has grown out or where product is losing adhesion. This process is called "a fill" or "rebalance." These tools are often used to nip off tags of dead skin in the areas surrounding the nail. Some are especially designed for toenails. Cutters are jaw-like blades operated by a spring mechanism and used to sever the free edge of the nail or artificial tips. They are available in many sizes.

#### *Emery Boards*

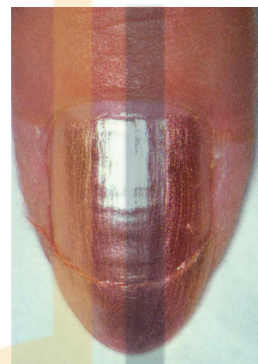
Flat, disposable, "paperboard" wands, coated with abrasives emery powder and used to shape, reduce the length, or smooth sharp or jagged edges on the nail's free edge. These types of abrasive files are inexpensive and often used by nail salons offering low-cost services and are considered single-use, disposable items.



**Figure 30.6** Nail art. (Courtesy of Dr. A Batistini).



**Figure 30.7** Onychoschizia (splitting into layers).



**Figure 30.8** Complete transverse breaking.

#### *Other Abrasive Nail Files*

Other types of abrasive nail files are made by coating an elongated board made of metal, glass, fiberglass, hard plastic, or wood with abrasive particles ranging in size and hardness. The "grit" of abrasive nail files is measured by counting the number of abrasive particles per cubic centimeter. Low-grit boards (60–120 grit) are used to quickly remove layers of the artificial nail and should not be used directly on a natural nail. Medium-grit boards (180–240) are for smoothing and shaping both artificial and natural nails, fingers and toes. High-grit boards (800–2400) are used for buffing, polishing, and finishing. Grit is not the only determining factor. Nail files can be coated with either crystalline aluminium oxide or silicon carbide. Silicon carbide is 20% harder than aluminium oxide, making these materials significantly more aggressive. Fine particles of diamond dust are electroplated on to a metalized fiberglass board, giving these abrasive files great durability and longevity. The high cost and extreme hardness of diamonds (10% higher than silicon carbide) is the reason these files have limited value for this application.

#### *Blocks*

These are also abrasive nail files and they take the form of a larger, rectangular foam block that fits comfortably into the hand. Blocks are the most widely used primarily due to their improved comfort, ease of use and efficiency.

### Nail Buffers

Specialty nail files designed to create very smooth and high shine surfaces on natural or artificial nails. They are normally used as “three-way buffers,” having three different grit sizes used in succession from coarsest to finest particle size which remove scratches and create smooth, uniform surfaces.

Padded chamois buffers used in conjunction with mild pumice-type abrasive creams or waxes and oils will produce a very high shine on the nail surface. Such systems have been used for generations. This is not a widely used mainstream technique, but is considered a more traditional technique used by more traditional practitioners

### Pushers

These devices are used to gently push back the eponychium and expose more of the lunula. They can also be used to carefully loosen, scrape, and remove cuticle tissue from the nail plate. Pushers can be polished, metallic probes with variously shaped rounded ends and relatively dull edges to minimize the potential for soft tissue injury. Another type is a pencil-shaped plastic or wooden implement used in the same fashion. The advantage of using wood is safety, since they are less likely to cause injury to the nail folds. Originally fabricated from orange wood, now they are primarily birch and are considered a single-use, disposable item.

### Cuticle Trimmers

The small, clipper-jawed scissors are used inappropriately for cutting frayed skin and living tissue (Figure 30.9). Devices that are curette-like, using a V-shaped blade mounted to a plastic handle, are used to shave living tissue. Nail technicians are using them for cutting the nail fold, which they mistakenly believe it is the cuticle. Trimming this living skin creates hardened tissue. When nail technicians cut this tissue away with any device, they often breach the skin barrier, increasing potential for infection, which is why government regulations usually forbid service providers such as nail technicians from intentionally cutting living skin as part of their professional services. Like all devices designed to shave the skin, they should not be used to perform salon services.

### Nail Whitener

This is a pencil-like device with a white clay (kaolin) core used to deposit color on the undersurface of the free edge of the nail. These should be avoided in professional salon settings because reuse can contribute to transmission of pathogenic organisms from client to client.

### Disinfectant Container and Cover

A disinfection container should have a cover and be large enough to contain disinfectant solution to completely cover all items intended for disinfection. Many have a tray for removing implements or provide tongs for reaching into the solution to withdraw implements and to help prevent skin contact with potentially irritating disinfectant solutions.

### Pedicure Implements

Pedicures require specialized implements. Toenails are larger and thicker than fingernails, and hardened callus can be tough to handle without the proper tools. Heavy duty toenail cutters with a squeeze-grip action are used along with abrasive files to smooth and shape toenails. Abrasive grit foot buffers are used on calluses. California has prohibited the use of metallic razors or abrasives on calluses in nail salons; the concern was that such tools were too aggressive and dangerous for use in nail salons.

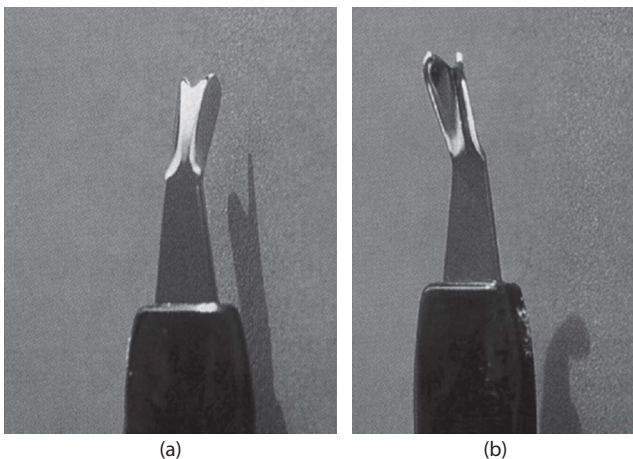
Aggressive tools and procedures, i.e. highly acidic or alkaline callus remover that completely removes the callus, are very concentrated and therefore have a higher potential to injure the underlying tissue. A risky practice performed by some nail technicians is to use razor-type devices to cut calluses from the foot. Complete removal of the callus is considered a medical procedure and is not appropriate for salon services and should be discouraged. Salon services should only smooth calluses, not remove them.

## Toiletries and Cosmetics

Items discussed include evaporation coatings, including base coats, top coats, and nail varnish.

### Nail Varnish Formulation

Whether referred to as nail varnish, polish, enamel, lacquer, varnish, or color, these products are identical in function and, for practical purposes, nearly the same in basic composition. These different terms are merely marketing terms used to establish brand identity. The varnish may be clear, opaque, or shaded with color. Varnishes may appear to be nothing more than a paint-like coating for the nail, but the chemistry is much more complex. In its simplest form, a nail varnish is defined as a durable film coating created by evaporation of a volatile solvent component. However, the coating must withstand severe abuse without losing color, gloss, or adhesion. Luckily, a clever formulator has a wide range of raw materials from which to choose, and through proper ingredient selection these properties can be greatly enhanced. However, the cosmetic appearance and durability of the final product are not the only considerations when choosing ingredients. Regulatory agencies and consumer perception also play important roles. For these and other reasons, we examine each ingredient type to provide a better understanding of these useful cosmetics.



**Figure 30.9** Cuticle trimmer.

## Nail Varnish Basics

A typical varnish formulation consists of seven basic types of ingredients:

1. Film formers
2. Film modifiers
3. Plasticizers
4. Solvents/diluents
5. Viscosity modifiers
6. Stabilizers
7. Coloration additives

Each of these ingredients contributes to the quality of the final product. If proper ingredient selections are made and correctly balanced, the varnish will be easy to apply and remove, quick-drying, waterproof, glossy, chip- and scratch-resistant, and flexible, and will adhere well to the nail. Ideally a properly applied coating should remain cosmetically attractive and intact for 5–7 days. The varnish coating also must have a low potential for toxicity and adverse skin reactions.

### Film Formers

The role of the film former is to create a smooth, continuous coating over the nail plate. The coating material of choice is an organic polymer called nitrocellulose (cellulose nitrate). The first natural polymer to be successfully modified by chemical manipulation, it was commercially produced in 1860 by treating cellulose with a mixture of nitric and sulfuric acids. Originally used in high explosives, dry powdered nitrocellulose is highly unstable and sensitive to light, UV energy, heat, atmospheric moisture, and oxygen, as well as an alkaline pH. It is so chemically reactive that it must be transported in a polar organic solvent, usually ethanol or isopropanol, to prevent explosive detonation. Formulators can choose from several viscosities and grades of material and use them alone or in synergistic blends. Non-nitrated cellulose such as cellulose acetate and derivatives are also used with varying degrees of success. Polyurethanes, polyamides, and polyesters have also been utilized, but can't match the surface gloss and hardness of nitrocellulose. Nitrocellulose also blends superbly with colored pigments, producing bright and vibrant colors. Still, nitrocellulose's disadvantages drive formulators to constantly seek alternative materials, since the coatings it produces are brittle, adhere poorly, discolor quickly, and shrink excessively to cause poor adhesion. Some newer formulations replace nitrocellulose with cellulose acetate butyrate because of its superior clarity, durability, and color stability. Examples of other film formers are meth(acrylate) polymers or copolymers.

### Film Modifiers

The purpose of a film modifier is to favorably offset some deficiencies of the primary film former. Specifically, film modifiers are used to improve adhesion. The most commonly used modifier used to be toluenesulfonamide/formaldehyde resin (TSFR) or tosylamide/formaldehyde resin (TF), the name listed in the *International Nomenclature of Cosmetic Ingredients* (INCI) dictionary. Due to the public's erroneous and unfavorable association with formaldehyde gas (see "Formaldehyde controversy," below), this resin has fallen out of favor and is being replaced by other resins, i.e. tosylamide/epoxy resin (4), polyvinyl butyral, ester sucrose benzoate, polyesters (5), acrylic ester oligomers, sucrose acetate isobutyrate (SAIB), and arylsulfonyle urethanes (6), and various copolymers, i.e. adipic

acid/fumaric acid/phthalic acid/tricyclodecane dimethanol. A multi-year independent study performed in salons at multiple locations in northern and southern California in both winter and spring, under the auspices of the California State Attorney General, was designed to address questions related to California Proposition 65. The study was commissioned by the Nail Manufacturers Council on Safety of the Professional Beauty Association. The study concluded that neither workers nor consumers in the salon had any risk from using cosmetics nail containing formaldehyde and toluene (7). The measured formaldehyde concentrations varied between 0.0012 and 0.0038 ppm, which is what would be normally expected in ambient air and far below the Occupational Safety and Health Administration (OSHA) 8-hour TWA of 0.75 ppm, and the more conservative ACGIH maximum concentration is 0.3 ppm.

### Plasticizers

Plasticizers add flexibility to polymer films and offer a useful way to improve the strength of nitrocellulose films. They reportedly increase separation between the cellulose chains and act as an internal lubricant (8). Film modifiers counterbalance the negative aspects of nitrocellulose, whereas plasticizers alter the properties of the entire film and can have profound, positive effects on flexibility and adhesion. The plasticizer must be compatible and remain in solution without negatively affecting viscosity, consistency, flow, color, or shelf life. It must not readily escape from the film through migration or volatilization and must be dermatologically innocuous. Camphor (b.p. 96°C) is the most common example of a low-molecular-weight, high-boiling-point plasticizer. Other examples of plasticizers are acetyl tributyl citrate, castor oil, sucrose benzoate, glyceryl tribenzoate, PPG-2 dibenzoate, ethyl tosylamide, glycerol, triacetin, and a polymeric plasticizer called NEPLAST (a polyether-urethane) (9).

Dibutyl phthalate (DBT) was for many years the preferred plasticizer for nail varnish. In 2003, DBT was reviewed in the United States and declared "safe as used" by the Cosmetic Ingredient Review (CIR) Expert Panel. However, in the same year, new regulations in the European Union (EU) required that any ingredient listed as category 3 on the EU Dangerous Substance List or CMR (carcinogens, mutagens, and reproductive toxicants) list must prove to the EU's Scientific Committee on Consumer Products that they are safe before they are allowed for use. DBT was listed as a category 3 (as an additive in spray paint), so rather than undergo exhaustive and onerous toxicological testing, nail varnish manufacturers around the world began to eliminate this ingredient since they did not wish to carry a separate inventory for the EU.

### Solvents and Diluents

Nail varnish solvents give these products their characteristic odor and flammability, but solvents are vital since they dissolve solid ingredients, and upon evaporation, deposit them on the nail plate. The most commonly used are alkyl esters (ethyl, amyl, and *n*-butyl acetate) and glycol ethers (propylene glycol monomethyl ether). Since each solvent has a different boiling point and evaporation rate, a skilful formulator can balance several solvents to achieve the desired drying time. Good solvents are those that easily dissolve solid ingredients and reduce viscosity or improve brushability.

Even though they are not solvents for nitrocellulose, ethanol, isopropanol, and butanol act as coupling agents and through hydrogen bonding act synergistically to increase the



overall solubility and flow of the system. Diluents are usually non-polar compounds that are also non-solvents for nitrocellulose, but help to regulate evaporation rates and stabilize viscosity to prevent uneven evaporation that may affect surface gloss, color, and clarity, especially in humid conditions. A great advantage of diluents is that they may be added in controlled amounts without reducing viscosity.

In the past, toluene was used without significant problems since the 1930s as a nail varnish diluent and often accounted for up to 25% of the formulation. Toluene was added to the list of chemicals for which California's Proposition 65 requires a warning label since is suspected of causing birth defects and cancer (10,11). However, study by the Nail Manufacturers' Council (NMC) performed under the auspices of the California State Attorney General indicates that nail technicians' salon exposure level was 769 times below U.S. federal safe limit set by OSHA (12). Toluene exposures were well below the OSHA exposure limit of 200 ppm TWA, and reported mean was 0.260 ppm TWA (7). Even so, in 2008 the California Air Resources Board identified toluene as an air contaminant that contributes ground level formation of ozone, and therefore most manufacturers have voluntarily discontinued use of the solvent, replacing toluene generally by increasing the levels of ethyl and butyl acetate (13).

### Viscosity Modifiers

Ideally, a nail varnish should have a gel-like consistency to keep pigments suspended, but thinner, more brushable liquids produce better and more uniform films. Luckily, both consistencies are possible in systems that display thixotropic behavior. Thixotropic systems become thinner as they are mixed and brushed, but while at rest they will reform a semi-gel structure. Examples of substances used to create this effect are cationic modified montmorillonite clays that are approximated by the formula  $(Al,Mg)_2(SiO_2)(OH)_2 \cdot nH_2O$ . Treating these clays with quaternary ammonium compounds will render them organophilic. Stearalkonium hectorite is the most frequently used of these clays. The main disadvantage of clay additives is that they lower surface gloss. This can be offset by the addition of various polymers, e.g. acrylate copolymers and nylon. These additives improve gloss, as well as toughness and scratch resistance. Amorphous (noncrystalline) silica is also used as a viscosity modifier, at very low levels.

### Stabilizers

Color stability is a very important property in nail varnishes. Special stabilizers are added to nail varnishes to prevent the colors from fading and shifting. Nitrocellulose is usually the culprit, since it is inherently unstable in ultraviolet (UV) energy, which is invisible ( $>400$  nm) and therefore not considered to be "light" ( $<400$  nm). Solutions of nitrocellulose will change from a clear to a yellow to a brown liquid with relatively little UV energy exposure. Pigments and dyes may also become unstable with longer periods of exposure. Lighter colored varnishes are less stable than darker colors. Sheerer varnishes with lower levels of colorant are more susceptible to UV energy than richer varnishes with higher levels of colorant. Generally varnishes with colors nearer to the blue end of the spectrum shift more readily and dramatically than colors found nearer to the red side. To help prevent discoloration problems, nail varnish formulations utilize UV energy-absorbing stabilizers that absorb UV energy and convert it into harmless visible light and infrared energy (heat). The most common of the stabilizers of this type are benzophenone-1 and etocrylene.

### Coloration Additives

Unless the nail varnish is clear and colorless, additives must be used to alter the opacity and shade. Color additives must have very low or no heavy metal content and must be certified by the U.S. Food and Drug Administration (FDA), and if sold internationally must also use be allowed in the EU, Canada, and Japan. Occasionally, smaller manufacturers will risk using non-approved colorants (e.g. "day-glo" colors) to satisfy the faddish demands of younger consumers, but for the most part these regulations are adhered to closely. Colorants must have relatively high light fastness and should not stain the nail plate. Colorants can be stabilized by creating precipitating a particular pigment with aluminium hydroxide to form a salt complex called a "pigment lake," i.e. D&C Red No. 7 Calcium Lake and D&C Yellow No. 5 Zirconium Lake. Pastel shades are achieved by the addition of titanium dioxide ( $TiO_2$ ). Ferric ferrocyanide (Prussian blue) is used in small amounts to enhance blues and alter other shades. In order to achieve complete pigment dispersion and suspension, high energy ball or roll mills must be used to ensure uniform colors and to reduce the amount of colorant needed, i.e. 2% dry colorant or less. Pearlescent pigments continue to be highly desirable commodities in modern varnishes. Bismuth oxychloride and mica coated with  $TiO_2$  and other colorants are used to create the many beautiful iridescent shades. More complete information on approved colorants can be obtained from the CTFA's (now known as the Personal Care Product Council [PCPC]) *International Color Handbook* (14).

Colorants are usually indicated on the label by their international color index (CI) number, which is accepted by most regulatory agencies around the world; however, sometimes both the CI and INCI nomenclature is used, e.g. CI 77891 (titanium dioxide), unless there is a lack of space on the label. The U.S. FDA does not currently accept CI numbers when used alone and requires the use of Food, Drug and Cosmetics (FD&C, or D&C) names.

### Other Additives

A variety of highly specialized additives are known to those skilled in the art of nail varnish formulation. Even tiny amounts of many of these special additives can give dramatic differences in performance. Some examples are surfactants to improve wetting and adhesion and organic acids to stabilize colorants. However, some additives serve no function other than to increase consumer appeal. These include proteins, minerals, pearl, gem dusts, and vitamins.

### Base and Top Coats

Base coats are applied to the nail plate before application of the nail varnish. They are usually of similar composition to varnish, but contain more additives which improve adhesion and protect against staining of the nail. Top coats utilize higher levels of ingredients that maximize surface gloss and hardness. Often, the top coat contains special UV-absorbing materials to prevent discoloration. Not all top coats are evaporative coatings, since some are UV-cured urethane (meth)acrylate oligomers and blends (15).

#### Film-Drying Accelerant

Silicone oil blends and silicone oil-in-water emulsions are often used to accelerate nail varnish dry times. Film-drying accelerant is sprayed or brushed over freshly applied nail varnish to give rapid drying and increased protection from minor dents or surface scratches while remaining solvent evaporates from

the coating allowing it to reach a hard set. Drying is accelerated because the thickener's microstructure collapses, allowing solvents to escape more quickly.

#### *Formaldehyde Controversy*

Formaldehyde is an anhydrous gas and cannot be utilized as a cosmetic ingredient for that reason. When formaldehyde is mixed with water, it does not simply dissolve, but undergoes a chemical reaction which converts it from the aldehyde into methylene glycol and trace levels of free formaldehyde, in equilibrium. Unfortunately all around the world, formalin is incorrectly thought of as aqueous formaldehyde, even by medical and scientific professionals. The so-called "formaldehyde-releasing" preservatives do not release formaldehyde gas, but instead release methylene glycol. The INCI dictionary, which cosmetic product manufacturers are supposed to use to name their ingredients, used incorrectly to require that formalin be called formaldehyde. No one worried about the misnaming until advocacy groups began saying that cosmetic products contain cancer-causing formaldehyde as an ingredient, which is clearly incorrect. The NMC petitioned INCI to address this issue and as of December 2008, the INCI name for formalin is methylene glycol.

The FDA allows the use of up to 5% formalin in nail hardeners and requires warning labels on nail-care products, as well as "nail shields which restrict application ..." (16). EU regulations require "formaldehyde" warning labels when concentrations exceed 500 ppm formaldehyde gas. These levels of free formaldehyde gas are much greater than what is found in nail hardeners which typically contain less than 12 ppm, based on equilibrium calculations for these products, which typically contain 1.5% formalin or less (17).

It is suspected that formalin cross-links proteins (18) in the nail, resulting in increase in surface hardness and decreased flexibility, which the user misinterprets as improved strength and durability. After months of continued use, nail hardeners may eventually increase nail plate hardness and rigidity to the point that brittleness becomes obvious. Users remember the early success of the hardener, and usually respond to the brittleness by increasing the frequency of application. This leads to further cross-linking and nails may end up in worse condition than before. Onycholysis and abnormal growth of the hyponychium may result of prolonged formalin overexposure, but this is probably uncommon (19). Typically, these problems are a result of aggressive manicuring services (20).

#### *Microbiological Contamination and Water-Based Varnish*

Microbiological studies commissioned in 2009 by the NMC show that traditional solvent-based nail polishes will quickly kill common pathogens without additional preservation and do not create the potential for transmitting pathogenic organisms (21). Water-based nail varnishes are available, but they cannot compare to the performance of traditional nail polishes, and being prone to bacterial contamination must be preserved with, for example, quaternium (16).

#### *New Developments*

New industry trends are turning toward UVA-curable top coats, base coats, and nail color, which applies like traditional nail varnish to form durable, long-lasting coating when exposed to low levels of UVA energy (400–450 nm). These new types of nail coatings are referred to as UV gel polish or manicures, and are used to coat the nail plate with a longer-lasting colored coating capable of camouflaging nail

defects such as nail bed bruises or splinter hemorrhages. UV gel polish is removed after 2–3 weeks and replaced with a fresh coating. Because these nail coatings are polymerized by UV energy, they produce superior scratch-resistant coatings with enhanced durability, but are more difficult to remove than traditional nail varnish.

One study has claimed UV gel polishes create nail weakness, brittleness, and thinning. Ultrasound measurements demonstrated plate thinning, however the researchers failed to account for the effects of filing of the natural nail with a coarse abrasive prior to application in order to improve adhesion. Nail thinning was erroneously attributed to the UV coatings, even though these formulations are not corrosive to the nail and have no ability to thin or weaken the plate (22). This type of thinning/weakening of the nail plate is commonly observed after aggressive filing and/or improper removal. When firmly bonded nail coatings are forcibly scraped or pried from the nail plate, surface damage becomes more likely. To prevent surface damage and thinning, more time should be allowed for the solvent removers to loosen the bonds and allow nail coatings to be gently removed. Less aggressive filing techniques using higher grit nail files will also reduce the potential for plate thinning.

Nail varnishes that improve nail plate health are currently the hottest topics of interest for consumers. There is therefore a tremendous economic incentive for manufacturers to discover ingredients that provide demonstrable benefits to the nail. Improved nail toughness and solutions for yellow, dry, brittle, and splitting nails would be enormously beneficial. When these technologies are developed, it may eventually lead to the Holy Grail of nail varnishes—one that truly prevents or helps treat common nail pathologies. A step in this direction was introduced in 2014 with the development of a UV-curable methacrylate formulation that penetrates only the uppermost layers of the nail plate and is then rapidly polymerized by UV energy. Because the nail plate has excellent barrier properties, it prevents the UV-curable formulation from penetration beyond the upper 10%–15% of the nail plate's thickness. Upon UV exposure, the material absorbed into the surface layers polymerizes into place to seal the upper layers of the plate and thus prevent them from peeling apart. The surface is thus hardened and more damage resistant, while the nail plate is rendered tougher. Once polymerized into the nail, it also seals the nail against additional absorption and prevents staining. This differs significantly from methods of hardening that increase keratin cross-linking and may lead to embrittlement with excessive use.

#### *Nail Varnish Solvents (Nail Varnish Removers)*

Acetone and/or butyl or ethyl acetate, methyl ethyl ketone, ethoxy ethanol, or similar compounds are used for rapid softening and solubilizing of nail polish as well as to remove oils and waxes to cleanse and prepare the nail for varnish application. Some varnish removers claim to minimize stripping of moisture and oil from already brittle nails. The removers are based on solvents such as lactones or dimethyl esters of adipic, glutaric, and succinic acids (dibasic esters).

#### *Nail Creams and Lotions*

Oil-in-water emulsion preparations aid in softening the keratin of the nail plate and contiguous skin. This is achieved initially by the addition of water and subsequently by the reduction in evaporation of water from the nail. Cosmetic preparations are of two basic types: those designed to deliver water-soluble,



hydrophilic substances into the nail plate, and those designed to deliver hydrophobic substances, such as natural jojoba or almond oil. Once absorbed, these ingredients have the potential to plasticize, increasing flexibility, toughness, and durability, and are claimed to be especially useful for dry and/or brittle nails.

#### Cuticle Removers

These are lotions or gels containing approximately 2%–4% sodium or potassium hydroxide (pH 12–13.5). They are applied in the vicinity of the cuticular ridge to eliminate the remnants of cuticle that adhere to the nail plate as it grows outward. The lotion is left in place for approximately 1–2 minutes and then washed off. In nail salons, the process is hastened by using a cuticle pusher. Creams containing low levels of  $\alpha$ -hydroxy acids are also used as cuticle removers. These usually contain 1%–5% lactic acid (pH 2.5–3.5). Besides their ability to soften and remove cuticle, daily use can eliminate hangnails.

### ADVERSE REACTIONS TO NAIL COSMETIC PROCEDURES

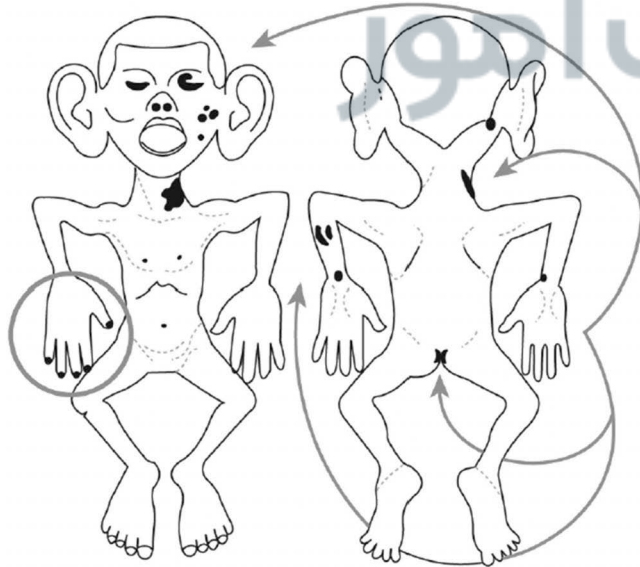
Adverse reactions to nail cosmetic procedures (21) may be divided into two main categories: reactions to applied cosmetics, and nail tool/implement damage.

#### Reactions to Applied Cosmetics

Cosmetics may produce reactions both at the site of application to the nail area and secondarily elsewhere on the body. The fingernails act as a reservoir for small amounts of cosmetic preparations transferred by the hand to other areas of skin, i.e. the face.

#### Nail Varnish Dermatitis

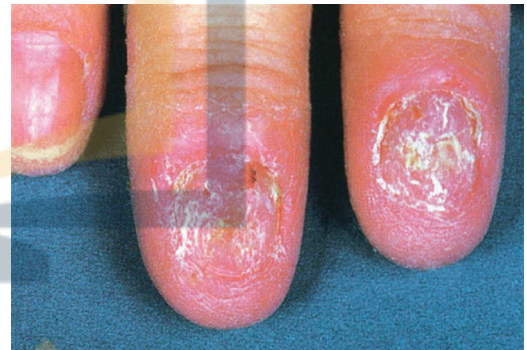
Nail varnish dermatitis of allergic origin can appear on any part of the body accessible to the nails (Figure 30.10), but often with no signs in the nail apparatus. Exceptions, however, may exist—mainly in the periungual area (24) (Figure 30.11). The



**Figure 30.10** Distribution of nail varnish ectopic dermatitis. (After Bonu G, Zina G, *Minerva Dermatol* 1959; 33:507–9.)

eyelids (Figure 30.12), the lower half of the face, the sides of the neck, and the upper chest are the most commonly affected areas, likely from scratching the skin in these areas with recently or freshly varnished nails (25,26). In addition to ectopic dermatitis, allergic airborne contact dermatitis caused by nail ingredients should be suspected when lesions on the face, neck, and ears are symmetrical (27). The allergen in nail varnish is usually thermoplastic resin. Diagnostic skin patch testing with nail varnish should be performed without occlusive covering, or with dry varnish films to avoid false-positive reactions from the solvent.

The thermoplastic resin used to promote adhesion is often the main culprit (28–31) but when the nail varnish is completely dry it is only a weak allergen (32). However, nail varnish that has completely dried on the fingernails contains water-soluble components that may reach the skin during extensive, transient contact (33). To make “formaldehyde-free” claims, cosmetic manufacturers have reformulated their nail varnish, replacing TF resin with other resins, i.e. tosylamide/epoxy resin (trade names Lustrabrite®, Nagellite®, a condensation product of bis-phenol A epoxy resin<sup>34</sup>), phthalic and trimellitic anhydride/glycol copolymer<sup>35</sup>, glycerophthalic polyester resin (PhaseTM), 4-methylbenzene sulfonamide-epoxy resin (CliniqueTM), phthalic polyester



**Figure 30.11** Rechallenge of nail varnish onto two fingers only, showing severe local dermatitis. (Courtesy of Dr. R Staughton.)



**Figure 30.12** Eyelid dermatitis.



resin (Shiseido™), or polyester saturated hydroxylated resin (Deborah™) (36). Unfortunately, some of these and related “hypoallergenic” resins (37,38) have already produced distant contact dermatitis. Another potential exposure source is the (meth)acrylates used in some UVA-curable nail products.

Reactions may also occur when improper colorants are mixed with UV gels to create special effects. A 37 year-old housewife with no previous history of allergic contact dermatitis purchased a glitter color from the Internet, mixed it with the UV gel and self-applied the mixture to her nails. After 7 months, she developed multiple intensely itchy, eczematous periungual and palmar lesions on both hands. Patch testing resulted in a strong positive for cobalt chloride. Cobalt was listed as an ingredient in the glitter and was responsible for the adverse reaction, which resolved when the nail coating was removed (39).

The following substances should be included in a test battery:

- Tosylamide/epoxy resin (10% petrolatum)
- TSFR (10% petrolatum)
- Glyceryl phthalate resin (polymer resin) 10% petrolatum
- Formalin (1%–2% methylene glycol in aqua)
- Phthalic and trimellitic anhydride (1% petrolatum)
- Colophony (resin) 10% or 20% petrolatum (40)
- Drometizole (Tinuvin P) 1% to 5% petrolatum
- Nickel (0.5% petrolatum) and 1% dimethylglyoxine spot test for nickel from varnish mixing beads
- Benzalkonium chloride (0.01 to 0.1% water)

#### Contact Urticaria

Recurrent urticaria involving the same area as contact dermatitis plus the distal phalanx of the fingers has been reported with isomorphic response to nail varnish testing, immediately after simple contact (41).

#### Nail Plate Staining

Nail staining from the use of deeper shades of red and brown nail varnish is most commonly yellow–orange in color (42) (Figure 30.13). Typically, it begins near the cuticle area, extends to the top of the nail, and becomes progressively darker from base to tip. Colorants such as D&C Red no. 6, 7, 34 and FD&C Yellow no. 5 Lake can penetrate into the nail too deeply to be removed, but this can be significantly avoided with the use of a base coat prior to nail varnish application. Fingernail

discoloration can be produced by chloroxine, an active ingredient in a shampoo used for control of seborrheic dermatitis which is highly reactive to metals such as iron oxides (commonly used as pigments in nail varnish) and it is likely that discoloration may result from a reaction between the two (43). Patients undergoing therapy with minocycline may develop discoloration of the nails (44). Analysis of the nail clippings from minocycline-treated women showed a large amount of iron concentrated only in discolored areas of the nail, which did not occur in women who did not varnish their nails or in men with nails free of a significant amount of iron. Nail discoloration may also result from the combined effect of nail varnish and dermatological treatment containing either resorcinol or resorcinol monoacetate (45).

#### Nail Keratin Granulation

Injury to the nail from nail varnish is rare. However, “granulations” of nail keratin (Figure 30.14), presenting as superficial friability (46), can sometimes be observed. In these cases individuals continually remove old nail varnish and apply fresh coats for periods of weeks. Nail keratin granulation may be avoided by lessening the frequency of removal and reapplication of nail varnish, i.e. no more than once per week, to avoid damaging the surface of the nail. These products contain acetone and/or butyl or ethyl acetate, methyl ethyl ketone or similar compounds which can dehydrate the nail plate and decrease corneocyte adhesion, extract lipids, and contribute to brittleness (47). Some polish removers contain significant amounts of water (17%) and/or conditioners for skin, to reduce tissue damage, making them the preferred solvents for varnish removal. Oils are sometimes added to prevent excessive drying of the nail. Nail varnish removal may cause inflammation of the paronychia area when the solvent remover solution is left in contact with the skin. Rarely, irritant and allergic contact dermatitis, blistering, onycholysis, and brittleness may occur (48).

#### Cuticle Removers and Softeners

Removers (see above) are designed to destroy keratin by attacking the disulfide bonds of cystine. After the nails have been soaked in soapy water, cuticle removers are applied and left in place for approximately 2 minutes before being washed off. The loosened cuticle tissue is usually removed from the nail by being rubbed gently with a wooden stick covered with cotton. Removers should not be applied to the fibrous cuticular ridge,



**Figure 30.13** Yellow-orange nail-plate staining. (Courtesy of Prof. A Tosti.)



**Figure 30.14** “Granulation” of nail keratin.

and this tissue should not be removed with sharp implements such as nippers or V-shaped curettes.

Cuticle softeners are often misnamed since they are actually designed to soften and moisturize the eponychium and lateral folds and not designed to remove cuticle tissue from the nail plate; the pH of these products is typically 4.5–6.0. Skin conditioners or softeners often contain substances such as quaternary ammonium or urea or  $\alpha$ -hydroxyacids at low concentrations and are used as emollient creams. Triethanolamine is used to adjust the product's pH and may act as a sensitizing agent (5% in petrolatum for patch testing).

#### Stick-On Nail Dressings

"Stick-on nail dressings" (decorative coatings) are thin, clear or colored synthetic films (Figure 30.15) with an adhesive that fixes them firmly to the nail. Decorative adhesive films have undergone a resurgence and become very popular again. In some cases, pathologic changes<sup>(45)</sup> of traumatic origin are said to be produced in nails by the occlusive nature of the film. In fact, they can be attributed to a temporary overhydration of the nail plate. The same is true for any occlusive or semiocclusive coating on the natural nail. For example, when artificial nails are removed, the nail may feel weaker and thinner because the higher moisture content increases flexibility. Surface damage with the appearance of whitish patches can occur if these films are peeled from the nail plate without first warming to soften the adhesive bond using a hair dryer or under an infrared lamp designed for the purpose. Press-on nail extender tips are also used for temporary extension of the nail. Generally, these are only used for special occasions, i.e. weddings, and are removed within a day or two.

#### Potential for Injury/Infection

Traumatic injuries from nail files, wooden or plastic sticks, and metal or porcelain spatulas may cause not only infection, but also onycholysis (Figure 30.16) and Beau's lines (transverse white streaks) from overzealous manicuring, pedicuring, or filing the nail (49). It is therefore wise to use wooden sticks covered with cotton or instruments with blunt edges. Nail technicians usually remove too much of the natural nail plate with coarse abrasives and powered electric files, which should only be used on artificial nails. Cutting the nail with dull or blunt tools may contribute to increased weakening, peeling, fracturing, or splitting. Tools should be sharpened regularly or the blades should be changed when they become dull. An alternative method is to shape the fingernail with an abrasive board,



Figure 30.15 Stick-on nail dressing.



Figure 30.16 Onycholysis due to overzealous manicure.

filing from the sides of the nail toward the center. Peeling any type of nail coating or tightly adhering film from the plate can cause nail cells on the surface to peel upward and create whitish-appearing patches that are often mistaken by nail technicians and clients for dry patches, when in fact they are surface disruptions. Such damage is completely avoidable if these coatings or adhesive films are carefully removed.

Procedures for consistent and proper hand washing by all hospital personnel should be reinforced, as well as the maximum 3-mm rule for end-of-finger nail lengths (50). The subungual space of the hand is heavily colonized with microorganisms, and contaminated instruments may lead to acute bacterial or chronic *Candida* paronychia and onycholysis. Overfiling of the nail plate with coarse abrasives or heavy-handed filing techniques with even the mildest buffing block can disrupt the thin tissue seal that holds the plate to the bed and lead to onycholysis and possibly subsequently *Candida* or other infections. Acrylic nail extensions are also implicated in *Candida* nail bed infection (51), but much more commonly the issue is increased bacterial carriage versus natural nails (52), particularly *Staphylococcus aureus* and *S. epidermidis*, which are not uncommon under the free edge and are more difficult to remove out of this area despite surgical scrubs (53). In contrast to fresh nail polish worn on short, healthy nails (54), chipped nail varnish is a known potential reservoir for bacterial growth on natural nails (53). In addition, bacterial carriage is higher in subjects with artificial nails than in those with natural nails, possibly because of the longer length of artificial nails and more surface area for colonization (55). Serious eye infections have also been reported following *Pseudomonas* involvement in the nail apparatus (56), as has subacute bacterial endocarditis after nail trauma (57).

Warts can affect mainly the periungual tissue and sometimes are found on the proximal nail fold as a result of nail biting or other insult (58). In the mid-1990s, a woman was awarded \$3.1 million after contracting herpes on all ten fingers at a salon (59).

Transmission of more serious diseases such as hepatitis B and AIDS (HIV) seems to be virtually impossible through nail services. The approximately 200,000 nail technicians in the United States perform on average about 20–25 services per week. Since 1985, very conservative estimates are that over six billion nail services have been performed without a single identified or suspected case of HIV or hepatitis B



transmission. If this type of transmission were possible, it would be expected that a noticeable increase in unexplained infections in women who frequent salons would have been noticed, but no such link as ever been discovered. Of course, all infectious complications can be avoided with effective sanitary practices, and this has become an increasingly important focus in nail salons.

#### *Sanitation and Disinfection Practices*

**Sanitation** It is generally not understood that “to sanitize” simply means low-level cleaning, in which potentially harmful micro-organisms are reduced to levels considered safe by public health standards. The simple act of wiping an object with soap and water is a method of sanitizing. Sanitation is often confused with disinfection. Sanitation/sanitizing is a primary method for infection control and must be properly performed or disinfection procedures will be less effective.

**Disinfection** Disinfection is the elimination of most potentially harmful microorganisms, except bacterial spores, on a surface. U.S. Environmental Protection Agency (EPA)-registered disinfectants are authorized for use only on hard, non-porous surfaces, since this is how their efficacy is verified. Bleach and alcohol are examples of effective disinfectants that existed before the creation of the EPA and therefore they do not require EPA registration. It is against U.S. federal law to use an EPA-registered disinfectant in a manner contrary to its registration, therefore soft or porous surfaces can be disinfected with appropriate bleach or alcohol solutions and meet EPA requirements if properly performed. In American nail salons, most state board of cosmetology regulations require that hard, non-porous implements be thoroughly washed and then disinfected for 10 minutes in an EPA-registered disinfectant that is virucidal, fungicidal, and bactericidal.

Wooden sticks, cotton balls, emery boards, and certain types of abrasive files are considered single-use items (i.e. they should be disposed of after one use). These items should not be stored or put aside for one specific client, since this can contribute to spread of microorganisms in the salon. Salon disinfection regulations nationwide are confusing and in many cases antiquated or use incorrect terminology, i.e. interchanging “sanitize” with “disinfect.” Each licensed nail technician receives training in the best practices of sanitation and disinfection during the course of their education and must pass an examination, but sometimes this information is not properly taught or fully understood.

Improper cleaning and disinfection probably account for the vast majority of salon-related infections which would be prevented by adhering to the proper guidelines. A comprehensive set of recommendations for cleaning and disinfecting both manicure and pedicure equipment in salons has been developed by the NMC and is available for free-will download in several languages (60). These recommendations have been accepted and endorsed by competent associations in the United States, the European Union, Australia, and Korea.

Some people prefer to keep their own clippers or other implements at their regular salon, but doing so means loss of all control over these devices. Patrons have been known to use them on other family members and even their pets. Nail professionals must clean and disinfect all implements prior to use on a client. Due to time constraints, this is almost never done; therefore the practice of leaving one's instruments at the salon should be discouraged.

#### *Sterilization*

Complete elimination of living organisms, including viruses, bacteria, and bacterial spores, from a surface is required to achieve sterilization. Ethanol 90% (effective against herpes) and 10% sodium hypochlorite (bleach) work well if instruments are fully submerged for 10 minutes. Some authorities are convinced that glutaraldehyde should not be recommended for salons, claiming that the risks of overexposure do not warrant using potentially unsafe, toxic, and highly aggressive pesticides.

Autoclaves are growing in popularity, but some experts estimate that less than 3% of the salons in the United States use autoclaves. Those that do often do not properly maintain the units or verify their efficacy by spore testing, as required. Due to the negative press and positive PR value of using autoclaves, this trend is expected to continue. Use of autoclaves is more prevalent in Australia, due to government regulations. An interesting development is that several companies have set up services that sterilize implements for salons. Sterilization will never replace disinfection and since many items that come in contact with patrons are too large to fit in autoclaves they therefore must be properly cleaned and disinfected before reuse.

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# Cosmetics for Abnormal and Pathological Nails

Douglas Schoon and Robert Baran

## INTRODUCTION

Medical or surgical nail disorders can sometimes be camouflaged by cosmetic nail techniques. This, however, covers up the underlying process, and proper diagnosis and therapy are therefore essential to correct the underlying condition (1).

There are limits to the use of cosmetics, such as acrylic chemicals, in some at-risk patients: those who have had a reaction to artificial nails in the past, for example, or individuals with circulatory disorders, particularly with scarring or ulceration around the fingertip. Any bacterial or fungal infection should be treated before applying any type of artificial nail. Individuals whose hands are in water for long periods will have difficulty keeping on artificial nails. Psoriasis produces an isomorphic reaction (2), so artificial nails should be avoided in persons affected by this condition, since just nicking the hyponychium or nail folds may provoke a Koebner reaction. Lichen planus and lupus erythematosus may also precipitate this type of reaction. If the condition is minor or temporary—that is, waiting for hangnails to heal—then artificial nails are acceptable; however, for potentially chronic conditions such as onycholysis, it might be prudent to avoid artificial nails until the condition is resolved. This will avoid medicolegal problems (3). For the same reasons, an ultraviolet (UV) energy-cured acrylic should never be used in people who are taking photosensitizing medications, or who are affected by photodermatitis.

## ARTIFICIAL NAILS

There are three major methods for applying artificial nails. Approximately 30% are sculptured on a form, 60% are molded on an acrylonitrile butadiene styrene (ABS) plastic tip, and 10% are applied as thin overlay on the natural nail. Each is popular for a variety of reasons.

- All three methods can correct the coloration of nail plates or beds.
- Each method can build a natural-looking curvature to flat natural nail.
- All will increase the durability of the nail plate and may be decorated with colors, designs, ornaments, and jewels.
- Each type can attractively replace a deteriorated nail plate, one reduced by onychophagia, affected by splitting for example, or one that is simply broken—acrylics can even cosmetically correct “ski-jump” nails or unsightly “racket” nails.
- Sculpting and plastic tips specifically add length beyond the free edge and can create the illusion of a longer nail bed.
- Plastic tips require less technical skill to create a natural looking nail.

- Natural nail overlays add no length and require lower maintenance while providing a protective coating. Artificial nail products are applied both in salons and in the home.

Since the sculpting technique requires considerably more skill, it is usually restricted to the salon setting.

There are three basic types of products used to perform these services. They share similar ingredients but feature different chemistries. All three are discussed in this chapter. They are “liquid/powder” (methacrylate based), “UV gels” ([meth]acrylate based), and “wraps” (cyanoacrylate based). In this chapter the term (meth)acrylates will be used to indicate methacrylates and/or acrylates.

A typical methacrylate liquid/powder based kit contains (4):

- A metallized paperboard template, placed on the natural nail surface to frame the new nail for sculpting
- A liquid methyl\*, ethyl or isobutyl methacrylate monomer blend containing proprietary blends of other mono-, di-, tri-, and tetrafunctional methacrylate monomers
- A powdered, spherical polymer made from poly(methyl methacrylate) and/or poly(ethyl methacrylate) polymer (or a copolymer of both methacrylates) with benzoyl peroxide as an initiator and possibly titanium dioxide as a white colorant
- A stabilizer such as resorcinol, eugenol, thymol, or (most commonly) hydroquinone (HQ) or methyl ethyl hydroquinone (MEHQ)
- A catalyst, such as N-N-dimethyl-p-toluidine, to catalyze the production of free radicals from benzoyl peroxide in the polymer powder
- Plasticizers such as tricresyl phosphate
- Very low levels of solvents to act as clarifiers or solubilizers
- Dyes

In salons, metalized nail forms (Figure 31.1) and mylar-coated nail forms predominate over reusable.

Teflon nail forms are shown in Figure 31.2a,b.

The nail is first thoroughly cleansed, by brushing with soap and water and often painted with antiseptic solutions.

The dried nail is sometimes coated with an adhesion-promoting primer. In the past, these primers were based on diluted methacrylic acid, but developed acid-free primers are designed to react covalently with the keratin and the acrylic, providing increased adhesion to both surfaces.

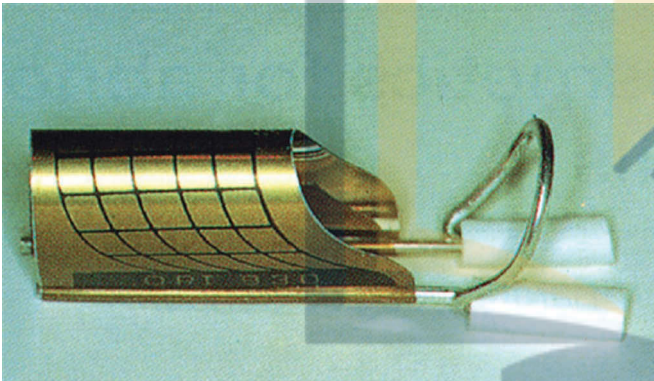
Primers are “adhesion promoters.” Nonmethacrylic acid primers are adhesion promoters in a solvent base, and are not

\* Methyl methacrylate monomer is specifically prohibited for use to create artificial nails in most U.S. states and Australia

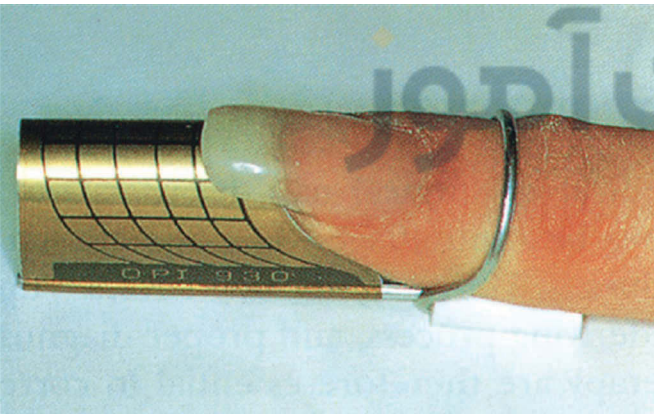




**Figure 31.1** Metalized paperboard template for sculptured artificial nails.



(a)



(b)

**Figure 31.2** (a,b) Teflon templates for sculptured artificial nails.

corrosive and therefore not likely to burn the soft tissue on contact, as is the case with methacrylic acid.

Newer artificial nail formulations no longer require pre-treatment of the nail with a primer.

Using a paper or Teflon nail form, the natural nail is coated with a fresh acrylic mixture to harden at room

temperature in less than 3 minutes. The prosthetic nail is enlarged by repeated applications. The sculpted nail can be filed and manicured to shape, and as the nail grows out, further applications of the acrylic mixture are added every 2–3 weeks to fill in the new growth of nail plate at the lunula. These types of services can be performed by sculpting either on a form or over a tip.

Colored polymer powders can create the illusion of a beautiful nail by using a combination of natural pink color over the nail bed and opaque white distally for the nail plate's free ledge. This combination perfectly simulates a natural nail and nail varnish need not be used. Various shades of pink powders are used to hide visible defects in the nail bed and plate.

### ALLERGIC REACTIONS

These may occur 2–4 months, and even as long as 16 months, after the first application (5). One of the first indications is an itch in the nail bed or redness of the soft tissue surrounding the nail plate. Paronychia, which is usually present in allergic reactions, can be associated with excruciating pain in the nail area, and sometimes with paresthesia. The nail bed is dry and thickened (Figure 31.3), and there is often onycholysis (Figure 31.4). The natural nail plate may appear thinner, usually due to overly aggressive filing techniques (Figure 31.5), split, and sometimes discolored. It takes several months for the nails to return to normal. Permanent nail loss (Figure 31.6) is exceptional, as is intractable prolonged paresthesia (6–8). Distant allergic contact dermatitis may affect the face and the eyelids (9), and is probably caused by touching the face with the hands. The arms and wrists of nail technicians may be affected if these areas are repeatedly exposed to filing dust. Filings may contain small amounts of monomer that has not yet reacted, since it takes 24–48 hours for the enhancement to cure fully.

Technicians should be instructed to wash their hands before touching the face or eye area. The area involved is usually the chin, where some technicians tend to rest their heads in their hands. They should also be warned to avoid skin contact with the dust of freshly applied product and to avoid using the product with too high a ratio of liquid to powder.



**Figure 31.3** Nail bed and hyponychium thickening from sculptured artificial nails.





**Figure 31.4** Onycholysis due to sculptured artificial nails.



**Figure 31.5** Thinning of the nail plate after use of sculptured artificial nails.



**Figure 31.6** Permanent loss of the nails due to sculptured artificial nails. (Courtesy of A Fisher.)

A medium dry consistency of the slurry is considered ideal. The filings from UV gel nails (discussed below) are also responsible for skin sensitizations.

The UV bulbs used to cure this type of artificial nail system lose about half of their original peak UV output after about 4–6 months, depending on usage. Newer LED-style UV nail lamps have been designed for use with UV gels specially formulated to cure with these more intense UV lamps. Curing can be up to 75% faster when LED-style UV nail lamps are utilized.

Technicians should be told to change the UV bulbs in their nail lamps three times per year, even though they will continue to emit visible blue light for many years. LED-style nail lamps are designed to continue to produce necessary levels of UV energy for 2–3 years, but then must be replaced since the diodes are soldered to a circuit board and can't be changed. Applying multiple thinner coats of products, rather than fewer and thicker coats, is preferred. The thicker the coating, the more difficult it is to cure the UV gel. UV penetration is low and the number of photons reaching beyond a depth of 2 mm is greatly reduced, and a complete cure cannot be assured if applied too thickly. Although sensitization to butylhydroxytoluene is possible, UV gels use acrylated oligomers and monomers. Acrylates are many times more likely to cause sensitization than methacrylates or stabilizers. These systems also rely on photoinitiators, such as benzoyldimethyl ketal or camphorquinone, (1%–3%) or benzophenone, which can all be sensitizing.

Gluteraldehyde and acrylic acid are also sometimes used in low levels (<1%), as keratin adhesion promoters and certainly contribute to adverse skin reactions. Since UV gels contain oligomers with high molecular weights, they tend to be very sticky and messy, which further increases the risks of overexposure.

### Patch Testing to Identify Reactions to Sculptured Artificial Nails

Patients who are allergic react strongly to the (meth)acrylate liquid monomer (10) (1%–5% monomer in petrolatum or olive oil). In the series of 11 patients of Koppula et al. (10), 0.1% ethyl acrylate in petrolatum detected 91% of the (meth)acrylate-allergic users of artificial nail. These authors proposed the following five chemicals be used as screens: ethyl acrylate, 2-hydroxy ethyl acrylate, ethylene glycol dimethacrylate, ethyl cyanoacrylate, and triethylene glycol diacrylate. The pattern of (meth)acrylate cross-reactivity among the most frequently positive (meth)acrylates suggests that a functional group that is a carboxyethyl side group may be requisite for allergic contact dermatitis to (meth)acrylates. Kanerva et al (11) reported that 6 out of 23 patients who were (meth)acrylate sensitive were sensitive to ethyl methacrylate which is consequently a significant allergen.

The powder contains ethyl methacrylate homopolymer or ethyl/methyl methacrylate copolymer, but also may contain small amounts of monomeric methyl methacrylate monomer and ethyl methacrylate (<0.1%) and up to 2% benzoyl peroxide. This explains why the powder may in some cases provoke an allergic patch test reaction (11–12). Benzophenone and other UV energy absorbers used in nail enhancement may produce eyelid dermatitis (13). UV absorbers are often used at low levels (<1%) to protect the coating from UV-related discoloration.

Since there are no real monomer-free (meth)acrylate resins, an adaptable nail prosthesis made of silicone rubber

is sometimes an alternative. This “thimble-shaped” finger cover takes nail polish well (14,15) (Figure 31.7). A further development is for UV gel nail systems to be used as prosthetic devices for toes (Dr Robert Spaulding, personal communication).

## NON ALLERGIC REACTIONS

With continued wear, the edges become loose. These must be filed or clipped and then rebuilt to prevent development of an environment prone to bacterial and, beneath the nail plate, *Candida* infection. This is a result of improper application and maintenance.

Failure to undergo filling every 2–3 weeks may result in creation of a lever arm that can predispose to traumatic onycholysis or damage to the natural nail. Older clients’ nails grow much more slowly and require regular maintenance less frequently.

Onycholysis (16) is more common with nail extensions that are too long (lever effect). It has also been said that the bond between the sculptured and the natural nail can be stronger than the adhesion between the nail plate and the nail bed. There is no evidence that occlusive prosthetic nail interferes with the nail’s normal vapor exchange. Irritant reactions to monomers are possible (4). These are manifested as a thickening of the nail bed’s keratin layer, which can sometimes cause the entire nail bed to thicken, with or without onycholysis. Still, without question, the overwhelming majority of cases result from physical trauma or abuse.

Damage to the natural nail is not unusual after 2–4 months of wear of a sculptured nail. If it becomes yellow or crumbly, this means that the product was applied and maintained incorrectly.

Higher-quality products which are properly prepared do not suffer from these problems. Therefore, instead of recommending wearing prosthetic nails for no more than 3 consecutive months with 1-month intervals before resuming application (17), the dermatologist should find a better-qualified

nail technician. It is probably unrealistic to assume that clients will remove their artificial nails for any period of time. The problem may not be the acrylic nail materials but rather the thinning of the nail due to overfilling with heavy abrasives. Acid-based primer (methacrylic acid) is a strong irritant, which may produce third-degree burns, so it is fortunate these are rarely used any longer. Acid-based primer is hazardous if one floods the soft, living tissue, neglects to clean up spills immediately, or ignores an individual complaining of burning. One must rinse the area immediately with water when the client says it is burning. Acid primer can permeate the plate and soak the nail bed if the nails are too thin. Soap or baking soda used with water are excellent neutralizers. If acid primer gets in the eyes, the eyes should be flushed with water for at least 15 minutes, making sure all traces of the chemical have been rinsed, then a poison control center should be called and emergency medical treatment sought. There is a general tendency to disregard manufacturers’ instructions and warnings, which causes the majority of disorders.

## Contact Urticaria

Butylhydroxytoluene has been reported as a cause of non-allergic contact urticaria (18).

## Removing Sculptured Artificial Nails and Nail Polish

Acetone is the primary solvent used to remove artificial nail coating of all types. Usually, 20 to 30 minutes is all that is required for methacrylate-based, liquid/powder systems. Most UV gels are extremely difficult to remove with solvent, so they are more frequently removed by filing with a coarse abrasive. The use of acetone-free nail polish remover on painted nails is not necessarily desirable. The alternative solvents, especially methyl ethyl ketone, have higher orders of toxicity and can damage the underlying polymer surface, requiring the nail coatings to be removed with greater frequency than would be typically performed. Acetone diluted with 10% water works well for removing colored polish without excessively drying the skin or damaging the underlying artificial nail coating. Reports have been published of severe and even fatal cases following ingestion or inhalation of acetonitrile-containing nail polish and acrylic nail removers, because the acetonitrile is metabolized into cyanide (19–22). Nitroethane poisoning from artificial fingernail remover has led to cyanosis and 39% methemoglobinemia (23). Due to toxicity concerns, acetonitrile and nitroethane are rarely used any longer for removal, if at all. Ethyl methacrylate monomer and polymer nails and the UV or photobonded variety may produce severe and prolonged paresthesia, even without associated allergic dermatitis (3), but this is relatively rare. Unfortunately, the product the patient was using could have contained methyl methacrylate monomer, since this ingredient is used in gray market products but not by mainstream manufacturers; it is often not disclosed in the ingredient listing.

## UV-Curing Gels (UV Gel Systems, Gel Nails, Light-Curing)

The word gel applies to the physical form of the product—not the product itself. Gels are made from oligomers, which are naturally thicker than monomers due to their much higher molecular weight. UV gel systems require no premixing of a powder component and either acrylates or methacrylate base (approximately 30% of the market, worldwide).



**Figure 31.7** Adaptable nail prosthesis made of silicone rubber.



Cyanoacrylate-based or so-called “no-light” gels are actually monomers that contain a thickening agent. They comprise 1% or less of the market worldwide and do not cure via UV energy nor do they undergo any photochemical reactions (11). These products cure via moisture and can be accelerated with internal or external applied catalyst, typically a tertiary amine. They have very little odor, which makes them popular in full-service beauty salons and spas seeking to create a relaxing environment. UV gels may also require the use of a nail primer. The thick UV gels are brushed on the nail and cured with via either UV energy. Visible light systems are impractical since the visible light lamps produce large quantities of heat and the photoinitiators produce an unattractive yellow discoloration of the nail coating.

UV energy-cured gels are what is used. If is not correct to refer to UV as light. “Light” is defined as electromagnetic radiation that is visible to the eye, e.g. wavelengths lower than 400 nm. UV is a higher wavelength (>400 nm) that is not visible to the eye; therefore UV is energy and not light. These UV gels often contain urethane (meth)acrylates and/or epoxy urethane (meth)acrylate oligomers and mono- and/or difunctional (meth)acrylate monomers for cross-linking and viscosity reduction, one or more photoinitiators, plasticizers, adhesion promoters, stabilizers, anti-yellowing agents, colorants, and a UVA energy source/unit that is typically referred to as a “UV nail lamp.” Many of the same types of ingredients used in two-part methacrylate systems are also found in UV gels, but oligomers are not used in two-part methacrylate systems.

Despite the fact that some products are marketed as not being “acrylics,” all two-part liquid/powder systems and UV gel systems are based on acrylic chemistry and use ingredients from the acrylic family of chemicals, which means they contain acrylic functional groups.

The UV gel remains in a semiliquid form until cured under a UV lamp. For traditional fluorescent-style UV lamps the cure time is 2–3 minutes per applied layer. For LED-style UV nail lamps, the cure time is 30–60 seconds. Due to significant difference in the UV wavelength and irradiance (intensity) produced by these different styles of UV nail lamps, UV gels are specially formulated to work with these very different energy sources. The molecular weight of the oligomeric resins as well as the ratio of monomers to oligomers helps determine the UV gel consistency. The chemical structure of each also plays an important role in determining the consistency. When the UV gel is exposed to energy of the appropriate wavelength (UVA) and the irradiance is sufficient, polymerization occurs, resulting in hardening of the UV gel. However, hardening occurs after only 50% of the oligomer and monomers polymerize, therefore hardening does not ensure the UV gels are properly cured. Proper cure occurs when polymerization exceeds 85%. Proper cure helps ensure the resultant polymers have sufficient durability to withstand the rigors that artificial nail coating must endure and also helps minimize exposure to uncured monomers and oligomers contained in fresh filings created when the nail coating is shaped with an abrasive file. UV gels do not require external catalysts and often do not require primers. Newer formulations also use very low levels of highly efficient photoinitiators and avoid reliance on methacrylic acid or glutaraldehyde for adhesion promotion.

Marketers may claim that some systems are visible light cure, but the photoinitiator used absorb and are chiefly activated by UV energy. Both fluorescent and LED-style nail lamps produce UV energy and visible light, but the visible wavelengths are not used to cure the coatings so it is misleading to refer to such systems as visible light curing.

No-light cyanoacrylate gels have a completely separate type of chemistry, utilizing a spray or a brush-on or dropper-applied activator, usually N,N-dimethyl-p-toluidine, which catalyzes a moisture-initiated cure. In curing these gels, a thermal initiator replaces the photoinitiator. UV nail lamps and primer are never used with cyanoacrylate gels.

Some individuals with distal fissure or men who bite their nails may want more attractive hands. This can be accomplished by liquid/powder systems, UV cured or no-light gel nailcoatings. Each can produce completely natural looking nails with a smooth hard finish that makes the nail more resistant to chewing. Liquid/powder systems are the preferred way to extend the length of the nail plate, but UV gels can also be used as tip overlays for individuals who want to extend their nail plate length. However, cyanoacrylate-based gels do not have the necessary strength or durability to extend beyond the tip of the fingernail. Some companies provide a thicker gel designed for building and sculpting nail extensions. Some UV gels may also be used as a “sealer” that can be applied over nail polish, making it more impervious to chipping, wearing, and fading. However, the difficulty in removing the sealer is a consideration when evaluating the usefulness of this technique.

### Colored UV Gels

Liked colored methacrylate powders, colored UV gels are sometimes recommended to persons who do not often change polish color, or to create nail art designs. They are noted for easy application, high shine, and durability. However if nail infection or onycholysis occurs, the permanently colored UV gel makes detection nearly impossible. Also, colored pigments and dyes usually block UV penetration, making them more difficult to cure completely.

### UV Gel Polish/Manicure

Newer types of nail coatings, often called UV gel polish or manicures, have become very popular. These types of nail coatings are used to coat the nail plate with a longer-lasting colored coating capable of camouflaging nail defects such as nail bed bruises or splint hemorrhages. UV gel polishes are unique in that they are designed to be removed and replaced after 2–3 weeks and then followed up with a fresh nail coating. Although they duplicate the look of nail polish/lacquer/enamel, these nail coatings are polymerized by UV energy (400–450 nm) to result in superior, scratch-resistant colored coatings with enhanced durability. However, UV curing makes these types of coatings more difficult to remove than traditional nail polish formulations that form film by solvent evaporation.

Because these are often more difficult to remove, improper removal can cause small, roughly round and symmetrical whitish spots, typically 2–5 mm, to appear on the nail plates surface after removal. When these are found on the surface of the nail plate immediately following removal of these types of coatings, forceable removal of residues of remaining nail coating is the most likely reason. The acetone remover is wrongly blamed for dehydrating the nail plate, but these areas are not a result of dehydration. The whitish spots appear following incorrect removal of any type of firmly adhering nail coatings. Although these areas sometimes resemble white superficial onychomycosis, they are not caused by any pathogenic organisms, but as result of surface damage created by overly aggressive removal techniques or when wearers forceably remove the nail coating by picking, peeling, or



chewing them from their own nail plates. When nail plate thinning is observed, excessive filing of the plate either before or after application of nail coatings is the likely cause.

Figure 31.8 shows several examples of white spots caused by improper removal. These scanning electron microscopy images show nail coating residues and surface damage magnified 186–3830 times. Examining the modes of failure indicates the damage is caused by prying and/or scraping off firmly adhered residual coatings. Often residual pieces of nail coating remains because of insufficient contact time with the solvent remover, which results in failure to sufficiently soften and loosen these firmly bonded areas. Typically 15–20 minutes is required for removal in acetone or other solvent removers. However, UV nail coating designed for removal every 2 weeks will become even more firmly bonded after 3 weeks or longer of wear. Longer wear times will require longer solvent soaking time, up to 25 minutes, as well as more gentle removal techniques to avoid nail surface damage.

Surface nail damage and the resulting visible white spots are avoidable when sufficient time is allowed to more completely soften the UV-cured nail coating and gentle removal techniques are practiced. Damage becomes more likely because the nail plates are softer and temporarily more susceptible to injury from removal implements used after soaking in acetone or other remover solvent blends. When wearing nail coatings of this type, users should be warned not to self-remove their nail coatings and to not allow their nails to be filed using overly aggressive techniques.

### Removing UV Gel Nail Coatings

Many gels slowly embrittle and degrade with continued UV exposure from tanning beds or excessive natural sunlight. Those with formulations susceptible to degradation must

usually be removed every 2–3 months. Better formulations are more stable and resistant to these types of continued UV exposure.

One good way to remove UV gels is to carefully file off the upper 75% of the coating with an abrasive file and remove the remainder with acetone or specially blended solvent removers.

Acetone will have no effect on some UV gels, depending on their composition and degree of curing. These types of UV formulation suffer serious disadvantages when compared to more advanced and easily removed UV gel formulations. There is no basis to the claims that these types of artificial nails are “safer” or healthier for the nail. In fact, they are no better for the nail than any other system. History has shown that nail technicians’ knowledge and skill are the primary determinants for successful application and avoiding nail damage or other adverse reactions.

### REMOVABLE UV GELS

The latest trend in UV-curable gels is to make products that can be removed easily. This is usually accomplished by either lowering the amount of cross-linking monomers, thereby lowering the cost link density of the artificial nail, or by using an insufficient amount of photoinitiator to lower the degree of curing and creating a partially cured artificial nail, or by softening the nail coating with an excess amount of plastizers or adding polymeric substances to make the nail coating more susceptible to solvents, or some combination of these. These types of UV gels generally require 30 minutes soaking in acetone to remove.

### ADVERSE REACTIONS

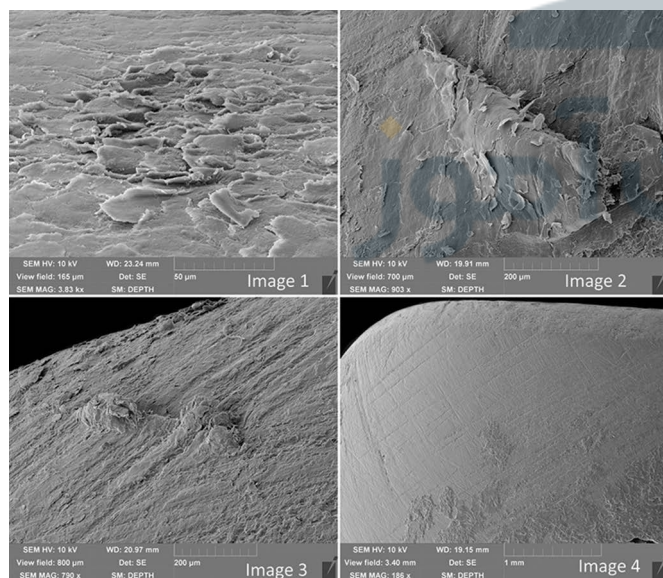
Artificial nails shrink when they polymerized. UV gel enhancements shrink up to 18%, which can result in adhesion loss and a tight feeling on the nail that causes throbbing, warmth below the nail plate. This may lead to tender, sore fingertips, but usually the condition resolves within a day or two. Adverse nail reactions, even with nail loss (24) and paresthesia with UV gels, have been observed (25).

UV-cured (meth)acrylates sensitize some individuals in many applications including inks, lacquers, composite dental resins, audiological ear molds, and nail cosmetics (26–30). In patients wearing UV-cured (meth)acrylic nails who had perionychial and subungual eczema with fingertips fissuring extending under the nails (31), Hemmer et al. (32) patch-tested “hypoallergenic” commercial products. The omission of irritant methacrylic acid primers from use with UV curable gels does not reduce the sensitizing potential of acrylate systems, since they still contain several other potential sensitizers. In contrast to the manufacturers’ declarations, all “hypoallergenic” products continue to include (meth)acrylate functional monomers and oligomers, and therefore continue to cause allergic sensitization.

UV gels and two-part liquid/powder systems (commonly called acrylic nails) share many similar ingredients but are compositionally distinct enough that they will not necessarily cross-react (11,24). The resistance of disposable latex gloves to penetration by (meth)acrylates is low (33,34). Disposable nitrile or vinyl gloves are a better alternative, as their resistance to methacrylates is much higher than latex.

### Preformed Artificial Nails

Plastic press-on nails are preformed by injection molding with the thermoplastic resin ABS and temporarily glued with



**Figure 31.8** Scanning electron microscopy images show nail coating residues and surface damage magnified 186–3830 times. Image 1 is clearly the result of upward prying forces. Images 2 and 3 are due to scraping forces caused by a wooden implement known as a “pusher.” Image 4 is a lower magnification that shows the residual coatings still firmly adhered to the nail plate after the solvent soaking removal procedure is completed.



**Figure 31.9** Preformed plastic "nail tips."

cianoacrylate to the nail (Figure 31.9). They are packaged in several shapes and sizes to conform to normal nail plate configurations. They come in two types:

1. Those that are adhered to the free edge of the nail plate to act as platform to support artificial nail coatings.
2. Those that are adhered over the entire nail plate as a temporary application for special occasions, such as weddings.

Decorative, preformed nails in gold plate (Figure 31.10) may be used in the same way as plastic nails. The application of preformed prosthetic nails is limited by the need for some normal nail to be present for attachment (Figure 31.11). When used alone (item 2 above) it is recommended that they are removed after 1–2 days as they are not very durable and can cause onycholysis and nail surface damage when improperly removed. Since these tips are adhered by using cyanoacrylate, in some cases they can produce allergic changes indistinguishable from dermatitis caused by so-called formaldehyde nail hardeners. Ectopic allergic or irritant contact dermatitis may affect the face and eyelids (35) and large areas of the trunk (36) but disappears with removal of the cause.

Allergic onychia and paronychia due to cyanoacrylate nail preparations require some comment (9,37). After about 3 months, painful paronychia, onychia, dystrophy, and discoloration of the nails may become apparent and last for several months (Figure 31.12).

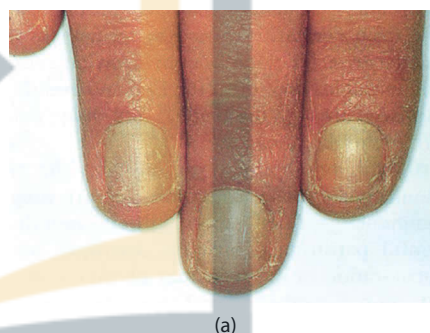
Eyelid dermatitis disappears with removal of the allergen. Shelley & Shelley (38) reported an isolated chronic allergic contact dermatitis simulating a plaque of parapsoriasis due to an allergic reaction to cyanoacrylate adhesive used on the fingernails.

Not surprisingly, patients react far more often on patch testing to the adhesive than to the plastic nails. Suggested allergens for patch testing are:

- *p*-*t*-butyl phenol resin (1% petrolatum) (39,40)
- Tricresyl ethyl phthalate (5% petrolatum)
- Cyanoacrylate adhesives (10% petrolatum)
- 5% methyl ethyl ketones
- MEHQ or HQ and
- The artificial nail itself



**Figure 31.10** Preformed nail in gold plate.



(a)



(b)



(c)

**Figure 31.11** (a–c) Different stages for shaping the tips of nails.





**Figure 31.12** Dystrophic nails with subungual hyperkeratosis due to preformed plastic nails. (Courtesy of P. Lazar.)

Most cyanoacrylate adhesive formulations contain HQ at concentration up to 1000 ppm, but more typically 200 ppm. Since higher levels of HQ are need to prevent premature polymerization of cyanoacrylate monomers used in artificial nail products, the European Union has been petitioned by several cosmetic associations to raise the allowable limit of HQ to 1000 ppm from a 200 ppm limit in artificial nail systems (after mixing if contained in a two-part system) (41). Therefore most investigators perform patch testing not only with cyanoacrylate glue or nail preparations, but also with HQ.

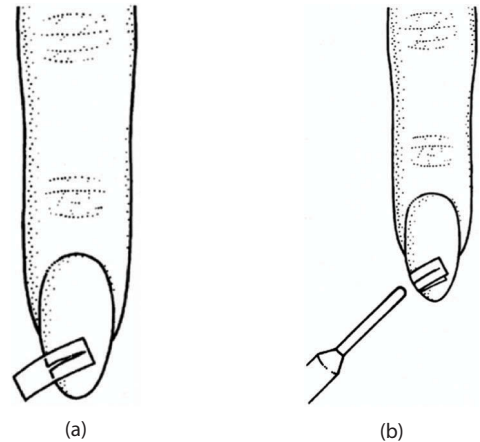
### Nail Mending and Wrapping

The purpose of nail mending is to create a splint for a partially fractured nail plate (Figure 31.13) or one longitudinal split extending the full length of the nail. The splint is first bonded with cyanoacrylate monomer. Then a piece of wrap fabric is cut and shaped to fit over the nail surface. This is then embedded within the cyanoacrylate monomer and several coats are applied; or the fabric is applied directly over the crack and subsequently sealed to the nail with cyanoacrylate monomer or no-light gel. In nail wrapping, or “wraps,” the free edge of the nail should be long enough to be splinted with paper, silk, linen, or fiberglass and fixed to the plate with cyanoacrylate monomer (Figure 31.14). The activator for cyanoacrylate wraps is a catalyst and contains N,N-dimethyl-p-toluidine in a solvent carrier. Methemoglobinemia with resultant cyanosis may follow its ingestion (22). DMPT is typically 0.5% of the formulation and HQ up to 1000 ppm. The ethyl acetate and trichloroethane in these products do not promote curing, but are instead solvents.

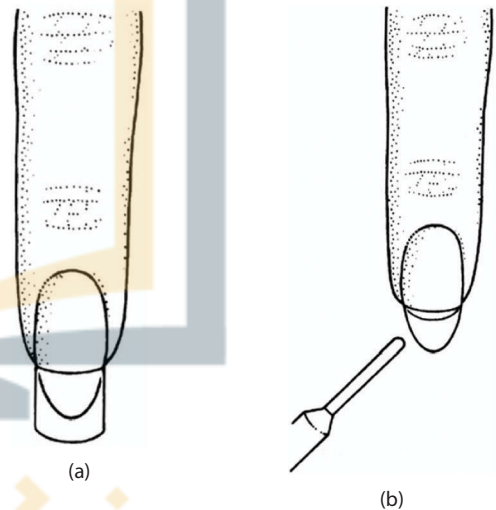
Paper is not very effective, but silk wraps are sheer, very thin, and work quite well. Linen is thicker and offers increased strength but inhibits cyanoacrylate penetration to the nail, thus lowering adhesion, and it does not have as natural an appearance as other materials. Fiberglass combines many benefits of both silk and linen and is the most universally used.

Most wrap systems consist of a few basic elements (42):

- Monomeric cyanoacrylate, polymerizing from moisture in the air or in the natural nail's surface, to form the hard nail coating that is both the base and top coats of the nail wrap. A mesh material, for example fiberglass or silk, is preferred.



**Figure 31.13** (a,b) Nail mending.



**Figure 31.14** (a,b) Nail wrapping.

- An activator or catalyst that cuts the hardening time to seconds.

See above for patch testing patients sensitized to cyanoacrylate.

### NAIL HARDENERS AND TREATMENTS

There are two main types of products which make nail hardening claims. In one group the products are modified nail polish containing, among ingredients, nylon fibers, (meth)acrylate resin, and hydrolyzed proteins. They function either as a base coat for nail polish or as a standalone treatment. These products provide a protective coating, therefore the implied benefits come from the added strength and durability of the coating itself rather than altering the physical properties of the nail plate. These products may also consist of polyesters and polyamides. These nail hardeners are essentially a modification of clear nail polish and the addition of nonfunctional ingredients for marketing purposes, that is, nylon fibers and amino acids or hydrolyzed protein.



Like nail varnish and base coats, they are applied to the clean nail plate. Some are designed with a dual purpose and also function as a varnish base coat (17).

The second type of hardener chemically alters the structure of the nail. The U.S. FDA allows these products to contain up to 5% formalin (International Nomenclature of Cosmetic Ingredients [INCI] name: methylene glycol) and kits are required to contain a skin shield to protect the eponychium and side walls from exposure (43).

The INCI dictionary no longer requires manufacturers to call formalin by the incorrect name formaldehyde. Formaldehyde is an anhydrous gas that upon mixing with water reacts to form a methylene glycol and residual traces of formaldehyde in equilibrium. Most products never exceed 1.5% methylene glycol since at higher concentrations both the nail plate and the surrounding tissue can quickly show signs of adverse changes. At these concentrations used products contain less than 12 ppm (0.0012%) free formaldehyde (44). Companies selling these products generally disregard requirements for skin shields, so accidental skin exposure can occur.

Most products never exceed 1.5% methylene glycol, since at higher concentration. Both the nail plate and the surrounding tissue can quickly show signs of adverse changes. At these concentrations products contain less than 12 ppm (0.0012%) free formaldehyde (44).

Companies selling these products generally disregard requirements for skin shields, so accidental skin exposure can occur. Using a concentration of less than 0.2% methylene glycol seems to have little or to no positive benefits on the surface hardness of the nail plate.

Methylene glycol permanently alters the structure of the nail plate by cross-linking the keratin (45), which can quickly lead to embrittlement since the cross-link density rises over time with continued regular use. Repeated use also allows a deeper migration into the plate, further affecting the bulk properties of the natural nail. Greater cross-link density increases the surface hardness of the nail plate, but it also lowers the flexibility while increasing the strength, resulting in an imbalance called brittleness (46). The property that people are unknowingly seeking is toughness. This occurs when there exists a favorable balance between strength and flexibility. Since the general public doesn't understand how or why these products work, the products are often applied to nails which are already overly brittle or rigid and therefore not suitable for further hardening. Even on nails which could benefit, these products are frequently misused. The preparations work so well on thin, weak nails that users see an almost instant improvement, which encourages repeated and frequently excessive use. After several weeks of success, the nails can eventually become overly hard and rigid. Continued use can cause splitting, cracking, and breaking which unaware users can misinterpret. This will often cause them to continue to use these products with even greater frequency, leading to the problems associated with overexposure to this ingredient.

Methylene glycol preparations may cause nail changes including a bluish discoloration (Figure 31.15), which may turn red, with intense throbbing (47). Resolving hemorrhages produce reddish-rust or yellow discoloration of the nail. Methyl glycol can also be responsible for paronychia, onycholysis (Figure 31.16), subungual hyperkeratosis, and dryness of the fingertips, but nail shedding is uncommon. Pterygium inversum (48) (Figure 31.17) has been observed, sometimes accompanied by severe pain necessitating systemic corticosteroid (43).



**Figure 31.15** Acute formaldehyde reaction. (Courtesy of P Lazar.)



**Figure 31.16** Longstanding onycholysis due to formaldehyde.



**Figure 31.17** Pterygium inversum due to formaldehyde.

Isolated onycholysis and ectopic contact dermatitis, even associated with hemorrhages of the lips in nail biters (49), have been reported. Airborne contact dermatitis of the face may also be seen.

Formalin (1%–2% in water) should be used for patch testing, but caution is necessary in interpreting the reactions, because the agent also acts as an irritant.

A new nail hardening ingredient has now been introduced which overcomes the objections related to formalin. The ingredient, dimethyl urea, is nonsensitizing and 2% concentrations in an basecoat preparation does not overcross-link the keratin. The higher molecule weight and relative increase in hydrophobicity prevent dimethyl urea from penetrating as deeply into the plate as methylene glycol. This effectively limits the cross-linking action to the surface of the plate, thereby dramatically reducing the potential for overhardening and embrittlement. In addition, the greater the cross-linking on the surface, the more restricted the dimethyl urea penetration will become, essentially creating a self-limiting cross-linking reaction while having the additional benefit of being non-sensitizing (50).

Other alternatives to formaldehyde hardeners are aluminium chloride (5% in water) tannin, and nail creams with a low water (30%) and high lipid content for minimizing nail fragility.

## Overall Risk

One study showed 9 out of 819 patients manifested a contact allergy to nail polish, while two persons reacted to artificial nails (51). The Cosmetic Ingredient Review Expert Panel reviewed 24 artificial nail enhancement methacrylate monomers used to create the two-part systems and declared all to be “safe as used,” while recognizing that it was important to avoid direct skin contact with the monomers to minimize the potential for skin sensitization.

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## Evaluating Hand and Body Lotions

F. Anthony Simion

### INTRODUCTION: LOTIONS AND THE SKIN

Skin, the largest organ of the body, plays a critical role as the interface between the human body and the environment. Originally it was thought mainly to have a passive role, being a physical barrier to environmental threats such as low humidity, biological pathogens, UV radiation, physical trauma, and environmental pollutants. It has always been recognized as playing a vital role as a sensory organ alerting us to the presence and nature of objects surrounding us, extremes of temperature, and noxious chemicals. More recently its active interactions with the environment have become better appreciated. It is physiologically active: the immunological response involves keratinocytes and Langerhans cells as well as the T and B-lymphocytes. Melanin in the skin protects it from UV damage. Upon exposure to light, melanin can be redistributed to the keratinocytes protecting the cells of the lower epidermis. The skin has significant levels of enzymes such as cytochrome P<sub>450</sub> that can metabolize xenobiotics. Unsurprisingly, it is most effective as a barrier if it is intact. Hand and body lotions play a vital role in helping to maintain the integrity and plasticity of the SC in the face of many outside threats. They have the ability to prevent or reduce dryness and the impact of many irritants (1,2) as well as improving the skin feel and consumers' quality of life (QoL).

Dermatologists continue to see many patients suffering from dryness and itching often associated with visible scaling and flaking of their skin. Many more consumers treat themselves without medical intervention. There are many causes for the increasing prevalence of dry skin. Shifting demographics, specifically the aging population in combination with increased usage of low-humidity central heating and air conditioning systems has significantly contributed to the increase in dry skin complaints. The prevalence of atopic eczema is increasing in children although the reasons are not clear. Additionally, skin is exposed to household detergents and personal cleansers, which can quickly damage the SC proteins, extract small hygroscopic molecules, and deplete or rearrange the bilayer structure of key intercellular lipids. This causes a dramatic decrease in the skin's ability to act as a barrier. Detergent-induced dryness and primary irritation has made dermatitis one of the most common forms of occupational disease in developed countries. Health care workers, hairdressers, nurses, and food handlers are especially at risk (3,4).

During the last 100 years hand and body moisturizers have been used to provide relief to dry skin sufferers by increasing the skin's softness and smoothness (plasticity) while eliminating skin scaling. There are several mechanisms to achieve this. They include increasing the skin's water content by forming an occlusive barrier, by binding more water more effectively (humectancy), and by supplementing the water

with other plasticizing materials. Indeed individual moisturizing ingredients may deliver benefits to the skin by several mechanisms. For instance glycerin works as a humectant, a fluidizer of the lipid bilayer, and an activator of SC enzymes. It also is a skin softening agent (emollient). Today's moisturizers are far superior to those of the past. This is due to new materials that are capable of mimicking the skin's biological moisture holding mechanisms and the ability to deliver these materials effectively to the skin. Ingredients such as ceramide analogs, pro-vitamins, and essential fatty acids have been successfully incorporated into hand and body lotions. When moisturizers are used regularly, they help to mitigate dry skin. Furthermore, advances in formulating have produced aesthetically superior products that consumers enjoy using, yet maintain their efficacy. The leading hand and body lotion brands in the United States are shown in Table 32.1.

A second role for hand and body moisturizers is as a vehicle for functional and imagery ingredients including over-the-counter (OTC) drug actives such as sunscreens and anti-puritics as well as cosmetic ingredients such as dihydroxyacetone (DHA), alpha-hydroxy acids (AHAs), and a multitude of botanical extracts. Sunscreens are a good example. As a result of increased awareness regarding the negative effects of the sun, recent marketplace trends show an increase in the number of hand (and facial) moisturizers that have the ability to block UVA and UVB exposure. Hand and body moisturizers serve as an effective and convenient vehicle for the delivery and application of sunscreen ingredients. Optimization of the vehicle not only provides consumers with much preferred tactile benefits such as a non-sticky, non-greasy, and non-oily skin feeling—attributes which encourage usage (i.e. compliance)—but can impact the efficacy of the sunscreen molecule to absorb UV light (5).

Sunless tanners are lotions containing DHA and/or erythritol. These ingredients react with amino acids and small peptides in the skin via the Milliard reaction to provide a brown-yellow color reminiscent of a suntan. Nguyen and Kochevar have shown that the water content of the skin greatly affects the rate at which color develops (6), and the balance between DHA and erythritol impacts how natural the color appears to the consumer. In this way, consumers can appear to have tanned skin, which is aesthetically desired in many Western countries, but without sun exposure that can lead to skin damage and possibly eventually skin cancer. Additionally, the reaction products are strong UV-A absorbers that can give the skin some protection.

The efficacy and aesthetics of hand and body lotions depend on several factors. Gross chemical composition is not the only factor that effects both efficacy and skin feel. Most lotions are emulsions, heterogeneous mixtures of oil- and

**Table 32.1** Leading Hand and Body Lotions in the United States

Brand*	Manufacturer	Emulsion Type	Key Moisturizing Ingredients
Jergens	Kao USA Cincinnati, OH	Oil in water	Glycerin, cetearyl alcohol, petrolatum stearate
Vaseline Intensive Care	Chesebrough–Ponds (Unilever) Tumbrall, CT	Oil in water	Glycerin, sunflower seed oil, C11-13 paraffin, petrolatum TEA stearate
Aveeno	Johnson&Johnson Los Angeles, CA	Oil in water	Glycerin, cationic surfactants, petrolatum
Eucerin	Beiersdorf Norwalk, CT	Water in oil	Petrolatum, mineral oil, lanolin alcohol, hydrogenated castor oil
Curel	Kao USA Cincinnati, OH	Oil in water	Glycerin, cationic surfactants, petrolatum
Gold Bond	Chattem Chattanooga, TN	Oil in water	Glycerin, cationic surfactants, petrolatum
Nivea	Beiersdorf Norwalk, CT	Oil in water	Mineral oil, glycerin, isopropyl palmitate, glyceryl stearate SE, cetearyl alcohol
Jergens Natural Glow	Kao USA Cincinnati, OH	Oil in water	Glycerin, zeamays (corn) starch, cetearyl alcohol, dihydroxyacetone, erythritol, mineral oil, petrolatum
Cetaphil	Gelderma Laboratories Fort Worth, TX	Oil in water	Glycerin, hydrogenated polyisobutene, cetearyl alcohol, cetareth 20

\*Several brands have many variants—the “key ingredients” are a composite between variants.

water-soluble materials in aqueous solution. How ingredients are distributed between the oil and aqueous phases plays a significant role in how they are delivered to and partition into the skin to have their moisturizing and possibly physiological effects, as well as their impact on skin feel during and after application.

Although many skin characteristics are genetically determined, the environment also has significant effects. Negative effects from both sources can be ameliorated by regular use of moisturizers. The regular use of moisturizers, a healthy diet, protection from the sun, and regular exercise will contribute to significantly healthier, younger looking skin.

## INGREDIENTS FOR HAND AND BODY LOTIONS

As anyone who has looked at the ingredient statement for a typical hand and body lotion can attest, the number and variety of ingredients used in these products can be somewhat intimidating. Consequently, it is clear that a full review of all of these ingredients is beyond the scope of this chapter. However, this chapter covers the major categories of ingredients, organized according to their putative function in the product.

Before we begin the review of specific ingredients, a few general comments about ingredient disclosure may be helpful. In many countries including the United States and those of the European Union (EU), manufacturers of cosmetic products are required to provide a full disclosure of the ingredients used in their products. This requirement was established to allow consumers to monitor ingredients and to make an informed purchase. Package labeling guidelines require listing ingredients in descending order of weight percent. One exception is for ingredients used at levels of 1% or less. In the United States, such minor ingredients can be listed in any order. Since many of the ingredients used in hand and body lotions are complex or ill-defined chemical entities, a standard nomenclature has

been developed by the Personal Care Products Council, (PCPC; formerly the Cosmetics, Toiletries, and Fragrance Association). Under the PCPC guidelines, manufacturers are obliged to use the assigned International Cosmetic Ingredient (INCI) name for all ingredients used in their products. Information about these ingredients, manufacturers, and chemical structures are compiled in the PCPC INCI Dictionary, which is a highly recommended resource for cosmetic formulators. The European Union follows a similar system, although there are a few differences. For example, there are some fragrance ingredients that must be listed if their concentration exceeds 10 parts per million (ppm) in a leave on-product (7). Additionally, the manufacturer is only free to follow their preference for ingredient order on the Ingredient Statement below 1% concentration.

## Ingredient Classes

### Water

This ingredient can be found at the beginning of many ingredient statements for hand and body lotions since it can make up 70% or more of the formula. Water has an important function in a hand and body lotion—it is the vehicle by which many other ingredients are delivered to the skin. However it should not be viewed as an active ingredient, as any hydration it produces is short-lived, usually less than 15 minutes, before evaporating. Although the PCPC does not recognize the use of any particular type of water (such as “purified water”) for the purpose of ingredient labeling, most manufacturers do use treated water. They use water that is softened, or deionized (demineralized) in their products to avoid any interactions between calcium, magnesium, and iron ions with components in the formula. It is also customary in the industry to process the deionized water to remove problematic microorganisms.

Since the water concentration is transient after application and quickly evaporates, water-soluble materials become more concentrated. As a result, minor components of the

formula may penetrate more easily, a fact that is sometimes forgotten when determining the benefits and risks of these ingredients.

### *Emollients*

An emollient can be defined as an ingredient that softens the SC. Emollients used in hand and body lotions are frequently oily materials that help plasticize dry skin either by direct interaction with the SC or by providing an occlusive barrier that traps water from the underlying skin strata. However, glycerin and other polyols have a similar skin softening effect, although they do not fulfill either characteristic. The plasticized SC exhibits more flexibility and a surface that is more uniform which consumers perceive as smooth and soft. Also the skin surface may be less likely to crack, especially in body areas that are constantly flexed, such as knuckles, thereby reducing possible penetration of irritants and allergens.

Lanolin was one of the first commercial emollients used widely by the industry. It was a prominent ingredient in apothecaries from the 1920s to the early 1960s, where pharmacists used lanolin as a base for compounding ointments. It has the unique ability to adsorb up to 30% of its weight in water. Lanolin is a refined portion (deodorized and bleached) of raw wool wax—the fatty, waxy residue separated from the washings of sheep's wool, yielding a complex mixture of semisolid oils, fats, and waxes. As an emollient, lanolin provides a strong occlusive effect when applied to skin and may also directly plasticize the SC. Lanolin, its constituents and derivatives are still used in some hand and body lotions, but have largely been replaced by other emollients because of its reported sensitization potential. These adverse reactions may be due to the presence of low levels of alkane- $\alpha$ ,  $\beta$ -diols, and alkane- $\alpha$ , $\omega$ -diols (8). Pesticide residues are an additional potential issue for lanolin, and the United States Pharmacopoeia monograph specified limits for organic pesticides (9). This change in the monograph has forced manufacturers to commercialize a higher purity of an old emollient.

Two of most common emollients used today are mineral oil and petrolatum, which are hydrocarbon-based materials derived from petroleum. Petrolatum, in particular, resembles lanolin in its physical characteristics and its occlusive effect on the skin. In addition to forming an occlusive barrier, Ghadially et al. (10) showed that petrolatum penetrates into the intercellular lipids of the SC. Kligman (11) reported that petrolatum was very effective at reducing observable skin dryness and preventing its reappearance. Compositionally, the only difference between mineral oil and petrolatum is the number of carbons in the hydrocarbon chains, with mineral oil having fewer carbons. Mineral oil is less occlusive but has better spreading properties and is generally believed to have a less greasy feel on the skin compared with petrolatum.

Silicones are a class of synthetic polymers that are also widely used in hand and body lotions. Most silicones are used for their good spreading and de-tackifying properties as well as their emolliency. They also form good barriers to environmental agents and often are the principal active ingredient in skin protection OTC drug products. Common examples are dimethicone and cyclopentasiloxane. However they have recently come under additional scrutiny in the European Union for their human and environmental safety profiles (12,13).

Another class of emollients is triglycerides, which are derived from animal fats or vegetable oils. These oils are usually named according to the original source, such as sunflower seed oil. Triglycerides (oils) contain approximately 5% free

fatty acids. Many triglycerides contain essential fatty acids (EFAs). EFAs are unsaturated fatty acids that cannot be synthesized by the body yet are required for maintaining the SC barrier function. Experiments have shown that when EFAs are withheld from rats' diets, their skin becomes scaly and water barrier function is compromised (14). Healthy skin can be restored by topical application of triglycerides that are rich in EFAs. Similar results were observed in human patients whose lipid intake had been reduced due to stomach surgery (15).

Processing of triglycerides is a major industry. Triglycerides as neutral fats and oils are hydrolyzed to form fatty acids, fatty alcohols, and a predominant raw material in the manufacturing of soap. The hydrolysis produces monoglycerides and diglycerides, which are also used in emulsion products as a secondary agent for stabilizing the suspension.

The use of unsaturated triglycerides in a hand and body lotion can create problems due to the susceptibility of these oils to oxidation, producing discoloration and off odors (rancidity) over time. To prevent this oxidation, stabilizers such as antioxidants, e.g., tocopherol (vitamin E), BHA, or BHT, and chelators, e.g., EDTA salts, are often added to the formula.

Triglycerides may be processed to provide a large variety of other emollient ingredients. Glycerides are used not only for emolliency, but also for their ability to stabilize the lotion by emulsification. Examples of glycerides are glyceryl stearate (monoglyceride), glyceryl dilaurate (diglyceride), and palm oil glyceride (mixed glycerides). Fatty acids are also processed to produce fatty esters. Numerous types of fatty esters are used in hand and body lotions. Some common examples are isopropyl myristate, isopropyl palmitate, octyl hydroxystearate, and cetyl palmitate.

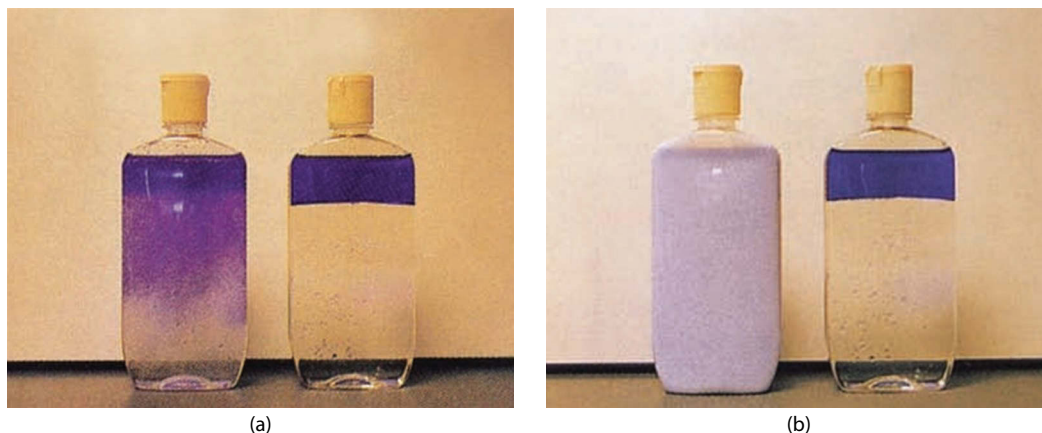
### *Humectants*

Humectants are water-soluble organic compounds, typically polyhydric alcohols, which have an affinity for water. The most common humectant is glycerin; others include sorbitol, propylene glycol, dipropylene glycol, and butylene glycol. Once it was believed that these ingredients helped to moisturize the skin simply by binding additional water in the SC, thus plasticizing the SC and reducing the rate of evaporation. However within the last 20 years, two additional mechanisms by which glycerin may moisturize have been proposed. Froebe, Matti, and their colleagues showed that glycerin at low humidity can prevent the conversion of liquid crystals in the intercellular lipids to a solid, rigid crystal within the SC (16,17). The solid crystal is more readily removed by environmental factors, e.g., cleansing, and provides less resistance to water loss. This effect may be responsible for the improvement in barrier function observed when glycerin (and possibly other humectants) is applied to the skin. Additionally, Rawlings et al. showed that glycerin is able to promote normal desquamation by enhancing the activity of the proteases that degrade desmosomes (18). It is likely that all three mechanisms occur in the SC simultaneously.

### *Emulsifiers*

Classically, these are surface-active ingredients, which are used to promote temporary mixing, and suspension of the discontinuous phase (often oils) into the continuous phase (usually water). This will significantly slow the rate at which the two phases separate (Figure 32.1). Conventional emulsifiers are often classified on the inherent charge associated with the surface-active ingredient. The classes are anionic (negative charge), cationic (positive charge), and nonionic (no charge/neutral).





**Figure 32.1** (a) Emulsifiers reduce the rate at which the oil and aqueous phases separate. The left-hand bottle contains an emulsifier with the oil and aqueous phases and the right-hand bottle contains no emulsifier. After mixing, the system on the left separates more slowly. (b) Emulsions are opaque systems (left) compared with an unemulsified two-phase oil/water system (right).

The anionic emulsifiers were widely used for many commercial moisturizers. Most early oil-in-water (o/w) emulsions utilized a soap (anionic) formed in situ by neutralization of a fatty acid with a metallic hydroxide, an amine, or basic amino acid. The common material used as a soap emulsifier is neutralized stearic acid. The anionic emulsifiers especially with chain lengths around  $C_{12}$  could be somewhat irritating so their level of use should be balanced to produce a stable product that is proven to be nonirritating to skin.

Nonionic emulsifiers are probably the most commonly form used today. They produce stable and non-irritating emulsions or suspensions. The cosmetic formulator again will use these materials judiciously to maintain the desired properties without adding excess amounts of the surface-active agents. Since these emulsifiers are surfactants, they have the potential to be irritating to the skin and any excess adds more cost to the formula. Common nonionic emulsifiers are ethoxylated or propoxylated fatty alcohols, such as cetareth-20 or steareth-2.

The third class, cationic emulsifiers, has become more widely used for moisturizers that are more therapeutic or long-lasting. The cationic products are substantive to the stratum corneum (SC) as it is negatively charged from the fatty acids and acidic amino acid residues. Hence a positively charged emulsion droplet is attracted to the skin, especially when it is damaged, and the pH increases from its usual 4.5 to 5.5. These types of emulsions resist wash-off with water and possess different aesthetics. Common cationic emulsifiers are the long-chain fatty alcohols bonded to quaternary nitrogen, such as distearyldimonium chloride or behentrimonium chloride.

Recent advances in polymer science have now created a quasi-fourth class of emulsifiers. The new emulsifier is based on a hydrophobically-modified polymer based on poly(2-acrylamido-2-methylpropanesulfonate) (AMPS). It is an amphipathic and when a nonionic emulsifier is added the emulsion yields a stable o/w emulsion. Most suppliers of polymers for personal care products now have variations of the AMPS copolymers within their product line (19).

#### *Water-Soluble Polymers (Thickeners and Conditioners)*

Common additives to emulsion products are water-soluble polymers. The polymers can be synthetic or natural. The most

common class is the acrylates. These polymers are synthetic with an anionic charge. Some common examples are acrylates/C10-30 alkyl acrylates cross-polymer and carbomers. Acrylic acid polymers often require neutralization with a base in order to realize their benefit in emulsion products. The base neutralizes the pendant acid chains allowing them to “unfold,” creating spoke-like projections which form an intricate network. The network builds viscosity and resists separation of the emulsion phases.

Many water-soluble polymers are derivatives of cellulose. The cellulosic polymers possess an anionic or nonionic charge and numerous grades with various properties are commercially available. The commercial grades vary in molecular weight, ether linkages and electrolyte tolerance. Examples of common cellulose are sodium carboxymethyl cellulose, hydroxypropyl methylcellulose, and hydroxyethylcellulose.

There are also cationic water-soluble polymers both synthetic and natural. They are derivatives of cellulose, polysaccharides, and modified acrylic acids. These polymers are usually restricted to cationic and nonionic emulsions due to the inherent instability that arises when added to anionic systems. Polyquaternium-10, chitosan, and derivatized guar gum are three such polymers frequently found in hand and body lotions.

The water-soluble polymers enhance emulsion stability by preventing the small, suspended droplets from fusing together. The polymer network sterically restricts the movement of the dispersed phase and enhances the formula's apparent viscosity.

#### *Minor Components*

##### *Preservatives and Fragrances*

Although they make up a very small percentage of the total formula, these ingredients are of concern to dermatologists as they are the predominant cause of the few sensitization reactions from lotions that require medical attention (20). Indeed the mixture of fragrances used in patch testing has one of the highest incidence rates observed for cosmetic ingredients. Consumers' recognition of the association between fragrance and adverse reactions has prompted many manufacturers to develop unscented products. Their increasing popularity has

led some manufacturers to include various botanical extracts which technically are not fragrances, yet still include many of the same chemicals found in certain fragrances. The European Union requires common fragrance ingredients that are believed to be allergens to be declared in the ingredient statement when exceeding minimum thresholds are established. This is 10 ppm for leave-on and 100 ppm for wash-off products. Additionally, three fragrance ingredients (HICC; Lyrall®, atranol, or chloroatranol) may be banned from cosmetics in the European Union in the near future (21). Additionally, the level of almost 20 other ingredients and extracts may be restricted.

Preservatives are included in hand and body lotions to prevent the growth of microorganisms, specifically bacteria, mold, and yeast. These are necessary for several reasons. First, most hand and body lotions contain a high percentage of water, which makes them a good growth media for these microorganisms. Inadvertent contamination by consumers during use can lead to rapid bacterial growth and spoilage of the product. Further, if the growth of opportunistic pathogenic bacteria such as *Pseudomonas* is not prevented, infection or blindness could result when applied to compromised skin or near the eye, respectively.

Given the need to prevent growth of microorganisms in their products, manufacturers use a variety of biocide and biostatic agents together in sufficient concentrations to provide a product that does not allow for active growth. In other words, the preservative system is designed and tested to withstand multiple contaminations by consumers so that any microorganisms introduced into the product are either killed or prevented from actively multiplying. These agents are most often used in various combinations to provide effective preservation against a broad spectrum of bacteria and mold.

The preservatives are also an ingredient class which receives a significant amount of consumer concern. In today's environment, misinformation can often be represented as scientific data. The majority of consumers do not have the training to scrutinize data and determine its scientific validity but they do understand fear and wish to avoid harm to themselves and their loved ones. This has been the situation with the common biocides in personal care products, especially parabens. With consumer access to the internet, many materials can be erroneously classified as harmful. Our challenge as an industry is to counter and correct consumer misperceptions. To help in this process the PCPC has launched a website ([www.Cosmeticsinfo.org](http://www.Cosmeticsinfo.org)) that provides accurate information on the safety of cosmetic ingredients.

The following is a partial listing of some of the common biocides used in hand and body lotions.

Preservatives that work by releasing formaldehyde (formaldehyde donors):

- DMDM hydantoin
- Diazolidinyl urea
- Imidazolidinyl urea
- Quaternium-15 (soon to be banned in the European Union)

Preservatives that work by mechanisms other than releasing formaldehyde:

- Parabens (esters of p-hydroxybenzoic acid)—only the straight-chained (not the iso-) parabens are permitted in the European Union.
- Methyldibromo glutaronitrile (not permitted in the European Union).
- Benzyl alcohol.

- Benzoic acid/sodium benzoate.
- Methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) (Kathon™ CG; not permitted in the European Union in leave-on cosmetics).
- MI (probably will not be permitted in European Union leave-on skin care products in the near future).
- Ethanol.
- Phenoxyethanol.
- Iodopropynyl butylcarbamate (should be avoided in products that are readily inhaled).
- Chlorphenesin.
- Salicylic acid.

#### Skin Care Additives

Many lotions have cosmetic materials added at low levels for marketing purposes, i.e. to persuade consumers of additional benefits from these ingredients and to buy the product. In these cases the moisturization benefits that consumers require are delivered by the overall product. However in the last few years, additives have been identified that improve the appearance and feel of the skin over the vehicle alone. These include AHAs, retinol, and ceramide analogs (22,23). Imokawa et al. showed that ceramide analogs increase the water holding capacity and barrier function, especially of damaged skin. Addition of 3% pseudoceramides has been shown to reduce erythema in atopic dermatitis lesions (24). Stiller et al. showed that 8% lactic or glycolic acids reduce the signs of photodamage compared with a vehicle after 22 weeks of treatment. However these levels of AHAs have also been shown to increase the skin's sensitivity to the sun (25).

### ASSESSING MOISTURIZER EFFICACY

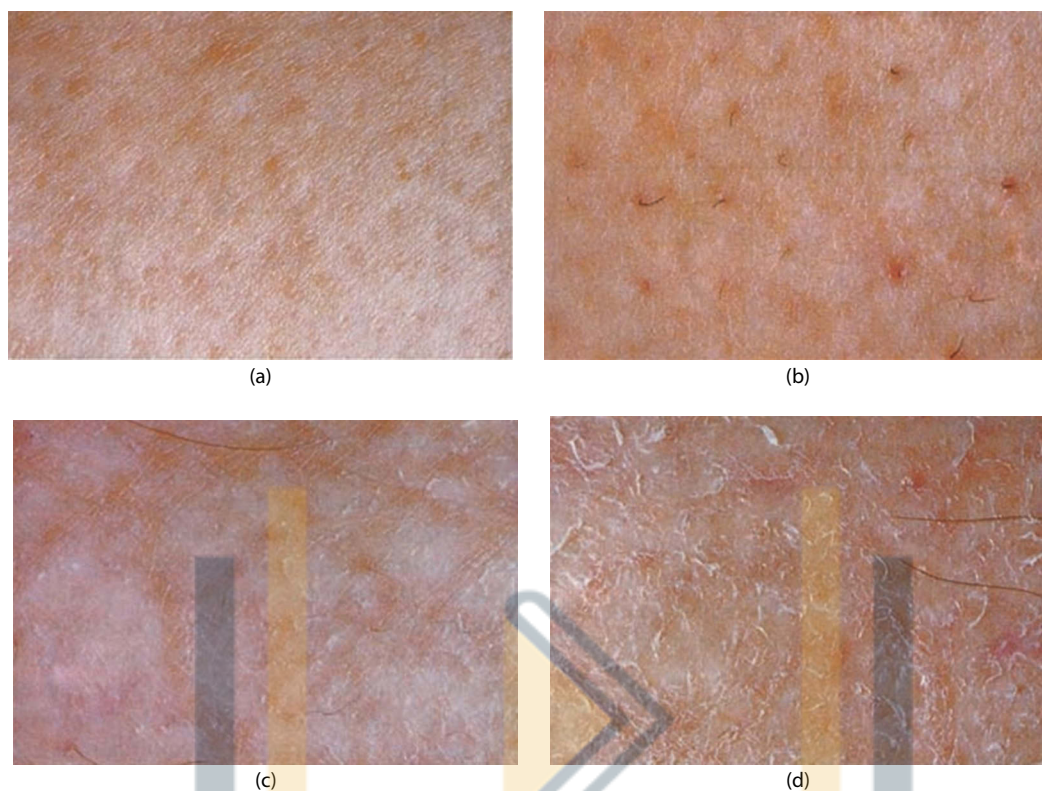
The primary use of hand and body moisturizers is to alleviate skin dryness and irritation and prevent their return. Measuring these effects both from a consumer and an objective perspective are primary objectives for assessing a moisturizer's efficacy. Clinical methods with controlled application have been developed that assess dry skin and irritation or their absence by visual scoring by a trained observer, using biophysical measurements of the skin and by panelists' self-assessments. However, clinical efficacy alone is not sufficient to make a product commercially successful. To appeal to consumers, the lotion must be both efficacious and aesthetically pleasing, i.e. pleasantly scented (or unscented) and have acceptable tactile characteristics during and immediately after application.

#### Clinical Evaluation of Moisturizer Efficacy

##### Alleviating Dry Skin

Clinical evaluation is an important component in the measurement of moisturizer efficacy. The visual assessment of skin dryness is a direct link to the perceivable benefits of "moisturization" that consumers readily recognize such as skin flaking and scaling, fine dry lines, rough texture, ashiness, and skin cracking. Figure 32.2 shows the different levels of skin dryness observed in clinical studies. In many studies, visual assessments are supplemented with instrumental measures of skin hydration, surface topography and/or elasticity. These instrumental measurements are more easily standardized than observer assessments and each provides an objective assessment of a particular cosmetic benefit.

There are a variety of methods used to assess the ability of moisturizers to alleviate dry skin. These include the single-application test and the regression test developed by Kligman



**Figure 32.2** Different levels of skin dryness observed in a moisturization study, utilizing a 0 to 4 scoring scale: (a) Grade 0; (b) Grade 1; (c) Grade 2; (d) Grade 4.

(11) and later modified by others (see below). Typically these studies start with dry skin, frequently on the legs, and then the skin is treated. In the single-application test, the condition of the skin can be reassessed as little as 2 hours after product application and at subsequent time points, some even beyond 24 hours. It is important to include time points of 2 hours or greater post-application, as many lotions contain water that rapidly evaporates. This is usually completed within 2 hours. In these studies, moisturization is frequently assessed by electrical and imaging measurements as well as observer scoring.

The regression test was developed by Kligman to assess the effects of both products and their constituents on dry skin. Test material ( $2 \text{ mg/cm}^2$ ) is applied to the lower legs of 12 to 30 female panelists twice daily for up to 3 weeks (see Figure 32.3). Visual dryness was assessed prior to treatment (baseline) and at the end of each week. Panelists started with dry skin and the improvement in dryness from baseline was the measure of moisturization efficacy, or the relief of dryness.

Using the regression test, Kligman showed that hydrophobic oils, such as mineral oil or olive oil, alone had little ability to alleviate dry skin. The efficacy of these oils was enhanced when they were formulated with hydrophilic materials into cold creams. Kligman's data suggested that the moisturizer's composition could have a greater influence on its efficacy than the number of applications (dosage). He demonstrated a large range in the ability of ingredients to alleviate dryness, but increasing the dosage had limited effects, especially beyond four applications a day.

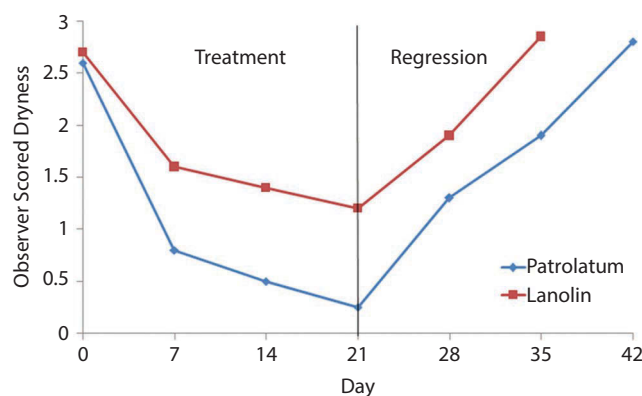
The Kligman regression protocol has been modified by several groups to meet different assessment needs. Boisits et al. (1989) (26) applied more rigorous conditions to reduce the experimental variability and increase test sensitivity. The most notable modifications include:

1. Conducting the test only if the temperature and humidity were below  $45^\circ\text{F}$  and 40%, respectively.
2. Throughout the study, washing test sites with a true (fatty acid-based) soap prior to lotion application to increase the propensity of the skin to dry out.
3. Requiring leg shaving no more than two times a week and no later than 30 hours before an observation.

Biosits et al. claimed the refined methodology allows for better differentiation between products.

Other modifications can be grouped together as "mini-regression methods." Prall et al. (27) reported a regression study using a 4-day treatment followed by a 6-day regression phase. The protocol was similar to Kligman's method as the applications were conducted twice daily with a dose of approximately  $2 \text{ mg/cm}^2$ . Product efficacy differences were determined during regression in the following descending order of efficacy: 5% lactic acid lotion > o/w lotion > placebo. Grove et al. (28) utilized the same mini-regression time frame to examine the efficacy of marketed moisturizers. Clear differences between each of the two products and between the treated sites and the untreated site were observed. In this study skin conductance was used to confirm the observer scored dryness.





**Figure 32.3** Relative efficacies of petrolatum and lanolin reducing dry skin and preventing its return in the regression test. The regression test shows that under similar conditions and dosage, petrolatum is more effective at alleviating dry skin and preventing its return. Test material was applied to the lower leg daily for 3 weeks (treatment phase). After treatment stopped, the legs were observed until the skin condition returned to its original level of dryness (regression phase). (Adapted from Kligman AM, *Cosmet Toilet*; 93:27–35, 1978.)

In a similar study of moisturizers containing glycerin, Appa et al. (29) utilized a 7-day treatment period followed by a 7-day regression. These investigators demonstrated that the moisturizer efficacy increased with the concentration of glycerin. A plateau was reached with a 25% glycerin lotion being similar in efficacy to a 40% glycerin cream. These results could also relate to the ability of the products to deliver glycerin into the skin, or to the effect of ancillary ingredients.

A third adaptation of the regression test utilizes the lower arms, either the volar or dorsal aspect. Prall et al. (27) used a mini-regression design to compare the performance of two lotions on the legs using a controlled lotion dosage to performance on the outer aspect of the arms using ad-libitum dosage. They concluded that either method accurately predicted the directional efficacy of the lotions. Grove (20) originally used the mini-regression methodology to examine lotion efficacy on the volar forearm using the instrumental method of skin conductance to evaluate performance.

#### Preventing the Return of Dry Skin

Effective moisturizers are able to prevent the return of dry skin even after product applications have stopped. The residual effect is important to consumers who wish to maintain their skin's good condition until the next convenient application time, even though it is still exposed to the stresses that caused the dryness in the first place. Both the single application test and the regression test are able to measure this. When an effective product has moisturized the skin, the decay of the benefits can be measured after application ceases. A slow return to baseline is indicative of an efficacious product with lasting effects. Figure 32.3 shows the data obtained by Kligman for two cosmetic moisturizing ingredients, petrolatum and lanolin. The data clearly demonstrate product efficacy during the treatment and regression phases. During the regression, persistent moisturizing effects are demonstrated 21 days after the last treatment with petrolatum but only 2 weeks for lanolin.

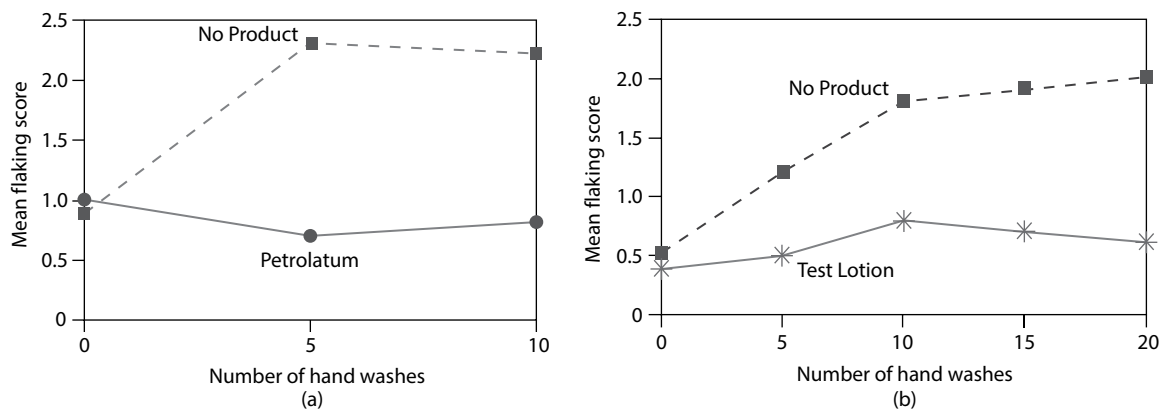
A more rapid approach to measure the prevention of dry skin was developed by Highley et al. (30). In the High-

ley Hand Wash protocol, the analysis begins with non-dry, healthy skin. The panelists wash their hands with bar soap for 1 minute five times a day over a 4-day period. One hand remains otherwise untreated, while lotion is applied to the other hand after the first four washes of the day. The condition of the skin is assessed by a trained observer and by instrumental measurements before the first wash of the study (baseline) and approximately 1 hour after the last (fifth) wash each day. Results show that ingredients such as petrolatum and effective commercial lotions can prevent the induction of dry skin, which can be considerable on the untreated hand (see Figure 32.4 and Table 32.2). Although panels as small as five were used by Highley, it is more usual to use panels of 15 or more to enable the data to be statistically analyzed. Recently the test has been modified so only a defined area of each hand is treated with lotion. The surrounding area acts as that hand's "no product" control. This enables more than one product or ingredient to be evaluated at a time, using a within-subject design.

#### Reducing Primary Irritation

An adverse environment or working conditions may drive the skin beyond dryness (xerosis) into the realm of primary irritation and dermatitis. In recent years many scientists and dermatologists have realized that moisturizers may reduce the propensity of the skin to develop primary irritation and help accelerate its reduction should it occur. A review by Zhai and Maibach gives a good overview on this subject (1). The prevention of irritation and its reduction when it does occur can be modeled separately.

Hannuksela and Kinnunen (1992) (31) performed a study using 1-minute washes with dishwashing liquid twice a day on the arms over a 7-day period. The authors evaluated cleanser-induced irritation using transepidermal water loss (TEWL) as a measure of SC integrity and laser-Doppler flowmetry to assess blood flow. They demonstrated that moisturizer application could prevent surfactant-induced skin damage and accelerate repair compared with no treatment, but were unable to differentiate between products.



**Figure 32.4** Ability of petrolatum (a) and lotion (b) to prevent the induction of skin flaking by repeated washing with soap, petrolatum, and lotion, and no treatment. (From Simion FA, Babulak SW, Morrison BM et al. Experimental method soap induced dryness in the absence of erythema. Scientific Exhibit in the 50th American Academy of Dermatology Annual Meeting, Dallas, Texas, 1991.)

**Table 32.2** Ability of Ingredients to Prevent Induction of Skin Dryness Due to Repeated Hand Washings with Soap

Ingredient	Ability to Prevent Dryness*
Petrolatum	54
Mineral oil	49
Glycerin (25% aqueous solution)	34
Sorbitol (25% aqueous solution)	14
Propylene glycol (25% aqueous solution)	–1

Source: Data from Highley DR et al., *J Soc Cosmet Chem*; 27:351–63, 1976.

\*The higher the score, the more effective the ingredient.

Another approach was that of Loden and Andersen (32). They used occlusive patching with 0.5% sodium laurel sulfate (SLS) to generate primary irritation, then applied different lipids to accelerate that rate at which the skin repaired itself. They utilized observer and instrument measures of erythema to assess the skin’s response and hydrocortisone as the positive, effective, control. They found that soy sterols had a beneficial effect. This model has been modified using 5% soap to cause the irritation and evaporimetry to assess SC barrier function. In this case 1% hydrocortisone (an OTC drug in the United States), was highly effective at reducing erythema but the results on barrier function appeared to be dependent on the vehicle rather than the hydrocortisone itself. Some high glycerin-containing moisturizers are also very effective at accelerating erythema reduction and restoring barrier integrity (2). This is consistent with the observations of Fluhr and his colleagues on the restorative properties of glycerin.

Preventing Primary Irritation

The ability of moisturizers to prevent detergent-induced skin dryness has important public health implications. Dermatitis is a leading occupational disease and professions that involve frequent hand washings are at particular risk (3,4). Frequent, effective moisturization may provide a significant preventative benefit. However moisturization or using a barrier cream does not provide sufficient benefit alone. Their use must be part of a

comprehensive education program to be able to provide a tangible benefit in the workplace (33).

The two main models that assess the ability of a moisturizer to prevent primary irritation are the occlusive patch test and a repeated washing test. In the former, test lotions are applied to the skin and allowed to dry—about 30 minutes. Then occlusive patches with dilute SLS or soap solutions are applied for 24 hours and then removed. Twenty-four hours later, primary irritation is assessed by a trained observer, by colorimeter, and by evaporimetry. A no-product site is used as a control. High glycerin cationic emulsions were shown to be more effective at preventing the induction of primary irritation than hydrocarbon-based moisturizers that form occlusive barriers (2).

Similar methods have been utilized to assess the ability of barrier creams to reduce or even prevent the induction of irritation (34). For instance, Schentz et al. (35) standardized the repeated short-term occlusive irritation test (ROIT) to assess the ability of test barrier creams to reduced irritation induced by toluene or 0.5% aqueous solutions of SLS. Sites on the volar forearm were pretreated with the test creams at 25 mg/cm<sup>2</sup>. Ten minutes later the irritants were applied under occlusion for 30 minutes. This was repeated 3.5 hours later, and the twice a day treatment was used for 2 weeks (excluding the weekend). All four test centers found that the petrolatum-based creams were effective at reducing SLS induced irritation as measured by TEWL rates and erythema measurements (both trained observer and colorimeter), but appeared to have a less consistent protective effect against toluene, possibly due to the hydrophobic nature of both petrolatum and the solvent. Wigger-Alberti et al. (36) used a similar approach. They applied petrolatum to sites on the ventral forearm of 20 volunteers. Thirty minutes later, test irritants such as aqueous solutions of lauryl sulfate, sodium hydroxide, and lactic acid as well as toluene were applied for 30 minutes. This process was repeated daily for 2 weeks. Assessments of skin condition by erythema scoring, chromameter, and TEWL demonstrated that petrolatum could reduce irritation although it appeared least effective against lactic acid.

### Instrumental Evaluations of Moisturizer Efficacy

Instrumental evaluation of skin condition is often used to supplement visual assessments in clinical (controlled application) moisturization protocols. They provide an objective method to evaluate a specific skin parameter. For example, TEWL measures SC barrier function by measuring water flux from the skin. Conductance/capacitance have been used as indirect measures of skin hydration. It must be emphasized that a single instrumental parameter should not be used alone as a measure of moisturization. Instruments measure defined physical parameters, which may not always correlate with moisturization or skin condition. For instance, hydrophobic materials such as petrolatum, silicones, or mineral oil that can be effective moisturizers reduce skin conductance immediately upon application. This contradicts the usual interpretation of this parameter that correlates increased conductance with moisturization. Instead the bioinstrumental measurement should be used to support and expand on observer scoring and panelist self-assessment, and if possible multiple bioinstrumental measures should be used simultaneously.

Several bioinstrumental methods are briefly reviewed in this chapter. More thorough reviews of bioinstrumentation are available in the literature (37–41).

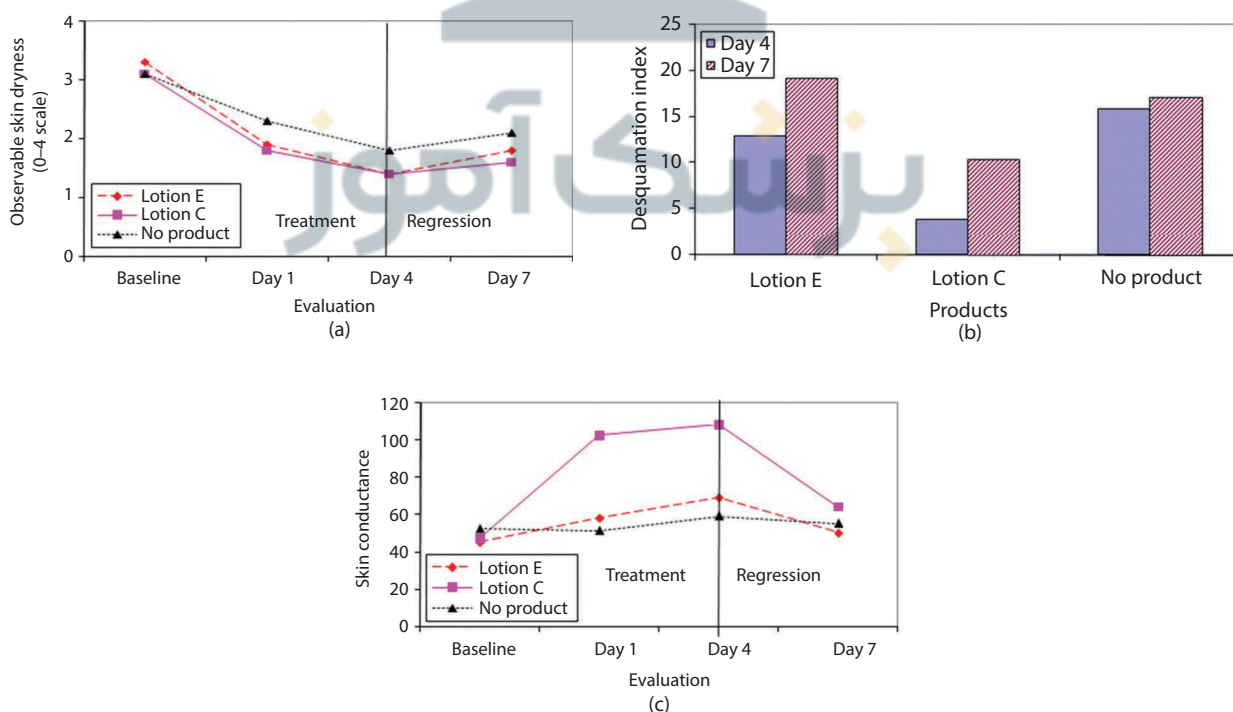
### Measuring Skin Dryness

Biophysical methods have been developed as alternatives to assessments by a trained observer. Chief among these is assessing skin dryness harvesting skin scaling with D-Squame® sticky tape strips, then quantification by computerized image analysis (42). D-Squame tapes are clear plastic disks with a homogenous layer of adhesive on one side.

The disk is pressed against the skin using uniform pressure. Scales on the surface of the SC are removed along with the disk. These scales can be readily visualized when placed on a black background and scored with a 0–4 analog scale or image analysis (27,40). Prall's quantization utilized reflected light from the scales. The analysis of Schatz et al. included both the area of scale coverage and the thickness of the scales to yield a desquamation index (DI). The Corneofix® (Courage + Khazaka, Cologne, Germany) uses a similar approach in a commercially available instrument. The DI is technically superior to observer scoring, as skin scales are readily obscured from the trained observer by high humidity or products that mat the scales down but do not cause desquamation. However as they are still present on the skin's surface, they still can be harvested by the sticky tape and quantified. An ingredient or product that causes desquamation will lower the measured DI. This was demonstrated in a regression test that compared lotions C and E, with a "no product" control. While the trained observer perceived few differences, both D-Squames and conductance demonstrated that product C was significantly more effective than lotion E or the "no product" control (Figure 32.5).

### Assessing the Integrity of the Stratum Corneum's Surface

Squamometry has been developed as a method of assessing the integrity of the corneocytes on the SC surface. D-Squame tape is used to harvest the surface corneocytes, which are then treated with a hydrophilic stain, such as Polymultichrome stain (PMS). If the corneocytes are in good condition, they do not take up stain and remain relatively clear, and in large



**Figure 32.5** Effect of lotions E and C on (a) observable dry skin in a miniregression test; (b) the skin's desquamation index in a miniregression study; and (c) the skin's hydration as measured by conductance.



sheets. Damaged corneocytes appear in smaller clumps, and take up stain to give a purple color (Figure 32.6). This can be quantified by colorimetry.

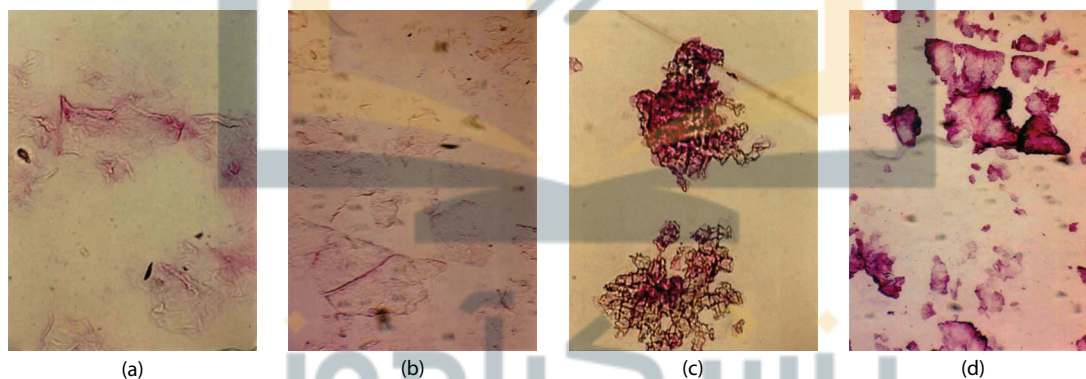
Since it is measuring damage to the surface corneocytes, squamometry is a very sensitive measure of damage to the skin's surface. Previously Simion et al. showed that increased color uptake correlated to the sensory irritation produced by different cleansing bars before any clinical signs were observed (43). Similarly Pierard et al. showed that squamometry could demonstrate that fabric softeners could reduce damage on sensitive skin (44).

Dry skin is a failure of the normal desquamation process, resulting in damaged corneocytes remaining on the surface. Hence there is much staining of the harvested cells. An effective moisturizer causes desquamation, removing the damaged cells, so that the surface corneocytes are in good condition and staining is reduced. Under the microscope the corneocytes appear to have taken up less stain, indicating less damage (Figure 32.7).

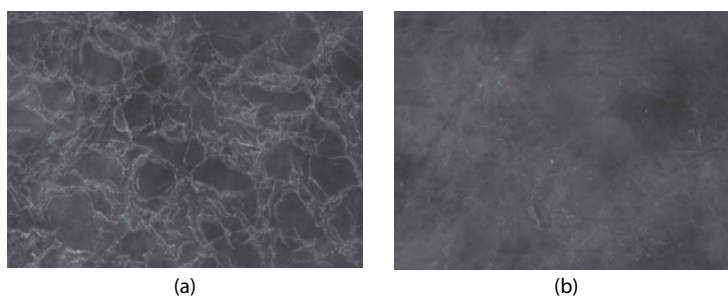
**Measuring Skin Surface Topography** New optical methods have been developed to assess the changes that a lotion can produce on skin topography more quickly and with less

damage than the silicone replicas previously used. One example is the Visioscan® (Courage + Khazaka, Cologne, Germany) that rapidly measures and quantifies skin dryness, smoothness, volume, and texture utilizing the "Surface Evaluation of Living Skin" (SELS) parameters (45). It uses UVA light sources to illuminate a small area (6 x 8 mm) uniformly and then the light reflected by the SC is captured by CCD camera with a high resolution black and white chip. When skin is hydrated using a moisturizer the flakes at the skin's surface are no longer observed (Figure 32.7). Furthermore, the skin can appear smoother and less uneven with repeated treatments.

**Spectroscopic Methods for Evaluating Skin Micro-structure** Different components of the skin such as hemoglobin, melanin, and collagen have the ability to absorb light at different wavelengths. By capturing and analyzing the reflected light for the different chromophores at different points across a large area, it is possible to build a map of the underlying surface structure down to a depth of about 2 mm. Using this method, Matts et al. showed that as the skin ages the distribution of both hemoglobin and melanin in sun-exposed skin becomes more diffuse, less homogenous. This could be used to measure the effects of antiaging lotions on the skin (46).



**Figure 32.6** Effect of lotion in preventing the reduction of surface corneocyte integrity. With lotion: (a) stained D-Square disk (x20 magnification) and (b) magnification of x100. Without lotion: (c) stained D-Square disk (x 20 magnification) and (d) magnification of x100.



**Figure 32.7** Effect of lotion on the appearance of the skin's surface. (a) Before lotion application and (b) 8 hours after lotion application.

### Measuring Water Content of the Skin

Direct measurements of the skin's water content have been developed. Unfortunately the leading method, confocal Raman microscopy, is no longer commercially available.

Assessing the skin's electrical properties is an indirect measure of its water content. Both conductance and capacitance can be measured. These parameters, while not identical, have been shown to strongly correlate to each other in moisturizer studies ( $r^2 = 0.92$ ) (Morrison and Scala [47]). Conductance measures the ability of a very small electrical current to pass through the skin, whereas capacitance measures the ability of the skin to hold a charge. However in practice the two parameters increase with hydration level produced by increasing the water content of the skin. Conversely, artificially or weather-induced dry skin holds less water and also has air pockets between the scales, the latter causing a white appearance. These lower both conductivity and capacitance. Measurements are rapid, in the order of seconds, and reproducible. The four most common instruments for measuring electrical properties are the Skicon® and DermaLab which measure skin conductance, while the Corneometer and Nova™ Dermal Phase Meter measure skin capacitance.

The procedures for taking these electrical measurements must be carefully controlled. It is important that the subject is in a relaxed state in a climate-controlled environment, to remove any contributions from sweat or from variable environmental conditions. Another source of error can result from product residue (48). Measurements taken immediately after lotion application will be especially high before the water from the vehicle has completely evaporated. In an opposite scenario, the presence of a hydrophobic product residue, such as petrolatum or silicone, will lower the measured value even if the hydration level of the skin is increased.

### Skin Elasticity

The elasticity of skin is widely recognized to decrease with chronological and photodamage-induced aging and has been shown experimentally to increase with hydration level (49). Though many devices have been used to measure elasticity in vivo, only a few are commercially available (50,51). The Dermal

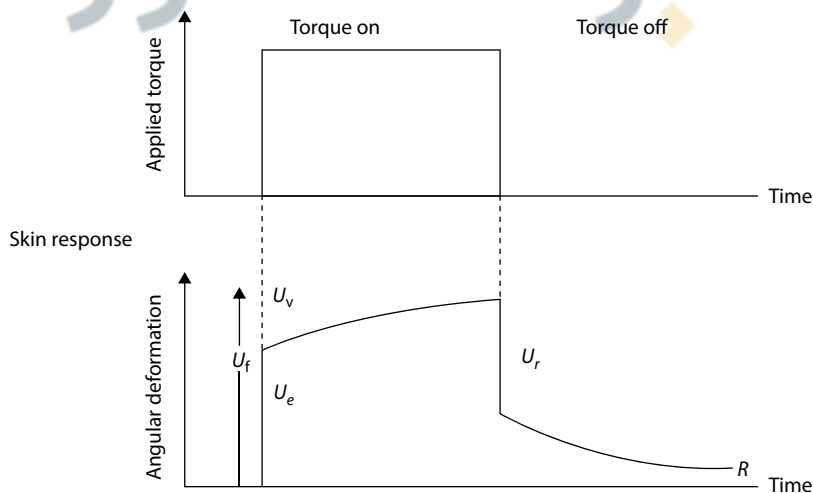
Torque Meter™ was designed to apply a torsional force (twist) in the plane of the skin and measure the deformation and relaxation of the affected skin. The Cutometer® and Dermaflex® both apply a vacuum suction perpendicular to the skin surface and measure the resultant displacement and relaxation of the skin, i.e. snap-back. Regardless of whether the force is applied perpendicular or parallel to the plane of the skin, the data are described by the same standardized parameters (Figure 32.8). That different instruments deform the skin in different ways but use the same parameter names has caused much confusion in the past.

There is a rapid initial deformation ( $U_e$ ) as the force is applied followed by a slow viscous “creep” ( $U_v$ ) if the applied force remains constant. As the skin is moisturized and becomes more plastic and flexible, both parameters increase. The final (total) deformation is termed  $U_f$ . When the force is released, the skin relaxes rapidly ( $U_r$ ), though only partially, toward the pre-force position. Over a longer period of time the skin relaxes back to its original state by viscous flow. All of these parameters are dependent on the thickness of the skin assessed. This in turn is dependent on the size and geometry of the probes utilized and how well the probe isolates the measurement site. Ideally for moisturizers the probes should assess the SC only. As this is difficult to accurately assess, the elasticity data are frequently presented as ratios. The ratio  $U_r/U_f$  is called the biological elasticity.

Although measurements can be completed within seconds, the techniques are not completely noninvasive. The skin may take up to an hour to completely return to its baseline values and in the case of the Dermal Torque Meter will undergo alterations from a tape-stripping effect of the adhesive used to attach the probe.

### User Evaluation of Moisturizer Performance

Ultimately whether the consumer or patient experiences the benefits of the moisturizer in relieving and preventing dry skin, continued usage, i.e. compliance, is a critical factor in determining efficacy. Not only must the lotion be pleasant to use, but the benefits should be quickly apparent. Methods have been developed to assess the benefits of lotions, especially in dermatological patients. There are several ways of doing this.



**Figure 32.8** Parameters used in assessing the viscoelastic properties of the skin. Abbreviations:  $U_f$ , total stretch;  $U_e$ , immediate deformation;  $U_r$ , immediate relaxation;  $U_v$ , viscous stretch;  $U_r/U_f$ , skin elasticity ratio;  $R$ , residue.

For immediate effects, panelists can assess the efficacy of a lotion at different times after application, using a questionnaire. Equivalent studies using cleansers showed that self-assessment of sensory effects was a more sensitive method at detecting product differences compared with observer-assessed signs (52).

For longer term effects (days to weeks), QoL measures use questionnaires so patients can assess the signs and symptoms of their skin condition and also the impact on their lives both in their ability to function normally and their emotional well-being. Some of the questionnaires can be very general, being able to compare the impact of diseases of different organs to each other (e.g., Short Form 36 and the Psychological General Well-Being Index) , while others are designed for skin in general (e.g., Skindex-29 and Skindex-16) (53–55). The Skindex-16 assesses how much the consumer/patient is bothered by the sign or system rather than the magnitude of the effect. It consists of 16 questions divided into three domains: Symptoms, Emotions, and Functioning (see Table 32.3). Each question is evaluated on a 7-point scale, where 0 is “never bothered” and 6 is “constantly bothered.”

Finally, there are questionnaires focusing on a specific disease or condition such as acne or atopic dermatitis (Acne Disability Index and Eczema Disability Index, respectively). Effective moisturizers have been shown to improve the QoL for

**Table 32.3** Skindex-16 Questionnaire

During the past week, how often have you been bothered by:	Domains*
1. Your skin condition itching	Symptoms
2. Your skin condition burning or stinging	
3. Your skin condition hurting	
4. Your skin condition being irritated	
5. The persistence/recurrence of your skin condition	Emotions
6. Worry about your skin condition (e.g. that it will spread, get worse, scar, be unpredictable etc.)	
7. The appearance of your skin condition	
8. Frustration about your skin condition	
9. Embarrassment about your skin condition	
10. Being annoyed about your skin condition	Functioning
11. Feeling depressed about your skin condition	
12. The effects of your skin condition on your interactions with others (e.g. interactions with family, friends, close relationships etc.)	
13. The effects of your skin condition on your desire to be with people	
14. Your skin condition making it hard to show affection	
15. The effects of your skin condition on your daily activities	
16. Your skin condition making it hard to work or do what you enjoy	

Sources: Copyright MM Chren, August 1999; Chren MM et al., *J Invest Dermatol*; 107(5):707–13, 1996; Chren MM et al., *Arch Dermatol*; 133:1433–40, 1997; Chren MM, et al., *J Cutan Med Surg*; 105–110, 2001; reproduced with permission from Mapi Research Trust, Lyon, France (PROinformation@mapi-trust.org; www.proqolid.org).  
\*Response choices for all items are a continuous bipolar scale with seven boxes, anchored by “never bothered” and “constantly bothered”

patients experiencing atopic dermatitis and non-pathological but extremely dry skin (56). As an example of the latter, two small groups of panelists (< 20 panelists each) used lotion in parallel for 12 weeks during the Minnesota (U.S.) winter. Those panelists using a high-efficacy test lotion containing synthetic ceramides and elevated levels of glycerin reported a significantly higher QoL compared with panelists using their usual lotion, even after the first week (Figure 32.9).

It is interesting to note that QoL is not always strongly correlated to the clinical observation of symptoms and their reduction by treatment. This suggests that these skin conditions and the products used to alleviate them have effects that go well beyond what is observed by the clinician (57).

As companies develop new lotions, they want to ensure that the target consumers recognize that the new product delivers its intended benefits and has the appropriate aesthetics such as fragrance and appearance as well as tactile properties including optimal levels of greasiness and spreadability. Prior to launch in the marketplace, this will be achieved by consumer usage testing. These studies can vary greatly in size, ranging from small exploratory studies to those that utilize large panels of several hundred volunteers. Usually panelists use the test moisturizer(s) for a designated period according to their normal routine. Afterward, they are debriefed with questionnaires and/or with individual interviews in focus groups. Feedback on product attributes such as greasiness, stickiness, and after-feel enables the cosmetic formulator to optimize the products to the needs of the target consumers.

*Product Evaluation by a Trained Expert Sensory Panel*

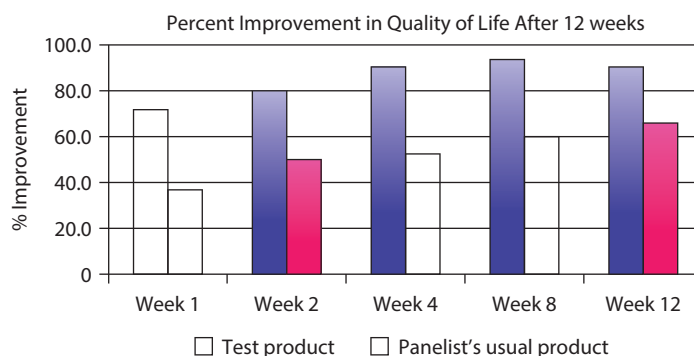
Since large scale consumer testing is time-consuming and expensive, the intensity of product attributes including stickiness, greasiness, and after-feel can be rapidly evaluated by a trained expert sensory panel. One such method is the Skin Feel Spectrum Descriptive Analysis (SDA) used by Meilgaard et al. (58). This method outlines the product attribute descriptors and scoring scales used to evaluate moisturizers. An expert panel of 8–15 persons is required to complete many hours of training to ensure they can reproducibly quantify moisturizer and skin attributes such as spreadability, amount of residue, and absorbency, which are scored using a zero (0) to ten (10) scale (see Figure 32.10). Once the panel is calibrated, they can be used to evaluate competitors’ products and optimize new formulas. It should be noted that these panels measure attribute intensity only, and do not assess the preferences of different consumer groups (hedonics).

*Advertising Claims Made for Moisturizers*

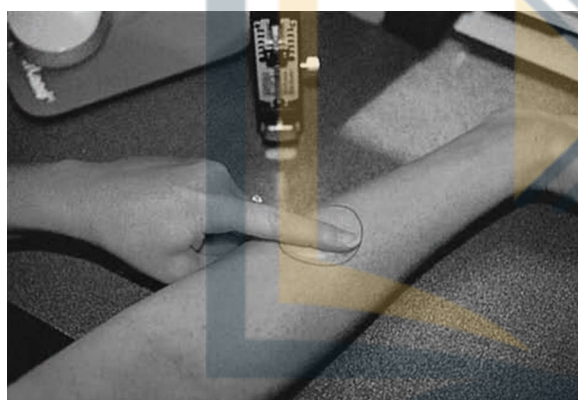
Modern-day hand and body moisturizers have been developed to meet the varied needs of today’s consumers. These products span the range from lightweight lotions designed to provide a pampered feeling for normal skin to heavy, greasy creams formulated to relieve severely dry skin. Communication of the benefits to consumers is known as advertising, which is made up of some or many claims, each elucidating an efficacy, sensory, or other benefit. Some of the common descriptors found on hand and body lotion packaging are listed in Table 32.4. Beyond package labels there are multiple other locations that claims may occur. These include TV, radio, and print advertising, as well as an increasing presence in the digital space.

In almost all jurisdictions, claims made must be supported by the manufacturer. For North America, the European Union, and Australia this is done by post-launch, in-market verification. In the United States if a label is found

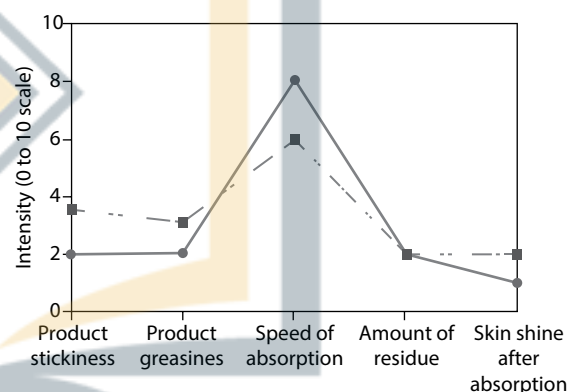




**Figure 32.9** Results: Quality of life (QoL) assessment indicated significant improvement for panelists' total QoL for the test product and the normal habits group. However, for those using the test product, the improvement was significantly greater (95% CL) starting at 1 week of usage. (Data from Adams L et al., Poster #86567 presented at: 72nd Annual AAD Meeting, 2014; Denver, CO.)



(a)



(b)

**Figure 32.10** Expert panel testing of lotions. (a) Product application. (b) Prototypical results for the sensory profile of two lotions: lotion A and lotion B.

to be false, the product is considered to be misbranded under Section 601 of the Federal Food, Drug, and Cosmetic Act. A deceptive act or practice, i.e. a false claim, is also illegal under Section 5 of the Federal Trade Commission Act. However, the U.S. industry is largely self-regulated by the National Advertising Division (NAD) of the Council of Better Business Bureaus, through which competitors or outside agencies can challenge claims made in advertising. In deciding whether a claim has been adequately substantiated the NAD assesses three types of support: rationale, subjective data, and objective data (59,60). The manufacturer will use a combination of test methods reviewed earlier in this section to provide the necessary support.

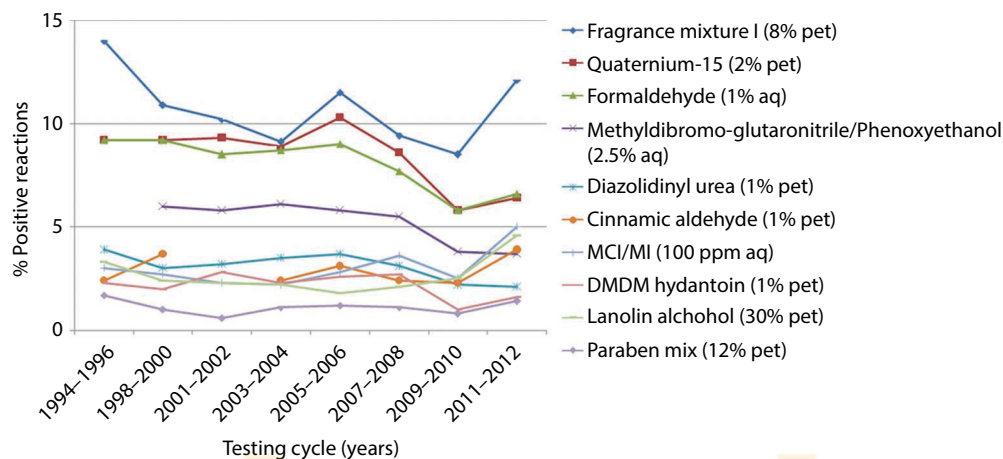
In the European Union, Proof of Effects is an integral part of the Product Information File (PIF) required for all cosmetic products. Expectations and requirements for claims made in different EU countries may vary with national expectations and sensibilities. To address this variation the EU commission has published guidelines (Common

Criteria) for what is expected from advertising (61). There are six principles:

1. Legal compliance
2. Truthfulness
3. Evidential support
4. Honesty
5. Fairness
6. Informed decision-making

The EU Commission is also developed guidelines for "Free-of" and "Hypoallergenic" claims.

Requirements to substantiate the claim are dependent on both the country and the venue for the claim. For instance, claims made on TV in the UK must be pre-cleared with Comcast before transmission. Comcast has a reputation for being extremely conservative in the substantiation standards they apply. Part of this is due to the requirement that in case of a challenge both they and the company must answer to the Advertising Standards Authority (60).



**Figure 32.11** The rates of positive dermal allergic reactions in patch testing run by the North American Contact Dermatitis Group (NACDG) for multiple testing cycles. (Data derived from Zug KA et al., *Dermatitis*, 2009; 20:149–60; Farnsway AF et al., *Dermatitis*, 2013; 24:10–21.; Warshaw EM et al., *Dermatitis*, 2013; 24:50–9; Warshaw EM et al., *Dermatitis*, 2015; 26:49–59; Marks JG et al., *J Am Acad Dermatol*, 1998; 38:911–18; Marks JG et al., *Am J Contact Dermatitis*, 2003; 14:59–62; Warshaw EM et al., *Dermatitis*, 2008; 19:129–36.)

**Table 32.4** Common Descriptors of Moisturizer Performance Used in Advertising

Action of Moisturizer	Description of Skin	Other Claims
Controls dry skin	Severe dry skin	Long-lasting relief
Heals dry skin	Extra dry skin	Fast relief
Protects dry skin*	Over-dry skin	Penetrates skin
Relieves dry skin	Rough, dry skin	Repairs moisture barrier
Soothes dry skin	Normal to dry skin	Soft, smooth, healthy skin
Ends dry skin	Itchy/tight skin	Firming/reduces appearance of cellulite
Nourishes skin		Reveals its natural glow
Younger-looking skin		Skin (color/appearance) evening
		Lasts up to x hours
		Dermatologist tested/recommended
		Lightweight/non-greasy
		Fragrance free

\*May be an over-the-counter (OTC) drug claim in the United States.

THE TOXICOLOGY OF HAND AND BODY LOTIONS

Although cosmetic hand and body lotions are leave-on products, they have a low rate of adverse reactions in normal usage. Adams and Maibach (20) reported that cosmetics caused 5.4% of the contact dermatitis cases studied by the North American Contact Dermatitis Group (NACDG) from 1979–1983. Hand and body lotions were probably a small group within this total, based on the observation that relatively few reactions occurred on the legs, arms, or torso, with many more reactions occurring on the face and in the eye area. This is supported by more recent data from the U.S. Food and Drug Administration (FDA). They reported that for the years 1991–1994, hand and body lotions caused approximately three “possible allergic or other severe irritation” reactions for the first million units distributed. In contrast, facial moisturizers caused six reactions for the first million units distributed, while bath soaps caused 45 (U.S. FDA’s Cosmetic Product Experience Report Summary). This

indicates that the hand and body lotions developed and sold by leading U.S. manufacturers deliver their benefits with little risk to consumers. However, even with minimal rates of adverse reactions, some consumers do experience reactions. This section reviews the types of adverse reactions experienced by consumers, the materials that may cause the reactions, and how lotion manufacturers test for them.

Most adverse reactions to hand and body lotions experienced by consumers are forms of primary dermal or sensory irritation. These are usually dependent on three factors:

- The lotion’s composition
- The condition of consumers’ skin
- How the consumer uses the product

Lotion Composition

The first point appears self-evident. If a product does not contain any potential irritants, then it is unlikely to cause irrita-

tion. Lotion manufacturers strive to market products that are both non-irritating and non-sensitizing.

However the addition of beneficial ingredients to lotions can cause adverse reactions. Such ingredients include sunscreens and AHAs that may cause sensory irritation such as facial stinging or burning in about 15% of the population. Preservatives can potentially cause very low rates of sensitization but are required to prevent microbiological contamination of products. Such contamination, especially by *Pseudomonas*, could pose a greater threat to a larger number of consumers. Some, such as quaternium-15 and Kathon CG, do cause higher sensitization rates than others and are not usually used in leave-on products. Indeed, Kathon CG is in the process of being banned for leave-on use in the European Union (regulation enforced for products on sale, July 2016). Novel delivery systems, skin protectants, and anti-irritants have been used to reduce adverse reactions by limiting release of, or penetration into the skin of, potential allergens or irritants. Responsible manufacturers seek to minimize these risks for lotions which consumers essentially regard as being “risk-free,” while maximizing the benefits to their consumers.

### Role of Consumers' Skin Condition

Most hand and body moisturizers are well tolerated by the general population as evidenced by the low adverse reaction rate reported to the FDA. However, there are groups of consumers that respond adversely to a product or ingredient more readily than the general population. There are several different reasons for this phenomenon. People with a damaged SC barrier due from pre-existing trauma or pathology may be more reactive, especially to marginal or cumulative irritants. This group includes people with atopic dermatitis or chronic pre-existing irritation.

A subset of the population experiences sensory irritation—stinging or burning—when they apply lactic acid or sunscreens to their facial skin. The mechanism by which this sensory irritation is induced is not clear, but it may involve the C-fiber nerve as the sensations can be inhibited by the “-caine” anesthetic/anti-pruritic and strontium salts which are known to affect this nerve type (62–64). Sensory irritation is distinct from detergent-induced irritation, as SLS, an irritating surfactant, does not cause stinging (65), and stingers to lactic acid demonstrate a wide range of irritation responses to lauryl sulfate (66).

Finally, people with a known allergy must avoid exposure to that allergen. Listing the ingredients on the package/label in all cosmetics sold in the United States, Canada, and the European Union as well as U.S. OTC drugs and Canadian Natural Health Products, provides consumers with this information. If there are any questions, especially about a fragrance component, the lotion manufacturer should be contacted via the toll free/free phone number shown on the package label. Additionally, U.S. dermatologists have access to the American Contact Dermatitis Society (ACDS) database that lists the composition of many personal care products (not just lotions), and can be searched to determine if a desired product contains the ingredient to which a patient has a sensitivity.

### How Consumers Use the Product

Usually consumers do not misuse hand and body lotions in such a way that they are harmed. Most lotions are relatively mild to the eyes and are not toxic when ingested. However this may not be the case for all products, and it is advisable

to contact the manufacturer or a poison control center in case of a question.

### Predicting Adverse Skin Effects

#### *Primary Dermal Irritation*

Erythema occurs when the concentration of an irritant and the time and conditions of skin exposure exceeds a critical threshold level: Malten's theory of traumatic irritation (67). Redness usually is localized to the exposure site. It may occur rapidly after the first exposure to an acute irritant or after several applications of a weaker or cumulative irritant. Diagnostically, irritation is frequently differentiated from sensitization reactions by factors such as:

- Composition and dose of the product
- If the response occurs after the first usage
- Rapid onset of irritation after usage
- The type and spreading of the reaction
- How long the erythema and/or swelling lasts

Lotion manufacturers routinely probe for this type of information when consumers complain concerning an adverse reaction and may utilize a dermatologist to diagnose or follow up as appropriate. This process often called cosme-to-vigilance, is now a requirement of the Cosmetic Regulations in the European Union. Ingredients that can cause primary irritation in lotions are frequently anionic surfactants used as emulsifiers, or AHAs that are added for their antiaging benefits.

A sensitive method to assess and differentiate lotions based on their primary dermal irritation potential is the cumulative irritation test (CIT) (68). Panelists are randomly selected from a general population, and the product is applied under an occlusive patch for 24 hours. The patch is then removed and the site is evaluated for irritation using a 0 to 3 scale. Traditionally this process is repeated for 21 consecutive days, with patches remaining in place over the weekend. However, Berger et al. modified this test to where applications are only made for 14 days (69) and showed that this modification gave the same lineup of irritation potentials as the 21-day study, but with a reduced chance of tape reactions. The cumulative irritation score in a panel of 10 to 25 subjects is a measure of primary irritation potential. The mean cumulative score per panelist for most lotions is low—frequently less than 5 out of a possible 45 in the 21-day test. In contrast, a 0.1% SLS solution, which is used as a positive control, usually yields a mean cumulative score per panelist that exceeds 20.

An alternative method for assessing irritation potential utilizes the induction phase of the human repeated insult patch (HRIPT). Although the primary purpose of this test is to identify sensitizing ingredients or products, it begins with an induction phase. During induction, usually 100+ panelists are occlusively patched with the product for 24 to 48 hours over a 3-week period. In the Jordan-King and Maibach-Marzulli variants of the HRIPT, the patches are applied for 48 hours and are removed shortly before scoring and repatching. As in the 21-day CIT, the skin does not have time to recover. This methodology resembles the 21-day CIT but on a larger scale, so it can be used to assess irritation potential, especially when an irritation benchmark such as SLS, is included. After the induction phase, the panel is rested. Two weeks later, sensitization potential is assessed when panelists



are challenged by patching with the product at a naïve as well as the original site.

Damaged skin may be more readily irritated than “normal” skin. This has been confirmed epidemiologically for occupations that involve repeated hand washings such as nurses and kitchen workers, who have significantly higher rates of hand dermatitis than their cohorts who do not do “wet” work, e.g., clerical workers (70). Atopy also increases the risks of occupational dermatitis. Such effects can be modeled. For instance, Freeman and Maibach showed a greater TEWL response on skin repatched with lauryl sulfate 2 weeks after the initial insult, even though the skin appeared normal (71). This suggested that there was a level of subclinical or “invisible dermatitis” still present at the cellular level. Indeed Kligman reported that patching with 0.5% lauryl sulfate can cause spongiosis even though the skin’s surface appears normal (72). Typically, subclinical levels of skin damage are caused by cold winter weather, repeated exposure to detergent solutions (i.e. “wet” work), or physical microtrauma. It has been suggested that the use of ancillary cosmetic or cleansing products can cause subthreshold levels of irritation. Subsequent application of a second product, such as a lotion, may elevate the irritation above the threshold, resulting in observable irritation (73).

To examine the role of the stratum corneum (SC) in preventing irritation, Frosch and Kligman developed a model (74). The SC is mechanically damaged by scratching with a needle and then is occlusively patched for three consecutive days. They showed that damaging the hydrophobic SC barrier reduced the concentration of water-soluble compounds required to cause irritation tenfold for SLS to 50-fold for nickel salts. The threshold concentration to cause irritation by lipophilic irritants such as triclosan or fatty acids was reduced less than sixfold by this method.

The final approach to assess the irritation potential of a product on a vulnerable group is to have individuals from that group, e.g., atopsics or lactic acid stingers, use the product under exaggerated conditions.

#### *Sensory Irritation*

This type of irritation is more common than many people realize. It may cover several different mechanisms, such as subclinical irritation and low-level contact urticaria as well as effects such as facial stinging, e.g., to lactic acid. Over half the adverse reactions to cosmetics involve sensory irritation in the absence of any visible signs (75). Frosch and Kligman (1977) examined many ingredients for their potential to cause facial stinging and showed that 5% lactic acid, and sunscreens such as PABA had the potential to cause facial stinging in approximately 15% of the population (65). It is unclear what the rate of reaction would be on other parts of the body, although it is probably lower. More recently, Christensen and Kligman showed that damaging the skin by repeated facial washing with soap would enhance the stinging response (76). Low levels of cinnamic aldehyde (0.01%) have been used to cause tingling in chewing gum. At higher concentrations it can produce visible contact urticaria and with repeated exposure, sensitization.

#### *Sensitization-Delayed (Type IV) Contact Hypersensitivity*

Typically, dermatologists are most familiar with this type of adverse reaction. From 1979 to 1983 the NACDG reported that 60% of adverse reactions to cosmetic products observed by the NACDG were allergic in nature (20). This differs from epidemiological data that indicate that most adverse reactions are

due to irritation (75). This difference may reflect the greater intensity and duration of allergic reactions that would require medical intervention. The leading allergens identified by the NACDG that are found in cosmetics include fragrances and preservatives such as fragrance mix, quaternium-15, and formaldehyde. With the exception of the 2011–2012 testing cycle, the sensitization rates for many of the key allergens has fallen with time (see Table 32.5 and Figure 32.11) (77–83). This includes fragrance mix 1, formaldehyde, and some formaldehyde-donning preservatives such as quaternium-15. The rates for other preservatives such as the parabens and DMDM hydantoin have not changed much with time. In the 2011–2012 testing cycle there appears to be a generalized increase in sensitization rates. The reason for this is unclear—does it reflect a change in methodology or is it a true epidemiological effect? An example of the latter may be the increase in Kathon CG (MCI/MI) allergy due to increased sensitization/crossreaction to one of its components—MI. Analysis of the data from the next testing cycle (2013–2014) will help resolve this issue.

Sensitization to ingredients may occur more readily on damaged or irritated skin. Previously parabens had developed a reputation as a potential sensitizer. However, this may be due to parabens being used to preserve mendicants for dermatitic skin. The parabens will penetrate the damaged SC more readily and be more available to induce sensitization reactions (84), as opposed to be hydrolyzed by full thickness skin. Recent epidemiological data has shown parabens to have a relatively low sensitization rate compared with other preservatives (Table 32.5).

**Preservatives:** Lotion manufacturers prefer to avoid potential allergens by excluding them from products. When this is not possible, such as in selecting effective preservatives, then the least sensitizing product that ensures microbiological integrity should be utilized. The preservatives frequently used in U.S. products are listed in the “Ingredients” section this chapter.

**Fragrances:** The Research Institute for Fragrance Materials (RIFM) is a fragrance industry organization that evaluates the safety of individual components. It recommends if a component should be used or not be used, or if there is maximum safe level. Most major U.S. manufacturers follow or exceed these guidelines. In the last few years the RIFM has updated its guidelines, basing them more on quantitative risk assessment (QRA) and the class of product and type of exposure consumers will experience (85).

#### *Sensitization Testing*

After reviewing the proposed ingredients for potential allergens, lotion manufacturers utilize the HRIPT to experimentally confirm that the product or its components do not sensitize. This testing involves occlusively patching a panel of over 100 individuals from the general population for up to 3 weeks with a test material. After a 2-week rest period, the panels are challenged with a 24–48 hour patch and the response is evaluated (86). If the responses at challenge are greater than during the induction phase, are long-lasting—increasing in the 48 hours after patch removal—or spread beyond the patch site, there is a possible sensitization that should be investigated further. Pre-damaging the SC with SLS can enhance the sensitivity of the test (87). This method, known as the maximization test, is not often used as it causes many false positives.

#### *Acnegenesis and Comedogenesis*

These two terms are frequently used interchangeably, although they likely represent different biological events. Acnegenesis

**Table 32.5** % Positive Patch Test Reactions to Ingredients Used in Hand and Body Lotions

Compound/ Mixture*	Years Tested							
	2011–2012	2009–2010	2007–2008	2005–2006	2003–2004	2001–2002	1998–2000	1994–1996
Fragrance mixture I (8% pet)	12.1	8.5	9.4	11.5	9.1	10.4	10.9	14.0
Quaternium 15 (2% pet)	6.4	5.8	8.6	10.3	8.9	9.3	9.2	9.2
Formaldehyde (1% aq)	6.6	5.8	7.7	9.0	8.7	8.4	9.2	9.2
Methyldibromoglutaronitrile/Phenoxyethanol (2.5% pet)	3.7	3.8	5.5	5.8	6.1	5.8	6.0	NR†
Diazolidinyl urea (1% pet)	2.1	2.2	3.1	3.7	3.5	3.1	3.0	3.9
Cinnamic aldehyde (1% pet)	3.9	2.3	2.4	3.1	2.4	NR†	3.7	2.4
MCI/MI (100 ppm aq)	5.0	2.5	3.6	2.8	2.2	2.3	2.7	3.0
DMDM Hydantoin (1% pet)	1.6	1.0	2.7	2.6	2.3	2.8	2.0	2.3
Lanolin alcohol (30% pet)‡	4.6	2.5	2.1	1.8	2.2	2.3	2.4	3.3
Paraben mix (12% pet)	1.4	0.8	1.1	1.2	1.1	0.6	1.0	1.7

Sources: Zug KA et al., *Dermatitis*; 20:149–60, 2009; Farnsway AF et al., *Dermatitis*; 24:10–21, 2013; Warshaw EM et al., *Dermatitis*; 24:50–9, 2013; Warshaw EM et al., *Dermatitis*; 26:49–59, 2015; Warshaw EM et al., *Dermatitis*; 19:129–3, 2008; Marks JG et al., *J Am Acad Dermatol*; 38:911–18, 1998; Marks JG et al., *Am J Contact Dermatitis*; 14:59–62, 2003.

\*Concentration tested and vehicle. Pet = petrolatum as the vehicle.

†NR, not recorded

‡In the 2011–2012 cycle, the concentration of lanolin alcohol was increased to 50%.

is the occurrence of breakouts, blackheads, and whiteheads, especially on the face but also on the back. Frequently there is a strong inflammatory component that is not observed in comedogenesis. Inflammation is due to *Propionibacterium acnes* proliferation and the body's immune response to them. Lesions seem to appear rapidly, although formation of the initial hyperkeratotic plug may occur subclinically over a longer period.

Comedogenesis originally was the term given to the formation of large hyperkeratotic impactions due to exposure to chlorinated hydrocarbons. Such exposure can result from industrial accidents such as in Seveso, Italy (88). These comedones do not have an inflammatory component, are larger, and develop more slowly than facial acne.

The interchangeability of the terms arose from two factors. The first is mechanistic—both events involve the formation of hyperkeratotic plugs. Secondly, the original models for assessing comedone formation—the rabbit ear test and comedone formation on the human back—are more convenient than for acneogenesis (89,90). This enables many products and ingredients to be readily assessed and claims can be made that a product is noncomedogenic. Comedogenic materials include branched and unsaturated fatty acids and esters. However, combining these ingredients into products can greatly modify their comedogenic potential. Indeed the oil used to dissolve a fatty acid greatly modifies its comedogenic potential (Table 32.6). In contrast, the test method for acneogenicity requires a panel of at least 40 normal and acne prone subjects to use a product on their faces for at least 6 weeks. A non-acneogenic product will

not significantly increase the level of acne over this test period (91). This is a more expensive test than patching especially since each panelist can test only one product at one time.

#### Contact Urticaria

Clinically defined contact urticaria is often characterized by the rapid formation of wheals or flares frequently within an hour of exposure to a causative agent. Either an immunological or a nonimmunological pathway can cause this. However, the exact molecular and cellular mechanisms are not well understood.

Potential urticants that may occur in cosmetic products include the preservative benzoic acid, and fragrance components—cinnamic aldehyde and Balsam of Peru. It should be stressed that the incidence of reaction to these ingredients is not known, so their epidemiological importance is not clear. At lower concentrations, many urticants can produce sensory irritation, especially itching or tingling, without observable clinical signs. von Krogh and Maibach proposed a cascade of increasing rigorous testing for contact urticarial (92). It must be stressed that this testing, especially invasive scratch or prick testing, should be carried out by an experienced physician who has resuscitation apparatus readily available.

#### Photoreactions

The interaction of UV radiation with certain ingredients can cause chemical changes that produce irritation or allergic reactions. Usually products are tested only if they contain ingredients that absorb UV light. This includes sunscreens and

**Table 32.6** A. Comedogenicity and Irritation Potential of Cosmetic Ingredients in the Rabbit Ear Model

Ingredient	Comedogenicity	Irritation
<b>Oils</b>		
Coca butter	4	0
Coconut butter	4	0
Evening primrose oil	3	2
Soyabean oil	3	0
Peanut oil	2	0
Castor oil	1	0
Sunflower oil	0	0
Mineral oil	0–2	0
<b>Lanolin and derivatives</b>		
Acetylated lanolin	0	0
Acetylated lanolin alcohol	4	2
Anhydrous lanolin	0–1	0
Lanolin alcohol	0–2	0
PEG-16 lanolin	4	3
PEG-75 lanolin	0	0
<b>Fatty acids and esters</b>		
Lauric acid	4	1
Myristic acid	3	0
Palmitic acid	2	0
Stearic acid	2–3	0
Butyl stearate	3	0
Cetyl acetate	4	2
Cetyl ester NF	1	1
Isopropyl isostearate	5	0
Isopropyl lineolate	4	2
Isopropyl myristate	5	3
<b>Alcohol sugars and their derivatives</b>		
Isopropyl alcohol	0	0
Cetyl alcohol	2	2
Isocetyl alcohol	4	4
Oleyl alcohol	4	2
Stearyl alcohol	2	2
Sorbitol	0	0
Sorbitan laurate	1–2	1–2
Sorbitan oelate	3	0
Sorbitan stearate	0	0
Oleth-3	5	2
Oleth-5	3	2
Oleth-10	2	1
Oleth-20	1	0

**B. Effect of Solvent on Comedogenicity Potential**

	Organic Solvent* (Grade 0–5)	Sunflower Oil (Grade 0–5)
Caproic acid	0	2
Lauric acid	3	4
Palmitic acid	0	2
Stearic acid	0	2
Behenic acid	1	1
D&C red #36	3 (in mineral oil)	0 (in PEG 400)

\*Ethyl ether or acetone

**C. Comparison of Human Back and Rabbit Ear Comedogenicity Scores**

Material	Mean Comedogenicity Score	
	Rabbit*	Human
Acetylated lanolin alcohol	3	2
Cocoa butter	3	2
5% Crude coal tar†	3	3
Isopropyl myristate	1	0.4
Safflower oil	1	0

Material	Mean Comedogenicity Score	
	Rabbit*	Human
5 or 8% sulfur†	3	2
2.5% sulfur†	2	1.2
Hydrophilic ointment	0	0

Sources: Fulton JE Jr., *J Soc Cosmet Chem*; 40:321–33, 1989; Mills OH, Kligman AM, *Arch Dermatol*; 118:417–9, 1982.

\*Comedogenicity scored on a 0–3 scale. n = 3 rabbits or 5 humans.

†These test materials were diluted with hydrophilic ointment. All other test materials used at full strength.

fragrances, although fragrance ingredients that cause photoallergy and phototoxicity have been identified by RIFM and are not used by most lotion manufacturers. Predictive test methods involve application of the test material to the skin, then exposure to UV radiation. A parallel site has the product applied but is not exposed to UV light. This accounts for normal irritation or sensitization reactions.

**Use of Testing to Assess Adverse Reactions**

It is apparent from the section above that there are many causes of the adverse reactions that consumers describe as “irritation.” In normal usage, any of these reactions may occur, but with drastically different frequencies. The reaction rates depend not only the product’s composition and the consumer’s skin condition but also on how the consumer uses the product. This last factor can only be determined by when a target consumer uses or misuses the product under normal conditions. Frequently such tests are run on panels that exceed 100, as the adverse reaction rate is low. To interpret the data properly, it is important to be able to benchmark the results, either from historical data for similar products or by including a standard—preferably a commercially available product with a known rate of adverse reactions in the marketplace. For those panelists who do experience an adverse reaction, follow-up is appropriate. Besides appropriate medical assistance, this may include a questionnaire to better understand the symptoms and their cause, and in a few cases diagnostic testing. Diagnostic tests include exaggerated usage such as the Repeated Open Application Test (ROAT) and the Provocative Usage Test (93,94). For contact urticaria, von Krogh and Maibach (92) suggested a cascade of open application on normal skin, then slightly affected skin, followed by open then occlusive patching on slightly or previously effected skin. Whealing or erythema and edema indicate contact urticaria. If indicated, diagnostic patch testing for suspected allergic reactions should be run. A dermatologist experienced in this issue should run all the diagnostic testing.

**Eye Irritation**

Accidental exposure of hand and body lotions to the eyes does occur, as a sizable minority of consumers uses these products on their faces. As with skin irritation, pre-marketing safety assessment has two major steps. First a review of the ingredients’ toxicological profiles—is this ingredient a known eye irritant (hazard evaluation), and at what concentration/how will the ingredient be used (risk assessment)? Second, testing. Traditionally the Draize test in rabbits was used to assess irritation potential. Recently this has been supplemented and in many cases superseded by predictive, in vitro methods. These include the Chorioallantoic Membrane Vascular Assay (CAMVA) in hen’s eggs, which models damage to the



conjunctiva, and the Bovine Corneal Opacity and Permeability Test (BCOP) which models corneal damage. Cell culture methods such as the STE and ICE are also used especially for assessment of ingredients (95,96). Human test methods such as direct eye instillation under the direction of an ophthalmologist may be used, but these are more common for facial care products.

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# Anticellulite Products and Therapies

Enzo Berardesca

## INTRODUCTION

The term cellulite, first used in the 1920s by Alquier and Paviot, defines a localized lipodystrophic disease which affects more women than men. Nodular liposclerosis, edematofibrosclerotic panniculopathy, panniculosis, and gynoid lipodystrophy are some of the other names proposed over the last decades instead of cellulite. The pathophysiological aspects of cellulite are still poorly cleared. Many predisposing factors seem to influence cellulite onset, including gender, heredity, race, body weight, and age. Hormones and impairment of both microvascular and lymphatic circulation are considered the most important etiological factors. Cellulite usually develops in particular anatomic sites such as lateral thighs and buttocks. The histopathological modifications that characterize cellulite involve the different “operational units” of the fatty tissue: the matricial-interstitial unit, the microcirculatory unit, the neurovegetative unit, and the energy-fatty unit. These alterations are accountable for the padded and orange peel appearance of the affected areas and induce irregular body profile. Cellulite represents a heartfelt aesthetic problem for millions of women around the world. Various systemic and topical products as well as numerous medical procedures have been developed to resolve cellulite, very often with poor results and little scientific basis.

## ETIOPATHOGENESIS

In 1922, Alquier and Paviot were the first to describe cellulite as a nonphlogistic dystrophy of the mesenchymal tissue, which implied accumulation of interstitial liquids. They suggested that the disorder was a reaction to stimuli of different origin: infectious, traumatic, toxic, etc. Since then, many contrasting theories have been expressed to explain the etiopathogenesis of cellulite, which is currently often considered a physiological phenomenon, especially in Anglo Saxon countries. Among the different hypothesis, the theory proposed by Curri has gained approval and remains one of the most popular (1). According to Curri, cellulite begins with alterations of the precapillary arteriolar sphincter, as described by Merlen, which induce capillary ectasia, increased capillovenular permeability with accumulation of interstitial liquid, and consequent intercellular edema. Edema provokes fibroblast activation and proliferation, which cause hyperpolimerization of glycosaminoglycans in the connective matrix of the subcutaneous tissue. This phenomenon increases hydrophilicity of the intercellular matrix as well as the interstitial osmotic pressure. These alterations induce tissue hypoxia, which results in collagen production and damaged adipocytes. Anisopoikilocytotic adipocytes are hence surrounded by thickened fibrosclerotic septae; groups of adipocytes gradually form micronodules and subsequently macronodules. Around 85% of postpubertal women suffer from cellulite, independently

of their weight. This is due to the anatomy of the subcutaneous fat, which is constituted by two layers divided by a superficial fascia. The areolar layer is located just underneath the dermis; in this layer adipocytes are large and arranged vertically. In the lamellar layer, which is the deeper one, adipocytes are small and arranged horizontally. If weight increases, the lamellar layer enlarges. When cellulite occurs it is the superficial part of hypodermis that tends to protrude into the dermis. The areolar layer is thicker in women than in men and is under the control of estrogen. Hormonal factors seem to play a significant role in cellulite onset as well as in its evolution. Cellulite affects predominantly women, appears after puberty, and worsens during pregnancy and contraceptive therapy; therefore cellulite is greatly influenced by estrogen. Estrogen acts on adipocyte increasing lipogenesis and hence causing adipocyte hypertrophy and anisopoikilocytosis; it promotes fibroblast proliferation and alterations in glycosaminoglycans and collagen, leading to fibrosclerosis. Insulin, prolactin, and thyroid hormones are also involved in the pathophysiology of cellulite. Furthermore, recent investigations on the adipose organ have highlighted that adipocytes release several substances which act in an endocrine or paracrine way as well as so-called adipokine (2). On the basis of the new knowledge, it is clear that adipose tissue is not a passive organ but rather a dynamic organ able to interact with and regulate other cells, such as endothelial cells. Cellulite development also depends on predisposing factors such as genetic, nutritional, lifestyle, and pharmacological factors. Gender is the most important genetic predisposing factor. On the basis of histological examinations, Nürnberger and Müller noticed that a female subcutaneous tissue presents some anatomical peculiarities such as fibrous bands whose course is perpendicular to the skin's surface and which could be responsible of the “hill” profile of the dermohypodermic border, whereas in men the fibrous septa present a different disposition, more oblique with reference to the skin (3). Furthermore, a recent study performed by means of magnetic nuclear resonance imaging (MRI) has confirmed that women with cellulite have constitutional characteristics of fibrous interlobular bands, which induce bigger and radial fat chambers (4). Caucasian women are more frequently affected by cellulite than Asian or Black women. Genetic influences the number and the sensitivity of hormone receptors on adipocytes as well as the tendency to circulatory insufficiency. Nutritional factors are very important. As hyperinsulinemia stimulates lipogenesis, excessive intake of fats and carbohydrates contributes to cellulite onset and worsening. But although adiposity is linked to cellulite, MRI has demonstrated that, among women with body mass index over 30, there are women who do not manifest cellulite at all. Salt favors hydric retention and alcohol stimulates lipogenesis. Sedentary lifestyle contributes to cellulite as it is associated with ponderal increase and decreased activity of the

muscular pumping in the lower limbs with consequent venous stasis. The muscular pumping activity is also influenced by the habit to wear tight clothes and high-heeled shoes. Among drugs, estrogens, antihistamines, and beta-blockers have shown to play a role in cellulite development.

## CLASSIFICATION

On the basis of clinical and histological modifications that occur in the subcutaneous tissue, four stages of cellulite are distinguishable (5).

### Stage I

In the first stage, the patient may be asymptomatic or only manifest a pale and pasty skin. Histologically, we can observe a thicker areolar layer, increased capillary permeability, anisopoikilocytotic adipocytes, capillary ectasia, and lipoedema.

### Stage II

Clinical alterations are not clearly evident at rest but only after skin pinching or muscular contraction we can observe an orange peel appearance, with decreased skin temperature and elasticity. Histologically, the fibril network, which surrounds adipocytes, appears hypertrophic; there is an important microvessel dilatation, and there are microhemorrhages.

### Stage III

In this phase, clinical changes are appreciable at rest with the characteristic orange peel aspect. On palpation, we can notice small lumps, hypoelasticity, and decreased skin temperature. This clinical picture histologically corresponds to anisopoikilocytotic adipocytes encapsulated in micronodules, neofibrillogenesis, and dilation of small veins.

### Stage IV

The clinical characteristics of stage III are more evident; in particular there are macronodules due to the agglomeration of many micronodules. In this stage, cellulite may be painful because of the compression of the nerves by the nodules. According to skin consistency, we can distinguish four types of cellulite: hard, flaccid, edematous, and mixed (6). Hard cellulite is characteristic of teenagers and young women who regularly practice sport; at rest skin appears firm and compact and orange peel becomes evident only after pinching. On the contrary, inactive subjects usually show flaccid cellulite, which is associated with muscular hypotonia. In the edematous form, lower limbs are globally enlarged and patients complain of a sense of heaviness, cramps, and swelling.

## NONINVASIVE TECHNIQUES TO EVALUATE CELLULITE

The variety of anticellulite products and professional (surgical or not) approaches to treat cellulite is quite huge, ranging from topical products to oral regimens, from manual or mechanical massages, to garments. We can find many strategies to contrast the condition, a borderline one with pathology. In general, the efficacy of cellulite treatments is often debated and objective studies are needed to claim support. Furthermore, it is very difficult to investigate cellulite by bioengineering methods.

### Thigh Circumference Measurement

This traditional measure indicates the reduction of thigh circumference, which can be due to both the reduction of edema and the

effect on the fatty layer. It is recorded on hips, ankles, and thighs as follows:

1. Hip: The tape measure is positioned around the hips, putting it finally on the superanterior iliac crest.
2. Thigh: The tape measure is placed around the thigh, marking the site of interest.
3. Ankle: The tape measure is placed around the ankle, exactly above the malleolar bone (7).

## Ultrasonography

Ultrasound is used to study the thickness and the quality of the connective tissue and the edematous component of cellulite. Frequencies between 10 and 15 MHz should be chosen for skin examination. With higher frequencies it becomes more difficult to view in depth (8).

## Laser Doppler Flowmetry

Laser Doppler flowmetry (LDF) is an optical technique used to evaluate skin microcirculation, which provides information on blood flow and erythema. The method consists of a Ne-He laser source of 632 nm wavelength applied to the skin via a small probe. The incident radiation enters the skin and is scattered and reflected by nonmoving tissue components and by mobile red blood cells encountered as the radiation penetrates to a depth of 1 to 1.5 mm. A portion of the scattered and reflected incident radiation exits the skin and is collected by a second optical fiber that carries the light back to a photodetector where it is converted to an electrical signal. Stationary skin tissue reflects and backscatters light with the same frequency as the incident source, while moving erythrocytes reflect the frequency-shifted radiation. The shift increases with increasing erythrocytes speed. The LDF extracts the frequency-shifted signal and derives an output proportional to the flux of erythrocytes of the blood flow. LDF is a reliable method for estimating cutaneous microcirculation (9).

## Thermography

Anticellulite products are meant to increase local skin blood flow. By increasing the blood flow, they increase the local skin temperature. Thermography is an electrooptical method for the imaging of temperature. The current technology used is based on the detection of the infrared radiation emitted by the skin. A conventional color thermogram uses a spectral color range, where blue is cold and red/white is hot. Intermediary temperatures are shown as shades of green, yellow, orange, etc. (10).

## Plicometry

The technique implies the use of the plicometer, a device that allows evaluation of the thickness of cutaneous plicae or folds to calculate the percentage of fat in human body. The measurement is usually performed on the thigh, on a defined point which can be determined by measuring the half distance between the iliac crest and the center of the knee as reference points. During measurement, the leg is relaxed. All measurements are performed in standard conditions, which guarantee reliability and suitability of collected data.

## Magnetic Resonance Imaging

Among in vivo skin imaging methods, MRI (11) is the most recent approach, being of high interest not only for its ability to distinguish structures at a submillimeter scale, but also

for its ability to describe the physiology of the different skin layers through the measurement of their intrinsic MR parameters. High spatial resolution MRI allows differentiation of the different skin departments—epidermis, dermis, and hypodermis—offering new and interesting opportunities for the evaluation of anticellulite treatments. Some authors (4) found that changes in skin architecture with cellulite can be well visualized by that method, pointing out clearly in the images the skin fat layers beneath the dermis and down to the level of muscles. Also, the diffuse pattern of extrusion of underlying adipose tissue into dermis is clearly imaged, and was found to correlate with cellulite grading. Other researchers (12) applying such a technique characterized the topography of the dermohypodermal junction and the three-dimensional architecture of the subcutaneous fibrous septae, giving a more clear frame of skin condition in areas affected by cellulite.

Since methods and guidelines to evaluate clinically and objectively cellulite are lacking, a multidisciplinary group has been created in order to define a valuable and reliable methodology for this purpose (13)

## THERAPIES

### Pharmacological Agents

Several proximate principles have been employed topically, systemically, or transdermally, in attempt to contrast the different pathophysiological aspects of cellulite. Notwithstanding, only a few scientific studies proving their real efficacy have been published. Methylxanthines such as theobromine, theophylline, aminophylline, and caffeine,  $\beta$ -adrenergic agonists such as isoproterenol and adrenaline, and  $\alpha$ -antagonists such as yohimbine, piperoxan, and phentolamine represent drugs with a lipolytic effect. Among these, topical aminophylline has been demonstrated to be the most effective, but the best results are obtained applying aminophylline together with yohimbine and isoproterenol (14). The use of coenzyme A and L-carnitine may contribute to improve the effects of the above-mentioned drugs, as they induce the discharge of free fatty acids. A 2.8-mm decrease in subcutaneous fat thickness has been observed after a month of application of a product containing caffeine, horsechestnut, ivy, algae, bladderwrack, plankton, butcherbroom, and soy (15). Extracts from *Centella asiatica* are active either on connective tissue or microcirculation, and they are commonly used orally and topically. In particular, 60 mg of *Centella asiatica* once a day for 90 days induces reduction of adipocytes' dimensions (16). Distant et al. have performed a prospective, longitudinal double-blind designed study aimed to test a plant complex on the basis of seed extracts of grape (*Vitis vinifera*), Ginkgo biloba, *Centella asiatica*, Melilotus (*Melilotus officinalis*), *Fucus vesiculosus*, fish oil, and borage oil. Data obtained from these trials have demonstrated that the oral intake of the mixture of plant extracts leads to significant improvement of cellulite (17). A 4-week oral intake of peroxisome proliferator-activated receptors (PPAR) agonists have demonstrated reduction in subcutaneous fat thickness in mice (18). There is no FDA-approved dietary supplement for cellulite treatment. The slimming effects of a cosmetic composition have been recently evaluated showing good efficacy and tolerability (19).

### Massage Treatment

Bayrakci et al. (20) have investigated the effects of mechanical massage, manual lymphatic drainage, and connective tissue

manipulation techniques on cellulite. They have reported improvement in all groups treated, with a decrease of thigh circumference and fat thickness. This study confirms previous data obtained by Lucassen et al. (21) who have monitored the effectiveness of electromechanical massage device by ultrasound imaging. A "smoothing" of the dermis-hypodermis interface has been observed but the improvement was transitory as the result regresses after treatments end.

### Endermologie

LPG endermologie is an electromechanical handheld massage device designed by Louis Paul Guitay, a French engineer and approved by FDA for cellulite treatment. The machine consists of two rollers and a vacuum chamber. The rollers rhythmically fold and unfold skin and subcutaneous tissue while the vacuum applies negative pressure. In the few published studies, the results obtained note that LPG induces fat mobilization and redefines body profile. In a recent trial performed on 33 women with cellulite grades 1 to 3, treated with LPG twice weekly for 15 sessions, Gülec (22) observed a circumference reduction in all patients but only 15% of the subjects had a reduction of cellulite grade.

### Optical Devices

Velasmoth (Syneron Medical Ltd., Yokneam, Israel) combines negative tissue massage, radio frequency (RF) and a 700-nm infrared light (IR). It has been approved by FDA for the treatment of cellulite. The mechanical massage improves microcirculation and facilitates lymphatic drainage while RF and IR heat the tissue, thus inducing collagen contraction and neocollagenesis. Improvement is usually obtained after eight or more treatments, delivered on a twice-weekly basis. Monthly maintenance treatment is recommended. Results decline within 6 months post treatment (23). TriActive (Cynosure, Inc., Chelmsford, Massachusetts) combines a 810-nm diode laser, contact cooling, suction, and massage. It represents another FDA-approved device to contrast cellulite and has shown to be as effective as Velasmoth in a randomized, comparative, prospective clinical study in which patients were treated twice a week for 6 weeks. Bruising was the only reported side effect, occurring globally in about 55% of the patients (24). Goldberg et al. have investigated the use of a unipolar RF device (Alma Lasers, Buffalo Grove, Illinois), which delivers a high-frequency electromagnetic radiation at 40 MHz, inducing heating of the tissue. Good results have been obtained with a treatment regimen of six every-other-week sessions and benefits were persistent for the following 6 months (25). More recently, a new bipolar RF system emitting variable frequencies, called automatic multifrequency and low impedance (AMPLI) RF, has been experimented for cellulite in the buttocks in a multicenter study. Enrolled subjects underwent one treatment per week for 12 sessions. An improvement in body profile was observed. With reference to the other types of RF devices already used, the AMPLI RF technology has the advantage of a continuous emission of different frequencies with consequent progressive heating, homogeneous thermal damage, sparing epidermidis (26). Manuskiatti et al. have tested a Tripollar RF device (Regen, Pollogen Ltd., Tel Aviv, Israel) for cellulite reduction. This novel system employs a three-electrode technology to deliver the RF. Patients were treated once a week for eight sessions. Good results were reached in terms of reduction of circumference and better appearance of cellulite (27).



## Surgical Subcision

The surgeon tears the connective bands that tie the dermis to the fascia by means of a needle (28). This mechanism is also exploited by liposculpture, which also reduces local adiposity.

## Mesotherapy

This method that delivers drugs directly into the dermis of the affected areas by means of several injections, was conceived by Pistor in 1958 and has been commonly employed since 1964. Despite its popularity, mesotherapy is lacking in scientific published supports. Several drugs are used such as aminophylline, enzymes, minerals, L-carnitine, and, recently, phosphatidylcholine.

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# Therapy of Telangiectasia and Varicose Veins and Their Complications

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## INTRODUCTION

One of the most commonly treated cosmetic disorders in dermatology is telangiectatic webs, or spider veins. The methods of treatment most commonly employed by the dermatologic surgeon include sclerotherapy and lasers. For larger varicose veins, dermatologic surgeons employ sclerotherapy with the newest improvement of foamed detergent sclerosing agents, ambulatory phlebectomy, and endovenous occlusion and ablation by intravascular radiofrequency or laser energy.

Sclerotherapy, which consists of the intravascular introduction of a sclerosing substance, is the most frequently utilized process. Sclerotherapy is actually endovenous chemoablation with subsequent fibrosis and concomitant vein wall collagen dissolution. The term "sclerotherapy" gained acceptance during the nineteenth century and has never changed. In the United States, sclerotherapy gained wide acceptance in the early 1990s and is regarded as a highly effective treatment for telangiectasias as well as veins of all sizes (1,2). It also serves as an effective addition to surgical techniques such as ambulatory phlebectomy for saphenous tributaries (3,4) and endovenous obliteration of refluxing saphenous veins (5,6). Knowledge of venous anatomy and physiology, principles of venous insufficiency, methods of diagnosing venous malfunction, uses and actions of sclerosing solutions, and proper use of compression are essential elements of successful venous therapy.

## EPIDEMIOLOGY

Bulging varicose veins and unsightly "roadmap" telangiectatic webs affect millions of patients and the number affected is increasing every year as the population ages. Telangiectases comprise one of the most common cosmetic complaints, affecting up to 50% of women, while larger varicose veins affect up to 40% of the population (7,8). Varicose veins may cause significant morbidity including chronic stasis dermatitis, ankle edema, spontaneous bleeding, superficial thrombophlebitis, recurrent cellulitis, lipodermatosclerosis, and skin ulceration on the ankle and foot.

The incidence of varicose veins increases with each decade of life. Increased incidence has led to increased demand for treatment of varicose and telangiectatic veins as the average age of the United States population grows. While 41% of women in the fifth decade have varicose veins, this number rises to 72% in the seventh decade (9). Statistics for men are similar, with 24% incidence in the fourth decade, increasing to 43% by the seventh decade. Six million workdays per year may be lost due to complications of varicose veins including stasis dermatitis, cellulitis, and ulceration (10).

## HISTORICAL ASPECTS

In the second century AD, Galen proposed tearing out the veins with hooks, a precursor to the modern day technique of ambulatory phlebectomy originated by Swiss dermatologist Robert Muller in the late 1960s. Primitive stripping and cauterization, however, were practiced by Celsus, while ligation was mentioned by Antyllus (30 AD).

In 1851, Pravaz attempted sclerotherapy with ferric chloride using his new invention, the hypodermic syringe. The foundation of modern sclerotherapy can be traced to World War I when Linser and Sicard both noticed the sclerosing effect of intravenous injections used to treat syphilis, which often resulted in vein sclerosis. Tournay greatly refined the sclerotherapy technique in Europe and wrote the gold standard of textbooks. French physicians were leaders in the field of vein diagnosis and treatment, which evolved into the subspecialty of phlebology. It was not until 1946, when a safe sclerosant, Sotradecol (sodium tetradecyl sulfate) had been tested and described, that sclerotherapy began to be seriously studied in the United States (11).

Another key to success and acceptance of the treatment of varicose veins by sclerotherapy was the addition of compression. The most significant contributions were European with Sigg and Orbach in the 1950s and Fegan in the 1960s emphasizing the importance of combining external compression immediately following injections. Starting in the 1980s, Duffy promoted the technique in the United States among dermatologists and advocated the use of polidocanol and hypertonic saline as safe and effective sclerosing solutions (12). The first endovenous obliteration technique utilizing radiofrequency, designed as a substitute for ligation and stripping of the greater saphenous vein, was researched and pioneered by dermatologic surgeons (6). Subsequent procedures, including 1320 nm laser ablation, were also pioneered by dermatologists, and these ablative procedures have now become accepted as the standard for treatment of saphenous vein reflux (13). Utilization began in Europe in 1998 on saphenous vein incompetence, and the technique was made available in the United States in March 1999. Goldman's first American textbook of sclerotherapy (now in its 5th edition) (14) integrated the world's phlebology literature, introduced new sclerosing solutions and validated dermatology's claim to expertise in vein treatment (15). Several additional textbooks by dermatologic surgeons have now firmly established phlebology, which includes the diagnosis and treatment of spider and varicose veins, firmly within the sphere of dermatology (16,17).

## VENOUS ANATOMY AND PHYSIOLOGY: THE KEY TO CHOOSING THE RIGHT TECHNIQUE

The superficial venous system consists of three primary territories: the great saphenous vein, the small saphenous vein, and the subdermic lateral venous system. Due to gravitational hydrostatic pressure, sequential retrograde breakdown of venous valve function often follows a leak at one point leading to propagation of a varicosity or spider vein. All veins regardless of their size contain valves (18). Increased diameter between valve leaflets with failure to oppose properly caused by genetically weak venous wall or venous valve structure may initiate these events (8). Calf muscle pump pressure plus gravitational hydrostatic forces are transmitted directly via the incompetent perforating vein or communicating veins to the surface veins. Venous pressure may reach as high as 300 mmHg in the cutaneous venules with the patient erect. Transmission of pressure may result in venular dilatation over a wide area of skin including the formation of telangiectatic webs.

When present in significant quantity, the volume of blood sequestered and stagnant in reticular veins and associated telangiectatic webs (particularly of the lateral venous system) may cause enough distention to produce symptoms (19). Symptoms are relieved by the wearing of support hose or with rest and elevation of the legs. Prolonged standing or sitting worsens symptoms. The size of the vessels causing moderately severe symptoms may be as small as 1–2 mm in diameter. Sclerotherapy has been reported to yield an 85% reduction in these symptoms as well as superb cosmetic results (19).

## Contraindications to Treatment of Spider Veins

A high rate of recurrence for sclerotherapy is commonly seen when reflux originates at the major saphenous junctions. When reflux exists at the saphenofemoral junction, this must be dealt with prior to treatment of distal varicosities or telangiectasias. Since the goal of sclerotherapy and other treatments is to eliminate reflux at its origin, the goal of noninvasive diagnostic evaluation is to reveal the primary source of reverse flow. The techniques of endovenous occlusion by radiofrequency or laser have been developed to address reflux occurring at the termination point of the saphenous veins. Over a decade of experience has shown that radiofrequency or endovenous ablation is as effective as the surgical techniques of ligation and stripping to eliminate saphenous and associated varicose veins (13,20).

Previous urticaria or suspected allergy to a sclerosing agent should serve as a relative contraindication to use of that particular sclerosing agent. A history of deep venous thrombosis (DVT) or previous trauma to the leg (e.g. auto accident) should preclude sclerotherapy until adequately evaluated by Duplex ultrasound. Venous treatment is contraindicated in a bedridden patient since ambulation is important for minimizing risks of thrombosis. Similarly, patients under general anesthesia for non-related procedures should not undergo simultaneous sclerotherapy. Severely restricted arterial flow to the legs necessitates postponement of vein treatment. During hot summer months, heat-induced vasodilatation and inability to comply with wearing of compression hose may also require postponement of treatment.

Pregnancy is no longer considered a contraindication to sclerotherapy and extremely painful or bleeding varices may be treated even in the last trimester in our experience.

Endovenous techniques may be employed to treat refluxing saphenous veins in pregnancy. However, treatment is typically postponed since many varicosities and telangiectasias will spontaneously clear within 1–6 months postpartum. Obesity should be considered a relative contraindication since maintaining adequate external compression is difficult.

## TREATMENT TECHNIQUES Sclerotherapy or Endovenous Chemo-Ablation Techniques

### General Principles

Progression from proximal to distal regions will focus initial treatment on vessels most likely to be proximal pressure sources. Thus a basic principle of treatment is to begin at the largest (reflux sources) and progress to the smallest varicosities. Sclerotherapy of telangiectasias is approached by combined injection of visibly connected reticular veins, venulectases, and telangiectatic webs or networks.

Reticular veins are treated only after all sources of reflux from major varicosities have been treated by sclerotherapy and/or surgery. When no clear feeder vessel is seen or identified by duplex ultrasound, transillumination (Veinlite, 3Gen, Dana Point, CA) may be used. Newer handheld, non-contact devices that project infrared light can also be used to locate and map feeding reticular veins that may be difficult to see with the naked eye (VeinViewer®, Christie Medical Holdings, Inc., Memphis, TN; AV400 Vein Viewing System, AccuVein, Inc., Huntington, NY) When unable to locate an associated reticular vein, then the point at which the telangiectasias begin to branch out is the site at which to begin injection. Injection of telangiectasias is simultaneously performed with injection of reticular veins in the hopes of decreasing the number of treatments (21).

### Technique of Sclerotherapy

#### Liquid Sclerotherapy

The preferred method for treating telangiectasia to reduce side effects is to use a liquid, as opposed to foamed sclerosant (see below). The American dermatology technique of sclerotherapy has been described in detail by Duffy and Goldman (12,22). The sclerotherapy tray is prepared with the necessary equipment, including a 30-gauge needle bent to an angle of 10–30 degrees with the bevel up, which is placed on the skin so that the needle is parallel to the skin surface. A 3-cc syringe filled with 0.5 cc of solution is held between the index and middle fingers while the fourth and fifth finger support the syringe against the leg in a fixed position, facilitating accurate penetration of the vessel (Figure 34.1). The nondominant hand is used to stretch the skin around the needle and may offer additional support for the syringe. Magnifying lenses or operating loupes on the order of 1.5–3× are recommended to aid in cannulation of the smallest telangiectasias.

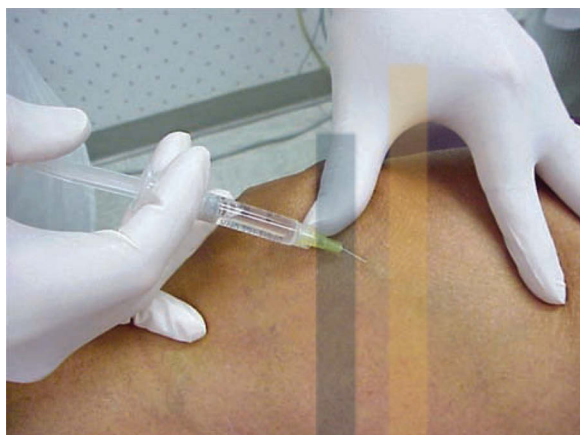
The initial treatment of telangiectatic webs begins with lowest possible concentration that will cause a telangiectasia to sclerose over a period of 1–6 months postinjection. This typically is 0.1% sodium tetradecyl sulfate, 0.2% polidocanol or compounded 72% glycerin diluted with 1% lidocaine with epinephrine (1:100,000). When ineffective sclerosis occurs judged at a subsequent visit, the concentration but not the volume per site of sclerosing solution is increased. Post-treatment compression consists of graduated 20–30 mmHg support hose for 2 weeks for telangiectasia associated with reticular veins and OTC 15 mmHg compression for telangiectasias only.



Treatment intervals vary between physicians, but allowing 4–8 weeks between treatments allows time for resorption of treated telangiectasias and therefore helps to minimize the number of necessary sessions. Typical results are shown in Figure 34.2.

#### Sclerosing Solutions

Sclerosing solutions have been classified into groups based on chemical structure and effect: hyperosmotic, detergent, and corrosive agents (chemical toxins – salts, alcohols, and acid or alkaline solutions). The distinct advantage of detergent agents is the ability to foam these solutions to use for larger varicosities. Table 34.1 summarizes the sclerosing agents.



**Figure 34.1** Position of the hands for sclerotherapy. While the dominant hand holds the syringe and creates a platform with the 5th digit, the nondominant hand stretches the skin and acts as a support for the needle hub so that fine changes in position are permitted.



(a)



(b)

**Figure 34.2** Typical results following sclerotherapy of telangiectasia. (a) Treatment with 0.1% STS. (b) Excellent clinical results at 4-month follow-up.

**Table 34.1** Comparison of Sclerosing Agents

Sclerosing Solution	Category	Advantages	Disadvantages	Vessels Treated	Concentrations (reduce concentrations for foamed solution by 50%)
Sodium tetradecyl sulfate (STS)	Detergent	May be foamed Low incidence of allergic reaction when used with Latex-free syringe	May cause skin breakdown at higher concentrations	All sizes	0.1%–0.2% telangiectasias 0.2%–0.5% reticular 0.5%–1.0% varicose 1.0%–3.0% axial varicose
Polidocanol (POL)	Detergent	May be foamed Forgiving with intradermal injection	May inadvertently be injected into arteriole without pain	Small to medium	0.25%–0.5% telangiectasias 0.5%–1.0% reticular 1.0%–3.0% varicose
Hypertonic saline (HS)	Hyperosmolar	Not allergic	Ulcerogenic Painful to inject	Small	23.4%–11.7% telangiectasias 23.4% reticular
Hypertonic saline + dextrose (HSD)	Hyperosmolar	Less painful than HS	Relatively weak sclerosant	Small	Undiluted–telangiectasias Undiluted–reticular
Sodium morrhuate	Detergent	None	Allergic reactions highest	Small	Undiluted–telangiectasias Undiluted–reticular
Glycerin (72% glycerin with 1% lidocaine)	Chemical irritant	Treats matting Low incidence of pigmentation	Very weak sclerosant	Smallest	Undiluted to ½ strength–telangiectasias
Polyiodinated iodine (Varigloban)	Chemical irritant	Powerful for largest veins	Avoid in iodine allergic patients	Largest	1%–2% for up to 5 mm veins 2%–6% for the largest veins

**Hypertonic Saline** Hypertonic saline (HS) is still a commonly employed solution (only in the United States) in spite of the extreme pain on injection and relatively low efficacy in vessels over 0.4 mm. We recommend against using this as a sclerosing solution. Used at a concentration of 23.4% (HS), a theoretical advantage of HS is its total lack of allergenicity when unadulterated. HS has been commonly used in various concentrations from 10%–30%, with occasional addition of heparin, procaine, or lidocaine. Additional agents typically provide no benefit. Therefore, HS is used either unadulterated or diluted to 11.7% with sterile water for smaller telangiectasias (23,24).

With hypertonic solutions, damage of tissue adjacent to injection sites may easily occur. Skin necrosis may be produced by extravasation at the injection site, particularly when injecting very close to the skin surface. HS is not one of our favorite solutions for this reason. Injection of hyaluronidase into sites of extravasation may significantly reduce the risks of skin necrosis with HS, although this has not been demonstrated in human clinical applications (24).

**Hypertonic Saline and Dextrose** Hypertonic saline and dextrose (HSD)(Sclerodex®, Omega Laboratories Ltd., Montreal, Canada) is a viscous mixture of dextrose 250 mg/mL, sodium chloride 100 mg/mL, propylene glycol 100 mg/mL, and phenethyl alcohol 8 mg/mL. HSD is a relatively weak sclerosant for local treatment of small vessels, with a total volume of injection not to exceed 10 mL per visit, with 0.1 mL–1.0 mL per injection site. HSD is marketed predominately in Canada. Although a slight burning sensation occurs, pain is far less than with HS. Efficacy has been seen by us in over 5000 patients with excellent results for treatment of telangiectasias and small associated reticular veins.

**Polidocanol** Polidocanol (POL) (Asclera®, Merz Aesthetics, Frankfurt, Germany) is the first new sclerosing solution introduced in the United States since 1946. It was FDA-cleared in 2010 in concentrations of 0.5% and 1.0%, and prior to this was available in the United States only through compounding pharmacies. The detergent-based POL, a urethane compound, was originally developed as an anesthetic but was found to have the property of sclerosing small diameter vessels after intradermal injection. POL contains hydroxypolyethoxydodecane dissolved in distilled water with 5% ethanol as a stabilizer. First used as a sclerosing agent in the late 1960s in Germany, POL is popular worldwide for smaller vessels due to painless injection and lowest incidence of cutaneous necrosis with intradermal injection. Lower concentrations of POL were initially suspected to have a lower incidence of hyperpigmentation than HS or STS, but recent clinical trials indicate a significant percentage of hyperpigmentation also occurs (25). Australian comparison studies have preferred POL over STS with increased efficacy with fewer complications (25).

POL is also available in a 1% foam that utilizes a proprietary canister device to generate a consistent, low-nitrogen, stable small-bubble foam which is FDA-cleared for the treatment of great saphenous vein incompetence (Varithena®, Provensis Ltd., A BTG International group company, Conshohocken, PA) (26). Notably, compounded formulations of POL have recently been found to have largely inconsistent concentrations and impurities, and such non-branded formulations should be avoided (27).

**Sodium Tetradecyl Sulfate** Sodium tetradecyl sulfate (STS) (Sotradecol®, AngioDynamics, Inc., Latham, NY; Fibrovin,

STD Pharmaceuticals, UK; Trombovar, Omega Labs, Montreal, Canada) is a long-chain fatty acid salt with strong detergent properties and is a highly effective sclerosing agent used worldwide. Approved for use in the United States since 1946, it has been popular with vascular surgeons since the 1960s and first described for use in telangiectasias in the 1970s. A relatively high incidence of postsclerosis pigmentation was reported at inappropriately high doses (1% STS). More appropriate concentrations for superficial telangiectasias are 0.1%–0.3%. Other concentrations are 0.2%–1.0% in reticular veins or small varicosities (1–3 mm diameter), and 0.5%–3% in larger varicosities related to major sites of valvular reflux. Recent use of foamed STS indicates that half the concentration may be utilized when the solution is frothed with air (see below).

**Sodium Morrhuate** Sodium morrhuate (Scleromate®, Palisades Pharmaceuticals, Inc., Tenaflly, NJ) is a 5% solution of the salts of saturated and unsaturated fatty acids in cod liver oil. Approximately 10% of its fatty acid composition is unknown and use is limited by reports of fatalities secondary to anaphylaxis (15). Although sodium morrhuate is approved by the FDA for the sclerosis of varicose veins, use in treatment of telangiectasias is not common and cannot be recommended due to the caustic qualities with potential for cutaneous necrosis and higher risks of allergy. This agent is reserved primarily for sclerosis of esophageal varices.

**Chemical Irritants** The chemical irritants include polyiodinated iodine (very caustic) and chromated glycerin (very weak) and are believed to have a direct toxic effect on the endothelium. After injection of polyiodinated iodine salt, the endothelium near the site of injection is destroyed within seconds. The corrosive action is limited due to rapid inactivation by blood proteins. At the sites of endothelial destruction the chemical can penetrate further and diffuse into deeper layers of the vessel wall causing further destruction. These agents are not commercially cleared by the U.S. FDA.

A preparation of 72% glycerin diluted 2:1 with 1% lidocaine with epinephrine can be prepared by local compounding pharmacies. Glycerin without the chromate salt may work primarily by osmotic injury. A commercial glycerin product has been reported to give a lower incidence of inflammation and subsequent pigmentation in smaller telangiectasias (28). In our experience in over 10,000 patients over the last 5 years, the 72% glycerin solution was the most effective at eliminating telangiectatic matting and resistant telangiectasias. It is particularly effective in treating smaller residual telangiectasias, with a greatly reduced incidence of matting and hyperpigmentation. Glycerin cannot be foamed.

**Foam Sclerotherapy** Detergent agents such as POL and STS may be mixed with air to create a foamed sclerosing solution (29–33). Typically, air is added at a ratio of one part solution to 3 to 4 parts air. Agitation is performed by rapid transfer from syringe to syringe via an IV stopcock or a two-way leur lock syringe connector. The advantages of foamed solutions for treatment of larger vessels include: (1) by displacing blood in the vein, the highest concentration of sclerosant is always contacting vessel wall, (2) the total amount of sclerosant injected is greatly reduced, (3) there is great persistence of sclerosant with very slow washout, and (4) foamed solutions can be used as a contrast agent under Duplex ultrasound, making Duplex-guided treatments easier and safer. An illustration of the persistence of

foam is seen is Figure 34.3. Recently it was reported with POL that the foamed version is far more potent on varicose veins than the non-foamed of equal concentration (34).

#### *Side Effects and Complications of Sclerotherapy*

##### **Postsclerotherapy Hyperpigmentation**

Postsclerosis pigmentation is defined as the appearance of increased visible pigmentation along the course of a treated vein of any size. Initially perivascular hemosiderin deposition and not increased melanin production causes this appearance (35). However, after several weeks to months the hemosiderin is replaced by melanin. The reason for persistence of pigmentation is unknown. The incidence of pigmentation is related to dilution and type of sclerosing agent as well as diameter of treated vessel (36). Pigmentation incidence ranges from 11%–30% using HS (18), 11%–30% with POL (12,37), and up to 30% with STS. The incidence of pigmentation may be reduced in varicose veins by expressing the dark, viscous blood thought to be a liquefied coagulum or intravascular hematoma, which may accumulate 1–4 weeks following sclerotherapy. For those patients highly susceptible to pigmentation, such as African-American patients, the use of glycerin as a sclerosant agent is highly recommended.

Pigmentation clears in 70% within 6 months but rarely persists for greater than a year (36,38). Attempts to hasten resolution of pigmentation have been mostly unsuccessful as the

pigment is dermal hemosiderin and not epidermal melanin. Bleaching agents, exfoliants such as trichloroacetic acid or phenol, cryotherapy, various lasers, and intense pulsed light have achieved limited success (39,40). The Q-switched ruby laser has been found to be the most consistently effective for treatment of postsclerosis pigmentation (41). Our experience with multiple wavelengths of Q-switched lasers indicate that ruby, alexandrite, or Nd:YAG Q-switched lasers may be successfully applied to clear the pigmentation more rapidly. We have also noted improvement with a picosecond alexandrite device utilizing a diffractive lens array handpiece.

##### **Telangiectatic Matting**

Telangiectatic matting is defined as the appearance of groups of new, fine (<0.2 mm diameter) telangiectasias surrounding or replacing a previously treated area in a blush-like manner. A retrospective analysis of over 2000 patients reports an incidence of 16% in patients treated with HS and POL (42). Resolution usually occurs spontaneously within a 3–12 month period with 70%–80% spontaneous resolution within the first 6 months (43).

Matting may also occur as a result of trauma to the leg in association with pregnancy or hormonal therapy, or in scars around previous sites of surgical stripping. Predisposing factors include predilection for certain areas of the leg, such as the medial lower thigh, obesity, hormonal therapy with estrogen, family history, and a longer history of telangiectasias (42). The relative risk factor for development of telangiectatic matting is 3.17 times greater for female patients taking hormonal supplements (44). Successful treatment of matting with a pulsed-dye laser (PDL) is reportedly accompanied by temporary hyperpigmentation (45). The use of enhanced visualization with a cross-polarized light source (Syris Scientific, LLC, Grey, MA) has been found to assist injection of sclerosing solution into telangiectatic matting. Treatment is often not required since matting will resolve spontaneously except when caused by a source of reflux superiorly.

##### **Cutaneous Necrosis/Ulceration**

Cutaneous ulceration may occur with all sclerosing solutions in spite of the most skilled technique. Unavoidably, a tiny amount of sclerosing solution may be left along the needle tract as the needle is withdrawn. Sclerosing solution may also leak out into the skin through the small puncture sites of vessel cannulation. The varicose vein may have a fragile, thin wall, with the injection causing rapid injury leading to sudden unexpected rupture with perivascular accumulation of sclerosant. Additionally, injection may inadvertently occur into a small arteriole associated with telangiectatic varicosities with resultant necrosis and ulceration.

When the dermatologic surgeon recognizes that extravasation has occurred the risk for necrosis can be minimized by injecting normal saline in a ratio of 10:1 into the extravasation site. Extensive massage of small subcutaneous blebs to spread the trapped sclerosing agent as quickly as possible will minimize prolonged blanching of the area. We have found that the application of topical 2% nitroglycerine paste applied immediately to the suspected extravasation site greatly reduces the risks of necrosis but will not always prevent it.

##### **Superficial Thrombophlebitis**

This complication is most commonly mistaken for the normal nodular fibrosis (endosclerosis) that occurs with proper sclerotherapy. After sclerotherapy, a nontender, nonpigmented, non-erythematous fibrotic cord may normally be palpable along the



(a)



(b)

**Figure 34.3** Foamsclerotherapy. (a) Injection of foam. (b) Persistence of foamed sclerosant at 2 minutes post-injection.



course of a treated 4–8 mm vein. This frequent finding is due to a liquefied intravascular hematoma with surrounding vein wall sclerosis and is not a thrombus. In contrast, superficial thrombophlebitis is characterized clinically by a very tender, indurated, linear erythematous swelling. Incidence of superficial thrombophlebitis is quite variable, estimated at 0.01%–1% following sclerotherapy (46), although some report that the incidence is higher than typically reported (47). Treatment consists of leg elevation and/or compression and regular administration of aspirin or other nonsteroidal anti-inflammatory drugs. Extension of superficial thrombophlebitis into the deep system is extremely rare, so aggressive anticoagulation is not the usual course of therapy.

#### Pulmonary Embolism

Pulmonary emboli probably occur from extension of a superficial thrombus into the deep venous system. Evidence of extension from superficial thrombus to deep thrombophlebitis should be treated promptly by anticoagulation. The incidence of pulmonary embolism has been associated with injection of large quantities of sclerosant at a single site. The incidence is extremely low with less than 1 in 40,000. We have not seen this complication in treatment of over 20,000 patients.

#### Arterial Injection

This dreaded medical emergency is fortunately extremely rare. Classic warning signs include immediate intense pain far beyond the normal discomfort at the initiation of injection, although leakage of sclerosant into the arterial circulation may present in an atypical fashion. Continuous intense burning pain with immediate bone-white cutaneous blanching over an area of several square centimeters is the usual initial sign. Progression to a sharply demarcated cyanosis within minutes is typical for arterial injection. Emergency treatment involves immediate application of ice, attempts to flush the inadvertently injected artery with normal saline and/or heparin, injection of 3% procaine to inactivate STS, and vascular surgery consultation for intravenous anticoagulation.

A major clinical problem is that arterial injection may rarely not be accompanied by any pain or cutaneous signs. The atypical cases are suspected to arise from arteriovenous malformations (AVMs), which allow sclerosant to enter the arterial system via the venous system (48). This is most commonly seen in the popliteal fossa. Arterial injection may lead to wide areas of skin necrosis and damage to subcutaneous tissue and muscle which take months to heal.

#### Neurologic Events

Neurologic side effects are a rare complication of sclerotherapy. In a recent review of 10,819 patients undergoing both liquid and foam sclerotherapy, 97 patients (0.90%) were found to experience neurologic events, including speech and visual disturbances, migraines, cerebrovascular accidents (CVAs) and transient ischemic attacks (TIAs) (49). In total, there were 12 reported CVAs and 9 TIAs, occurring both with liquid and foamed sclerotherapy, with the majority of symptoms resolving by the time of discharge. These events are hypothesized to occur secondary to sclerosant particles entering the cerebral vasculature via a patent foramen ovale (PFO), although not all affected patients have this malformation (49). In a separate study of 3259 patients who underwent ultrasound-guided foam sclerotherapy, seven (0.21%) experienced side effects including visual disturbances, migraines, and chest discomfort, all of which resolved by 2 weeks (50). Notably, five of these patients were found to have a PFO. Accordingly, a known

symptomatic PFO is considered a contraindication to foamed sclerotherapy by the 2nd European Consensus Meeting on Foam Sclerotherapy (51). When a TIA or CVA is suspected during treatment, the patient should be placed on oxygen in the office and transferred to a facility that administers hyperbaric oxygen (52–54).

## Modern Minimally Invasive Surgical Approaches for Varicose Veins

### *Endovenous Occlusion Techniques*

When it has been determined by ultrasound that the originating point of reverse flow or reflux is the great saphenous vein or small saphenous vein, endovenous radiofrequency or laser techniques are the treatment of choice. The efficacy for RF elimination of reflux is 90% at 2 years (55) and is now known to give similar results at 10 years follow-up (56). This method involves the placement of a catheter within the varicose vein through a small puncture or incision. The catheter is threaded up to the saphenofemoral junction typically under duplex ultrasound guidance. Following placement of tumescent local anesthesia between the vein and the skin and or in a perivenous location, energy is applied as the catheter is slowly withdrawn. This results in collagen shrinkage of the vein wall accompanied by complete occlusion (19). Midterm studies suggest a very favorable comparison to traditional ligation and stripping with far less morbidity without the risks of general anesthesia (55).

The other endovenous technique involves the use of laser energy. This technique is termed endovenous laser treatment (EVLT). Very similar to RF occlusion, this technique involves the placement of a laser fiberoptic via a small puncture. Wavelengths presently utilized are 810 nm, 940 nm, and 980 nm. The newest wavelength, 1320 nm, which is absorbed only by water, has been shown to provide superior results (57). The primary problem with wavelengths absorbed by hemoglobin is the requirement for blood for laser absorption (20). This leads to increased risks of bruising, pain, and skin burns. A wavelength absorbed by water only appears to eliminate these side effects by contracting the vein with far less heat generation. Accordingly, endovenous laser ablation with a wavelength of 1320 nm (CTEV™, CoolTouch Corporation, Roseville, CA) has become our preferred modality for laser ablation and recently published long-term results have confirmed its superiority over alternative modalities (58).

A promising future treatment option for great saphenous insufficiency is a recently FDA-cleared injectable adhesive that works to seal the great saphenous vein (VenaSeal™ Saphenous Closure System, Medtronic, Inc., Dublin, Ireland) (59). With this system, incompetent great saphenous veins are treated with an endovenous cyanoacrylate adhesive, injected under ultrasound guidance without the need for tumescent anesthesia or postoperative compression stockings. Two-year follow-up data has been promising, with a 92% occlusion rate (59).

### *Surgical Ligation and Limited Stripping*

For larger varicose veins, particularly originating from an incompetent valve at the saphenofemoral junction, ligation of the greater saphenous vein with short stripping of its proximal half in the thigh is the traditional surgical method but has been replaced by endovenous ablation by RF or laser. After proximal ligation without stripping of the saphenous vein, varicography has shown persistent mid-thigh perforator

incompetence in 34%, a patent portion of saphenous vein in 54%, and residual or recurrent femoral-saphenous communication in 80% (60). High ligation combined with sclerotherapy or with varicosity excision was inferior to high ligation and stripping of the saphenous vein (61). The technique of ligation and stripping has virtually been replaced by endovenous techniques.

#### Ambulatory Phlebectomy

This technique, originally described by Robert Muller and further refined by another Swiss dermatologist, Albert-Adrien Ramelet, involves the use of tiny incisions through which the varicose vein is removed by a small hook (62,63). This safe, outpatient local-anesthesia technique allows removal of almost any varicose vein except the saphenofemoral or saphenopopliteal junction. Ambulatory phlebectomy is used for primary or secondary branches of saphenous-related varicosities. Areas or veins that are resistant to sclerotherapy (axial) are particularly indicated for ambulatory phlebectomy (Figure 34.4). Risks minimized compared with sclerotherapy are DVT, postsclerotherapy pigmentation, skin necrosis, and superficial phlebitis. In many cases larger varicose veins coexist with smaller reticular veins and associated telangiectatic webs. It is reasonable to treat

larger varicose veins by various surgical techniques and follow up with sclerotherapy of the remaining reticular networks.

#### Lasers and Light Sources

New trends for improved results with lasers and light sources for spider veins include longer wavelengths, larger spot sizes, and cooling to protect the skin. The first report of 1064-nm Nd:YAG laser indicated that 75% improvement was possible after a single treatment at 3 months (64). These findings were confirmed and mechanism of action explained as heat-induced vessel damage and subsequent fibrosis (65). Recent reports also indicate the effectiveness of a 940-nm diode laser (66). Shorter wavelengths used in the past, like PDL, are useful on fine leg telangiectasias, such as telangiectatic matting, especially with longer pulse durations up to 40 milliseconds. A broadband, noncoherent intense pulsed light (IPL) has been reported to improve 70% of patients responding with up to five treatments per region (67). In our practice, the vast majority of laser treatments are performed using 1064-nm Nd:YAG in the millisecond domain on isolated telangiectasias, sclerotherapy-resistant telangiectasias, ankle telangiectasias, and suspected AVMs. We also use PDL for fine telangiectasias, especially using an elliptical optical spot at 10 milliseconds or longer which can be oriented along the long axis of telangiectasia of the leg (68).



(a)



(b)

**Figure 34.4** Ambulatory phlebectomy of a large truncal varicose vein. (a) Before. (b) After AP and endovenous RF occlusion of the great saphenous vein. It is important to eliminate the source of reflux into this vein concomitant with the ambulatory phlebectomy of this primary branch arising from the great saphenous vein.

#### SUMMARY

Phlebology is an integral part of dermatological surgery. The method of endovascular chemoablation or sclerotherapy is the gold standard for treatment of telangiectasias and small varicosities. A supplemental technique for telangiectasias is laser. Larger veins that originate from saphenous reflux require endovenous RF or laser techniques for effective treatment. These techniques replace traditional stripping and ligation. Primary or secondary branches of saphenous varicosities can be treated by ambulatory phlebectomy or the newest technique of foam sclerotherapy.

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# Management of Hirsutism and Hypertrichosis

Ralph M. Trüeb and Daisy Kopera

## INTRODUCTION

The queen [of Sheba] was brought before him, and when she saw the king [Solomon] sitting in his glass house, she thought within herself that the king sat on water, and so proceeded to draw up the hem of her dress so that she could pass over without getting wet. The king then saw her legs that they were full of hair, and when the queen had sat down beside him, he said unto her, "Thy beauty is the beauty befitting women, but thy hairs are the hairs befitting men. Hair on a man's body is comely, but uncomely on a woman's." Now the king greatly desired her beauty, but was taken aback by the hair on her legs, and so it was that he devised a method by which unwanted hairs may be removed, that is, by taking an admixture of lime and water and orpiment [arsenic trisulfide], which the king himself discovered and made known its usage abroad, calling it neskasir. When the queen had bathed herself that night in its solution, the hair on her legs fell off, and she found favor in the eyes of the king (from the Midrash Book of Proverbs).

Throughout the history of civilized mankind, overgrowth of hair has been of significant concern, the object of superstition and mystery, as well as of cosmetic and medical interest. Excessive hair may cause cosmetic embarrassment, resulting in a significant emotional burden, particularly if extensive. Sometimes the complaint of excess hair may pose a vexing problem for nonspecialized physicians, who tend to trivialize the complaint, though an individual's perception of abnormality is important in determining whether or not medical care is sought. Besides significant racial and ethnic differences in normal hair growth patterns, the role of society is to set the threshold level for "normality," which is much determined by advertisement for cosmetic treatments. With the advent of effective laser epilation devices this increasingly also applies to the perception of excess hair by men.

No single method of hair removal is appropriate for all body locations or patients, therefore patients should be adequately advised of the available treatment modalities for temporary or permanent hair removal. The method adopted for removal of unwanted hair will depend on the cause, the character, area, and amount of excessive hair growth, as well as on the age of the patient, and personal preference (1–3).

## CAUSES OF EXCESSIVE HAIR

### Hirsutism

Hirsutism is characterized by androgen-dependent, excessive growth of terminal hairs in women in a pattern more characteristic of adult men. Such androgen-dependent areas include the chin, upper lip, chest, breasts, abdomen, back, and anterior thighs (Figure 35.1). Objective assessment of

hirsutism can be obtained by evaluation and grading of hair growth and distribution according to the Ferriman-Gallwey (F-G) scale (Figure 35.2). The F-G score quantifies the extent of hair growth in nine key anatomic sites, which is graded using a scale from 0 (no terminal hair) to 4 (complete and heavy cover), for a maximum score of 36 (4). Hirsutism is defined by a score of 8 or more. Approximately 5% to 10% of women of reproductive age in the general population are hirsute, assessed as having a F-G score of 8 or more (5). A 1993 market survey of 25,000 women in the United States concluded that 41% of women remove unwanted hair and 22% remove facial hair on a weekly basis (6). But far from being only a cosmetic problem, hirsutism may be a marker for an underlying hormonal disorder, and identification of the underlying etiology helps to detect patients at risk for infertility, diabetes mellitus, cardiovascular disease, and endometrial carcinoma (6).

Hirsutism results from either an exogenous or endogenous increase in circulating androgens or from individually increased sensitivity (metabolism) of the hair follicle to normal serum androgen levels. Exogenous sources include androgenic medications, such as oral contraceptives with androgenic progestins (norgestrel, levonorgestrel, norethindrone), anabolic steroids (danazol), high-dose glucocorticoids, androgen therapy (testosterone), and valproic acid (raises plasma testosterone). Endogenous androgens in women arise from the ovary or adrenal glands, and peripherally from the skin and fat. There is thought to be considerable heterogeneity of responses among androgen-dependent follicles in different individuals (7). Therefore, the clinical severity of hirsutism does not always correlate well with expected levels of circulating androgens.

Seventy to eighty percent of patients with androgen excess demonstrate hirsutism (8), with the most common cause being polycystic ovary syndrome (PCOS). Up to 6% of women overall are affected by PCOS in whom hirsutism is the most common symptom. PCOS represents a syndrome of hyperandrogenic anovulation that is due to an intrinsic ovarian dysfunction, which is often aggravated by insulin-resistant hyperinsulinemia with its risks of diabetes mellitus, metabolic syndrome, and their complications (9). Other, less frequent causes of androgen excess are late onset congenital adrenal hyperplasia, Cushing syndrome, and hyperandrogenism, insulin resistance, and acanthosis nigricans (HAIR-AN) syndrome (Figure 35.3). Pituitary, ovarian, and adrenal tumors are important but rare causes of hirsutism.

Hirsutism is deemed idiopathic where it develops in the absence of detectable androgen excess and in conjunction with regular ovulation. It accounts for less than 20% of hirsute women (5). The definition of idiopathic hirsutism has been



**Figure 35.1** Hirsutism: excessive growth of terminal hairs in androgen-dependent areas: (a) chin, (b) chest, (c) abdomen, (d) thighs, and (e) lower back. (From Trüeb RM, *Haare Praxis der Trichologie*, Darmstadt, Germany: Steinkopff, 2003.)

an evolving concept, since normal androgen levels have been defined by conventional laboratory tests, while more sophisticated testing methods may uncover occult ovarian or adrenal functional hyperandrogenism in quite a number of these patients (10).

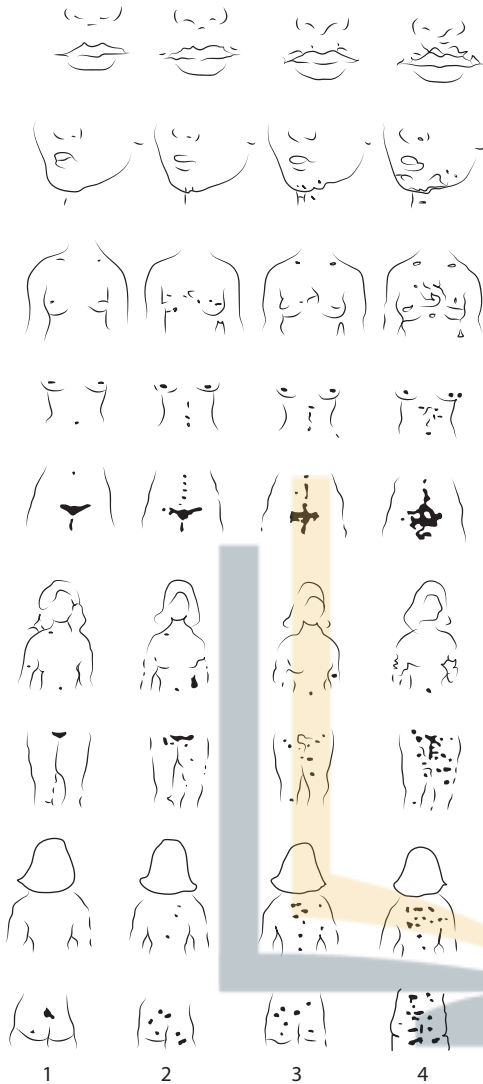
### Hypertrichosis

Hypertrichosis is the term used for excess growth of hair compared to the “normal” amount of hair in persons of the same age, ethnicity, and sex, in any part of the body, excluding androgen-induced hair growth (11). It may involve lanugo (hypertrichosis lanuginosa), vellus, or terminal hairs, and is classified on the basis of the age of onset (congenital or

acquired), the extent of distribution (generalized or circumscribed), and the site involved (12). In both its generalized and circumscribed forms, hypertrichosis may be an isolated finding, or be associated with other abnormalities. For instance, lumbosacral hypertrichosis (Figure 35.4) frequently indicates occult spinal defects, and it is essential that this possibility should be excluded as early as possible, if neurologic sequelae are to be prevented.

Universal congenital hypertrichosis (Figure 35.5) is a rare and particularly dramatic familial disorder. In former times such individuals were sought after by sovereigns to adorn their courts, or exhibited by showmen to satisfy the curiosity of the public. Since the first well-documented observation concerns a man named Petrus Gonsalvus, born in 1556, and





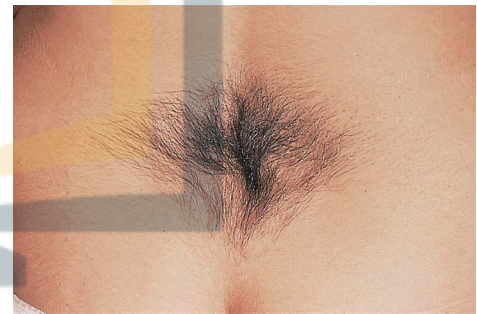
**Figure 35.2** Ferriman-Gallwey score: hair growth is rated from 0 (no growth of terminal hair) to 4 (complete and heavy cover) in nine locations, giving a maximum score of 36. (Adapted from Trüeb RM, *Haare Praxis der Trichologie*, Darmstadt, Germany: Steinkopff, 2003; Ferriman D, Gallwey JD, *J Clin Endocrinol Metab*; 21:1440–7, 1961.)

his family, whose portraits are shown in the castle of Ambras near Innsbruck, Austria, the term Ambras syndrome has been coined for this disorder (13). The whole body is covered with a remarkable amount of long, vellus-type hair, sparing only areas in which ordinarily no hair grows, including palms, soles, and mucosae. The forehead, eyelids, nose, cheeks and preauricular region are uniformly covered with hair, reaching a length of several centimeters. The hair is light-colored and silky; only the scalp hair, eyebrows, eyelashes and the axillary hair are darker. No decrease of hairiness during later life occurred in any of the well-documented cases.

Prepubertal hypertrichosis (Figure 35.6) is a generalized terminal hair hypertrichosis affecting healthy children (14). There is hair growth on the temples spreading across the forehead, bushy eyebrows, and marked growth of hair on the upper back and proximal limbs. The condition is usually noted



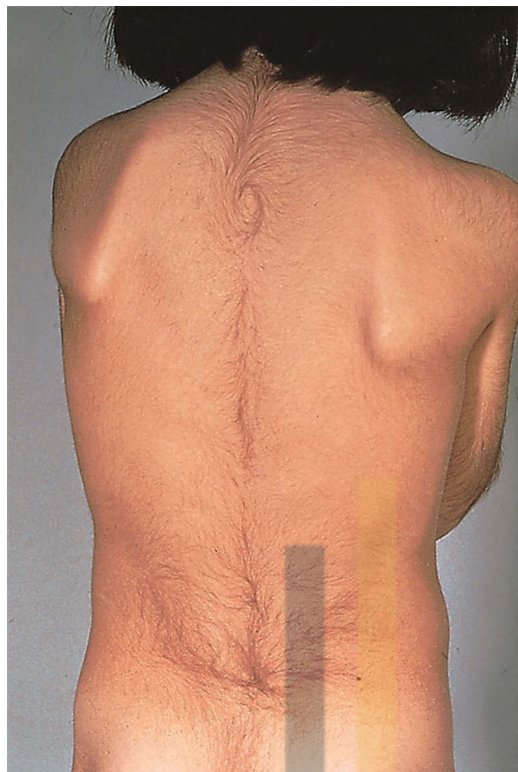
**Figure 35.3** HAIR-AN: woman with hirsutism and acanthosis nigricans of the lateral neck area. (From Trüeb RM, *Haare Praxis der Trichologie*, Darmstadt, Germany: Steinkopff, 2003.)



**Figure 35.4** Lumbar hypertrichosis should draw the attention to the possibility of underlying spinal dysraphism with potential neurologic sequelae. (From Trüeb RM, *Haare Praxis der Trichologie*, Darmstadt, Germany: Steinkopff, 2003.)



**Figure 35.5** Universal congenital hypertrichosis or Ambras syndrome: congenital generalized vellus-type hair hypertrichosis.



**Figure 35.6** Prepubertal hypertrichosis: congenital generalized terminal hair hypertrichosis. (From Trüeb RM, *Haare Praxis der Trichologie*, Darmstadt, Germany: Steinkopff, 2003.)

at birth, increases in severity during early childhood, and is not as rare. It probably often has been confused with “racial hirsutism,” though it is neither limited to a specific ethnicity nor androgen mediated.

Congenital generalized hypertrichosis has been associated with a number of abnormalities, such as gingival fibromatosis, osteochondrodysplasia, or congenital amaurosis, or may be a symptom of a more complex syndromatic disorder, such as Cornelia de Lange syndrome, the mucopolysaccharidoses, and porphyrias (11). In these cases, excessive body and facial hairs is either present at birth or develops during early infancy or puberty, and frequently involves terminal hairs.

A variety of underlying pathologic states may give rise to acquired generalized hypertrichosis. These include head injuries and other cerebral disturbances, malnutrition and anorexia nervosa, juvenile hypothyroidism, and juvenile dermatomyositis (11). Besides acquired trichomegaly (overgrowth of eyelashes), a more generalized form of hypertrichosis has also been observed in patients with AIDS (15).

Acquired hypertrichosis lanuginosa describes the sudden onset and rapid growth of long, fine, lanugo-type, white-yellow downy hairs over a large area of the body in association with an underlying malignancy. The growth of lanugo (so-called “malignant down”), particularly on the face of patients with malignant disease, is not uncommon (16). The lanugo may develop a few weeks or up to 2 years before an underlying malignancy is diagnosed.

Yet another form of paraneoplastic hypertrichosis may be found in the POEMS (peripheral neuropathy, organomegaly,



**Figure 35.7** Acquired generalized terminal hair hypertrichosis in POEMS syndrome. (From Trüeb RM, *Haare Praxis der Trichologie*, Darmstadt, Germany: Steinkopff, 2003.)

endocrine dysfunction, monoclonal gammopathy, and skin changes) syndrome (Figure 35.7) (17). This particular type of hypertrichosis producing terminal hair is most common on the extensor surfaces, malar areas, and forehead.

Finally, several drugs are well known to cause significant generalized hypertrichosis, the most frequent currently being corticosteroids, phenytoin sodium, cyclosporin A, and minoxidil (Figure 35.8) (11). Discontinuation of the offending drug leads to resolution of drug-induced hypertrichosis within several months to 1 year, depending on the hair cycling characteristics of the affected site (face, 3 months; arms, 1 year).

## MANAGEMENT OF EXCESSIVE HAIR

Accurate classification of excess hair must precede treatment and includes characterization of the type of hair involved (lanugo, vellus, terminal), characterization of the pattern of hair growth (generalized or circumscribed hypertrichosis, hirsutism), age at initial manifestation (congenital, acquired), patient history with regard to systemic disorders or drug intake, physical examination for associated abnormalities, and family history, including ethnic and racial background (11). It must always be kept in mind that hair overgrowth, either hirsutism or hypertrichosis, may be a manifestation of a more general medical problem that needs to be investigated. Testing of androgen levels is recommended in women with moderate to severe hirsutism (F-G score of 15 or more), and in women with any degree of hirsutism when it is sudden in onset, rapidly progressive, or when it is associated with any of the following: menstrual irregularity, central obesity, acanthosis nigricans, or clitoromegaly (18).

The current available treatment methods for removal of unwanted hair include cosmetic procedures, medical treatment, and hair removal using lasers and light sources. These treatments may be combined (11,19).

## Cosmetic Procedures for Removal of Excessive Hair

Unwanted hair may be masked by bleaching or removed by a variety of physical methods such as trimming of the hair, shaving, plucking or tweezing, and waxing, or chemical procedures (depilatories), and electrosurgical epilation (20).





(a)



(b)

**Figure 35.8** Drug-induced hypertrichosis: (a) cyclosporin A induced in a child and (b) topical minoxidil induced in a woman. (From Trüeb RM, *Haare Praxis der Trichologie*, Darmstadt, Germany: Steinkopff, 2003.)

#### Bleaching

Bleaching is a quick, easy, and painless process that can make unwanted hair less apparent through the partial or total removal of natural hair pigment, lightening the hair to a yellowish hue. A home bleach is made by mixing 40 mL hydrogen peroxide with 7 mL 20% ammonia. This is left in contact with the hair until the color is removed, usually 5 to 10 minutes. Bleaching can last up to 4 weeks. This method is best for treatment of localized excess pigmented hair on the face or arms of fair-skinned patients, because yellow-bleached hair may emphasize the hair when viewed against the skin of more darkly pigmented patients. Occasionally, bleaching results in skin irritation. The addition of a persulfate to boost the peroxide bleach in commercial products may occasionally result in anaphylaxis in the sensitized individual (21).

#### Trimming

Trimming of the hair is a recommended option for young children with either localized or generalized hypertrichosis, making the hair of involved areas less noticeable, while not resulting in acceleration of hair regrowth.

#### Shaving

While unacceptable for removing facial hair to many women, the majority accepts to shave excess body hair. It is perceived as being fast, effective, easy, and cheap. Contrary to popular belief, shaving does not affect the width or rate of regrowth of individual hairs (22), though the perception of the stubble as it grows out, without the finer tapered end of unshaven hair, may give this impression. As a consequence, daily shaving must be undertaken or the cosmetic result is worsened. The disadvantages of shaving are the need to shave daily, and skin irritation. Although dry or electrical shaves are not as close as wet shaves, a dry electric razor has been effectively used to treat generalized hypertrichosis during the neonatal period (23).

#### Plucking

Plucking is an effective temporary hair removal method, but it is slow, tedious, and painful. It is only appropriate for individual, small groups or scattered coarse hair and is performed with tweezers (tweezing). Adverse reactions to plucking include hyperpigmentation, folliculitis, scarring, ingrown hairs, and distorted follicles. A variation of plucking for treatment of more diffuse hypertrichosis acts as mechanized tweezers using a rotating, fine, coiled spring that grasps the hair shaft and pulls it out. The device is handheld and moved over the skin like a shaver. A drawback of this method is that it is painful.

#### Waxing

Waxing is performed with cold, warm, or hot wax. The wax is applied to hair-bearing areas and then stripped off, epilating the embedded hairs. Waxing methods are an efficient way of plucking vellus hairs in all areas of the body, and inexpensive when performed at home. The major disadvantages are discomfort, poor removal of short hair, and skin irritation or folliculitis. This method is too painful for use on children with hypertrichosis. As with hair plucking, the regrowth period is longer than that for shaving, and it needs to be repeated only every 2 to 6 weeks.

The Asian techniques of sugaring (24) and threading (25) remove hair in the same manner. Instead of using wax, the hairs are plucked out by a caramelized sugar mass or the scissoring action of a twisted thread, respectively.

#### Chemical Depilatories

Chemical depilatories function by damaging the hair to the point where it breaks at the skin surface. Substituted thiols form the basis of practically all contemporary preparations. Depilatories contain detergents to remove the protective sebum from the hair, adhesives that aid the depilatory in sticking to the hair shaft, swelling agents for better penetration of the bond-breaking agent, pH adjusters, and disulfide bond-breaking agents (thioglycolic acid, calcium thioglycolate). Thioglycolates are used in a concentration of 2% to 4%, and act within 5 to 15 minutes. Since thioglycolates attack keratin, and the hair shaft and skin are similar in their keratin composition, most chemical depilatories hold a high irritancy potential and may have adverse effects on the skin if the manufacturers' recommendations are not carefully followed. Additional adverse effects from the use of thioglycolates include allergic contact dermatitis, and with inadvertent eye contact, corneal alkali burns (26,27). Their application is messy, and they have an unpleasant odor and are relatively expensive, especially if treating larger areas. Chemical depilatories are most appropriate for weekly hair removal from small areas. In children with extensive



hypertrichosis, treatment with chemical depilatories should be limited to localized sites because of a theoretical risk of additional toxicity from systemic thioglycolate absorption (28).

#### *Electrosurgical Epilation*

In contrast to other cosmetic procedures for hair removal, electrosurgical epilation represents a permanent mode of hair removal. Treatment involves the insertion of a disposable fine wire needle into the hair follicle. Through this instrument a regulated and controlled electric current is transmitted from a sophisticated apparatus known as an epilator. The procedure has to be performed by a highly trained professional in order to be effective, as the hair bulb should not be missed by the fine needle. Three techniques are available: galvanic electrolysis, thermolysis, and the blend method (29,30).

Galvanic electrolysis uses galvanic current to destroy the hair growing cells of the hair follicle. This involves a direct current producing electrochemical sodium hydroxide formation, congealing the hair follicle. Thermolysis uses high-frequency electrocoagulation that cauterizes the hair follicle. Galvanic electrolysis is slower, but destroys more follicles in one treatment, while thermolysis is quicker, but more regrowth is seen with this method. The blend method combines both galvanic and high-frequency current from a single machine, and is considered by most users to be the most effective method of electrosurgical epilation.

Disadvantages of electrosurgical epilation are the length and number of treatments required for permanent removal of hair from a particular body site and discomfort during treatment, which is why the method is poorly tolerated in children. Generally, operators can only deal with 25 to 100 hairs per session, and individual treatments last from 15 minutes to 1 hour. Problems that may occur are perifollicular inflammation, postinflammatory hyper- or hypopigmentation, and less frequently, punctate scarring. The method is most suitable for treatment of localized, coarse hair.

#### *Medical Treatment*

Since abnormal hair growth in hirsutism is either stimulated by excess androgens or is related to hair follicle sensitivity to androgens, hormonal treatment of hirsutism is based on suppressing androgen production or counteracting the biologic activity of androgens.

Today, for the majority of women with hirsutism, a monotherapy with oral contraceptive pills (OCPs) that have antiandrogenic activity is recommended as first-line treatment. Antiandrogens may be the first-line therapy for postmenopausal women, or may be indicated in conjunction with OCPs in premenopausal women if clinical improvement is insufficient after 6 or more months of monotherapy. The choice between the different antiandrogens depends on patient preferences regarding efficacy, side effects, and costs (31). Antiandrogens including spironolactone, cyproterone acetate, and flutamide, or the 5 $\alpha$ -reductase inhibitors such as finasteride, should not be used in women of childbearing age unless they are strictly combined with a safe contraception method due to their potential feminizing effect on the male fetus (18, 32).

In women with hirsutism, hyperandrogenism, and insulin resistance, insulin sensitizers such as metformin and rosiglitazone are effective for the treatment of hirsutism as well as hyperinsulinemia, hyperandrogenism, and infertility, but there is no convincing evidence that they are effective for hirsutism itself (33,34).

Topical eflornithine cream is a medical treatment for slowing excessive hair growth but not removing excess hair (35). The compound is a specific and irreversible inhibitor of the enzyme ornithine decarboxylase that is important for hair growth and present in hair follicles. In clinical studies in women with facial hirsutism, twice daily application of eflornithine hydrochloride monohydrate 15% cream (Vaniqa, Bristol-Myers Squibb, New York, NY) was superior to placebo in reducing hair growth, as demonstrated by objective and subjective methods, after 2- to 8-week treatment. After 24-week treatment, 58% of eflornithine and 34% of placebo recipients had at least some improvement, and 32% versus 8% of patients were judged to be successfully treated (at least marked improvement) (36). Hair growth returned to pretreatment rates within 8 weeks of stopping treatment. Local irritation, characterized by burning, stinging, and/or tingling, occurred more frequently in eflornithine-treated patients. Use of eflornithine cream in combination with other therapies, including laser epilation, can be effective and results in a more rapid, visible hair reduction compared with laser monotherapy (37,38). The safety and efficacy of topical eflornithine treatment for widespread hypertrichosis and children has not been established.

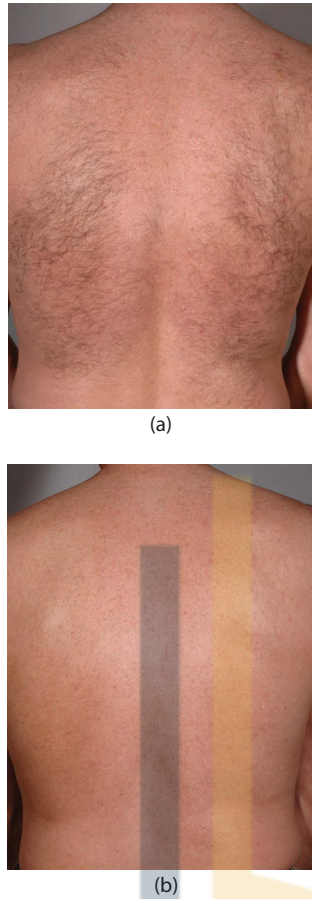
Topical application of finasteride 0.5% solution combined with intense pulsed light (IPL) treatment has also been tested (39). Various herbal oils have been shown to have some value in reducing hair in young women (40).

#### *Hair Removal Using Lasers and Light Sources*

The need for a rapid, noninvasive method for hair removal has led to the development of various laser and light sources for this purpose. An array of devices is now available. All are based on the principle of selective photothermolysis: melanin pigment in the hair follicle provides the chromophore for selective targeting of hair follicles, while the surrounding dermis is spared. Therefore, at deeply penetrating wavelengths in the 600- to 1100-nm range, melanin absorption may be used for selective photothermolysis of hair follicles (41).

Several hair removal systems have been shown to be effective in this setting: long-pulsed ruby lasers (wavelength 694 nm; pulse duration 1–3 msec), long-pulsed alexandrite lasers (755 nm; 2–20 msec), diode lasers (810 nm; 5–400 msec), long-pulsed neodymium:yttrium-aluminum-garnet (Nd:YAG) lasers (1064 nm; 5–250 msec), and IPL sources (590–1400 nm; 2.5–5 msec) (42–45). The physical parameters (wavelength, pulse duration, fluence, spot size, repetition rate) and cooling systems used with each system vary considerably. Regardless of the type of laser or light source used, all systems have been shown to temporarily reduce hair growth. Long-term, controlled hair counts have shown an average of 20% hair loss with each treatment, indicating the need for multiple treatments to achieve satisfactory results. Research indicates that in 80% of patients significant hair reduction can be achieved (Figure 35.9), while 20% will fail (42). Effectiveness for long-lasting hair reduction is strongly correlated with hair color. Blond-, red-, or white-haired patients are unlikely to experience a permanent reduction. In contrast, the patient with dark hair and fair skin may experience long-term hair removal after a single treatment (43). Regrowing hairs are often thinner and lighter in color, contributing to the improvement in the overall appearance.

Although there is no obvious advantage of one laser system over another in terms of treatment outcome, laser parameters may be important for choosing the ideal laser for



**Figure 35.9** Terminal hair growth of the back in a male patient (a) before and (b) 6 months after fifth in-office intense pulsed-light treatment (delivered at 2-month intervals).

a patient (44). A major drawback to using shorter wavelengths, for example, 694 nm ruby, is that a more deeply pigmented epidermis impedes laser radiation penetration of the dermis. The amount of light that reaches the hair bulb is therefore reduced with decreased efficacy. At the same time, unwanted epidermal injury may occur. Patients with darker skin tones should therefore receive laser treatment with either lower fluences of alexandrite and diode laser or with a long-pulsed Nd:YAG laser.

Adverse effects commonly include erythema and perifollicular edema, while crusting and vesiculation of treatment site, hypopigmentation, and hyperpigmentation are less frequent, depending on skin color and other factors (45,46). Most complications are usually temporary, and their incidence can be reduced by lightening of the skin and UV light avoidance prior to laser treatment, effective cooling of the skin during treatment, and sun avoidance and protection following treatment (44). Shaving the hair-bearing site is performed preoperatively to prevent conduction of thermal energy to the adjacent epidermis from overlying hairs. In a study conducted on 242 hirsute patients the mean hair plucking interval was extended 4.11 times, from a median of 3.69 days before treatment to an average of 15.19 days after diode laser epilation, inducing a well accepted increase in quality of life and selfconsciousness (47), as similarly in other studies (48).

One rare and peculiar unwanted effect of laser-assisted hair removal is the stimulation of new hair growth within previously treated areas or in close proximity. This “paradoxical effect” has occasionally been seen following treatment with each of the laser and light hair removal systems, and its development has been attributed to activation of previously dormant hair follicles by either the application of subthreshold fluences or the conduction of heat to surrounding areas (49,50).

As with traditional electrosurgical epilation, laser epilation is painful, which limits its usefulness in children with widespread hypertrichosis (6).

Since removing hair with in-office laser- or light-based treatments is expensive and requires multiple treatments sessions, a novel, low-energy, pulsed-light device for home-use hair removal (Silk'n1, Home Skinovations Ltd., Yokneam, Israel) (Figure 35.10) has been developed to overcome these disadvantages. The device is composed of two flashlamps in a handheld applicator (optical filter 475–1200 nm; fluences up to 5 J/cm<sup>2</sup>). A study was performed with 20 women with Fitzpatrick skin phototypes I to IV and dark terminal hair in nonfacial sites (axilla, forearms, inguinal region, legs) who self-administered three treatments at 2-week intervals using the device. Matched untreated skin sites were compared. Hair counts and clinical photographs were obtained pretreatment, and at 1, 3, and 6 months after the third treatment, side effects and patient satisfaction scores were recorded. All patients showed a positive clinical response to treatment, with reduction of unwanted hair, while no hair reduction was noted in untreated matched areas. Hair counts were reduced 37.8% to 53.6% six months after the three treatments. Lower legs exhibited greater hair reduction than arms, inguinal, and axillary areas. Mild erythema was experienced in 25% of patients, but no other unwanted effects were encountered. Patient satisfaction scores were high, with all patients stating that they would purchase the device for future home use (51, 52).



**Figure 35.10** Silk'n1: handheld intense pulse light device for home-based hair removal.

## CONCLUSIONS

Removal of unwanted hair is the most popular skin treatment worldwide. Excessive hair may cause cosmetic embarrassment, resulting in significant emotional burden. Sometimes the complaint of excess hair may pose a vexing problem for nonspecialized physicians, who tend to trivialize the complaint, though an individual's perception of abnormality is important in determining whether or not medical care is sought. Moreover, it must always be kept in mind that hair overgrowth, either hirsutism or hypertrichosis, may be a manifestation of a more general medical problem requiring investigation. The dermatologist seeing a patient with hirsutism or hypertrichosis must be prepared to look for physical clues and perform the workup that will help both define the extent of excess hair and suggest the presence of associated disorders. There is no single method of hair removal appropriate for all patients or body locations. The method adopted will depend on the cause, the character, the area, and the amount of excessive hair growth, as well as on the age of the patient, and personal preference. The best treatment of hirsutism is often a combination of medical therapy and the physical removal of unwanted hair. Over the recent past, hair removal using various efficacious lasers and light sources have been advocated for use in an office setting, although most people continue to remove unwanted hair with a variety of temporary physical methods in a home setting, presumably due to cost and convenience factors.

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## Pigmentation: Dyschromia

Thierry Passeron and Jean-Paul Ortonne

Variations of skin pigmentation are mostly due to quantitative or qualitative defects of melanin pigments (eumelanin and pheomelanin). However, dyschromia can also result from an abnormal increase or decrease of other endogenous pigments (hemoglobin, bilirubin ...) or from the deposit of exogenous pigments (heavy metals, cosmetic tattoos ...). This leads to a heterogeneous group with numerous causes. Although most of the patients will consult for the cosmetic disturbance due to the dyschromia, it is essential to determine the origin of the pigmentary trouble in order to propose the more adapted treatment and if necessary to ask for complementary investigations.

### PATHOPHYSIOLOGY OF DYSCROMIA

The color of the skin results from the presence of pigments in the epidermis and in the dermis. The melanins (eumelanin, dark brown, mostly produced by dark skin types, and pheomelanin, red-fair brown, mostly observed in fair skin types) are the most important pigments in human skin. However, other endogenous pigments such as hemoglobin and bilirubin also play a role in the color of the teguments. Dyschromia can result from a darkening, a lightning, and the occurrence of an unusual skin color (1). Quantitative or qualitative defects in the production or in the deposition of melanin explains most of the pigmentary disorders but abnormal variations of other endogenous pigments and deposit of exogenous pigments also lead to dyschromic lesions.

An increased amount of melanin in the skin is called hypermelanosis or melanoderma. A brown hypermelanosis is caused by excessive amounts of melanin within the epidermis whereas ceruloderma (or blue hypermelanosis) results from large amounts of melanin in the dermis. Mixed hypermelanosis is characterised by an excess of melanins in both epidermis and dermis may also occur. Epidermal hypermelanosis may result from increased melanin production by a quantitatively normal melanocyte density in the epidermis (melanotic hypermelanosis) or by an increased number of epidermal melanocytes (melanocytic hypermelanosis). Dermal hypermelanosis can be due to the production of melanin by ectopic dermal melanocytes (dermal melanocytosis) or to an abnormal transfer of melanin from epidermal cells to the dermis (pigmentary incontinence). In this situation, melanin granules accumulate within melanophages or may be free in the extracellular matrix of the dermis.

Skin lightening or whitening (leukoderma, hypopigmentation) is most commonly the result of decreased melanin content in the skin (hypomelanosis). Epidermal hypomelanosis may be the result of at least two different pathogenic mechanisms: partial or total absence of epidermal melanocytes (melanocytopenic hypomelanosis) or even melanin synthesis, melanosome

biogenesis, transport and transfer and melanosome transfer despite a normal number of epidermal melanocytes (melanopenic hypomelanosis). Increase of epidermal turn over can also induce hypomelanosis. Hypo- and hyperpigmentation disorders can be inherited or acquired (2,3).

Dyschromia that results from variation of the hemoglobin content within the skin (diffuse such as in anemia or in polycythemia, or localized such as in Bier spots) can be easily distinguished from other pigmentary disorders as the change in color disappears with pressure (Figure 36.1). Xanthoderma describes a yellow to orange macular discoloration of the skin. Jaundice and carotenoderma are the two main causes of xanthoderma. Although patients may consult for the cosmetic disturbance, drug and excessive dietary intake of carotenoids and search for underlying disease have to be performed (4). The treatment is etiological. Heavy metals (e.g., iron, silver, copper ...) and traumatic, medical, or esthetical tattoos are other sources of skin discoloration. An increased thickness of the epidermis can lead to diffuse, patchy or reticulated light to dark brown hyperpigmentation. The chronic avoidance of washing can also induce hyperpigmented and sometimes keratotic patches. Finally, the discoloration of the skin cannot only be due to pigment abnormality within the skin, but also to an abnormal coloration of the sweat (called chromhidrosis or pseudochromhidrosis).

### DEPIGMENTING AGENTS Phenolic Compounds

#### *Hydroquinone*

Hydroquinone (HQ) is the most popular depigmenting agent (5). Several studies have established the therapeutic effect of HQ in the treatment of hypermelanosis (6). HQ is still the "gold standard" of depigmenting agents. The effectiveness of HQ is related directly to the concentration of the preparations, the vehicle used and the chemical composition of the final product. Two percent HQ was reported to improve hypermelanosis in 14%–70% of the patients. However, HQ is most commonly used at a 4% concentration by dermatologists. At this concentration, HQ is very effective, but it can have a significant irritant effect. Concentrations as high as 6%–10% are prescribed extemporaneously for resistant cases, but may be a strong irritant effect. Because of the hazard of long-term treatments, the use of HQ in cosmetics has been banned by the European Committee (24th Dir. 2000/6/EC). Formulations are available only by prescription of physicians and dermatologists. A number of different vehicles can be used for HQ, but the most suitable for the formulation is a hydro-alcoholic solution (equal parts of propylene glycol and absolute ethanol). A nitro-oxidant such as ascorbic acid or sodium bisulphate is regularly used to preserve the stability of the formulation.



**Figure 36.1** Bier spots on the arm of a young adult.

The acute side effects of HQ include irritant and allergic contact dermatitis, nail discoloration, and post-inflammatory hypermelanosis (7). These adverse events are temporary and resolve after HQ discontinuation. Higher concentrations ( $\geq 5\%$ ) may induce persistent hypo or amelanosis (leukoderma en confetti). Exogenous ochronosis is a very rare complication occurring in dark-skinned or black individuals after chronic use. This irreversible disorder presents in the form of reticulated, ripple-like, sooty pigmentation affecting common sites of HQ applications (cheeks, forehead, periorbital areas). The lesions are typically localized on photo-exposed areas. Histological examination of these lesions shows banana-shaped yellow-brown pigment granules in and around collagen bundles in conjunction with giant-cell and melanophage-containing granulomas in the upper dermis.

The pathogenesis of HQ-induced ochronosis is unknown and no effective treatment is available. The mode of action of HQ is not fully understood. HQ seems to exert its effect mainly in melanocyte with active tyrosinase activity. Guidelines and radical oxygen species arising from the oxidation of HQ induce an oxidative damage of membrane lipids and proteins including tyrosinase and depletion of glutathione contributes to the lightening action (8). Other depigmenting pathways attributed to HQ include inhibition of tyrosinase through the covalent binding to histidine or interactions with copper at the active site of tyrosinase, inhibition of DNA and RNA synthesis, and alteration of melanosome formation and melanization extent.

## Hydroquinone Derivatives

### *Monobenzylether d'Hydroquinone*

The clinical use of monobenzylether d'hydroquinone (MBEH) is restricted for generalized depigmentation in patients with extensive vitiligo. MBEH should never be used for the treatment of melasma or post-inflammatory hypermelanosis. Indeed MBEH cause permanent depigmentation of the skin even at sites distant, for those of application. MBEH induced vitiligo depigmentation has been described in dark-skinned individuals. MBEH is metabolized to reactive free radicals inside the cells resulting in melanocyte destruction.

### *Monomethyl of Hydroquinone*

Monomethyl of hydroquinone (MMEH) is also called 4-hydroxyanisole (para-hydroxy-methoxy-benzene). This compound is oxidized by tyrosinase and exhibits strong melano-cytotoxicity. MMEH is used in France at 8% or 10%

concentration for the treatment of various acquired hypermelanoses including melasma and post-inflammatory hypermelanoses. Side effects include irritant and allergic contact dermatitis, post-inflammatory hypermelanosis and leukoderma en confetti at treated sites. Hypomelanosis at sites distant from the application areas have been reported.

## Others

### *4-n-Butylresorcinol (Rucinol®)*

4-n-butylresorcinol has an inhibitory effect on tyrosinase and tyrosinase-related protein (TRP-1). A lotion containing 0.3% 4-n-butylresorcinol has been demonstrated to improve melasma. This product also decreases post-inflammatory hyperpigmentation following laser therapy (9).

### *4-isopropylcatechol (4-IPC)*

This compound is considered as more potent and more consistent in his depigmenting effect than hydroquinone. A clinical trial involving 68 patients treated with 1% and 3% 4-IPC has demonstrated that this product is a potent depigmenting agent and is of use for the topical therapy of hypermelanosis (10).

### *Phenolic-Thioether*

N-acetyl-4-S-cystaminylphenol has been evaluated in a small number of patients with melasma (11). Marked improvement or complete clearing with minimal side effects were obtained in 75% of patients. These compounds are not widely used and large clinical trials to evaluate their safety and efficacy are not available.

### *Azelaic Acid*

Azelaic acid (AA) is a naturally occurring 9-carbon-dicarboxylic acid isolated from cultures of *Pityrosporum ovale*. AA is thought to play a key-role in the pathogenesis of hypomelanotic tinea versicolor. AA has been used at concentrations of 15%–20% for the treatment of melasma and post-inflammatory hypomelanoses. The best results demonstrated that AA is more effective than 2% HQ and equivalent in efficacy with 4% HQ for the treatment of melasma in dark-skinned women (12). Similar good results have never been obtained in European patients. AA is well tolerated. Adverse effects such as pruritus, transient erythema, scaling, and irritation are usually mild and disappear within a few weeks. Phototoxic and allergic reactions are rare.

AA may halt the progression of lentigo maligna and even induce its disappearance suggesting that AA exert an antiproliferative and cytotoxic effect mainly on hyperactive and abnormal melanocytes.

### *Soy and Soy-Based Products*

Soy and soy-based products may affect skin pigmentation. Several studies have demonstrated that soy-derived serine protease inhibiting PAR-2 mediated phagocytosis of melanosomes by keratinocytes. Thus soy-based products containing these serine protease inhibitors represent safe and effective products to treat hyperpigmentation (13).

### *Kojic Acid*

Kojic acid (KA) a fungal metabolic product is used at 1%–4% concentrations. In monotherapy, KA shows a modest effectiveness. Thus, it is mainly used in combinations. It is a potent tyrosinase inhibitor and functions by chelating copper at the active site of the enzyme. Long-term side effects of KA are not known. A high frequency of contact sensitivity has been reported (14). The use of KA in cosmetics has been banned in Japan.



*Ascorbic Acid*

A stable ester of ascorbic acid (ASA) (magnesium L-ascorbyl-2 phosphate) in a 10% cream base produced a significant lightening effect in patients with melasma after 3 months of twice daily application (15). ASA interferes with the different steps of melanogenesis by interacting with copper ions at the tyrosinase active site and reducing dopaquinone and by blocking DHICA oxidation. A randomized double-blind placebo-controlled trial of vitamin C iontophoresis in melasma has demonstrated that this strategy may be an effective treatment for melasma (16). A double-blind left/right randomized comparative study in melasma patients showed that 93% of good and excellent subjective results are observed on the 4% hydroquinone side compared with 62.5% on the 5% ascorbic acid side. However, colorimetric measures showed no statistical differences. Side effects were more common with hydroquinone (68.7%) than with ascorbic acid (6.2%) (17).

*Tranexamic Acid*

Tranexamic acid is known as an oral medicine for treating melasma. The antiplasma activity of tramexamic acid has been proposed for treating melasma by targeting the vascular component of this disorder (18–20). Although proposed also topically, best results seems to be achieved with oral administration. A prospective randomized study is still required to determine the usefulness of this approach.

*Arbutin*

Arbutin is a naturally occurring beta-D-glucopyranoside derivative of hydroquinone. It inhibits tyrosinase activity. In a clinical trial, an arbutin-containing formulation (3%) was shown to be effective for treating hyperpigmentary disorders (21,19).

*Ellagic Acid*

Ellagic acid inhibits tyrosinase activity by chelating copper atoms in its active site. In a clinical trial, a 0.5% ellagic-containing cream was shown to be effective for treating UVB-induced hyperpigmentation of the skin (22,19).

*Chamomilla Extract*

Chamomilla extracts have been shown to act as an antagonist for endothelin receptor binding which mediates cell-to-cell signaling between keratinocytes and melanocytes and leads to the inhibition of melanin synthesis in melanocytes. In a clinical trial, a 0.5% chamomilla extract-containing cream was shown to be effective for treating UVB-induced hyperpigmentation of the skin (23,24,19).

*Adenoside Monophosphate Disodium Salt*

Adenosine monophosphate disodium salt accelerates elevated intracellular energy metabolism that leads to the excretion of melanin from the skin, preventing the accumulation of melanin in the skin. In a clinical trial, a 3% adenosine monophosphate disodium salt-containing salt formulation was shown to be effective for treating hyperpigmentary disorders (19).

*5,5'-Dipropyl-biphenyl-2,2-diol (Magnolignan)*

Magnolignan inhibits the maturation due to the glycosylation of tyrosinase and thus induces decreased melanin synthesis. 0.5% Magnolignan-containing formulation was shown to be effective for treating UVB hyperpigmentation of the skin (25) and also is effective in treating hyperpigmentation disorders such as melasma and while lentigo (26,19).

*4-(4-Hydroxyphenyl)-2-butanol (4-HPB)*

4-HPB, a phenol compound, inhibits melanin synthesis due to its competitive inhibition of tyrosinase activity (19).

*Tranexamic Acid Cetyl Ester Hydrochloride*

The effect of tranexamic acid cetyl ester hydrochloride to treat hyperpigmentary disorders is due to the inhibition of UVB-induced inflammation that leads to the quiescence of active melanocytes (19).

*4-Methoxy Potassium Salicylate (4-MSK)*

4-MSK inhibits melanin synthesis through competitive melanin of tyrosinase activity (19).

*Cystamine and Cysteamine*

Cystamine and derivatives have been shown to inhibit tyrosinase activity as well as the level of tyrosinase protein (27,28). However, as it reduces eumelanin it increases pheomelanin synthesis. A recent prospective randomized study showed a moderate but statistically significant superiority compared to placebo for treating melasma (29). However, as it reduces eumelanin it increases pheomelanin synthesis (28). At the contrary to eumelanin, pheomelanin has no photoprotective properties. When pheomelanin is exposed to UV radiation it produces radical species (30,31). More recently it has been shown that pheomelanins without UVR may promote melanogenesis by the radical species they produce (32). Thus, great caution should be taken with such products and in light of the current knowledge it is very difficult to advise their use for treating pigmentary disorders.

**Retinoid Monotherapy***Tretinoin*

Tretinoin (all trans retinoic acid—ATRA) has been used in concentrations from 0.025%–0.1% to treat a variety of pigmentary disorders such as pigmented spots of photoaged skin, melisma, and postinflammatory hyperpigmentation in dark-skinned individuals (33–36). Erythema and peeling in the area of application are adverse events of ATRA 0.05%–0.1%. Post-inflammatory hyperpigmentation may also occur. Topical ATRA appears to exert its action by enhancing keratinocyte proliferation and increasing epidermal cell turnover. However ATRA, acting on retinoid-activating transcription factors, interferes with melanogenesis. ATRA does not inhibit melanogenesis in skin equivalent or monolayer cultures of melanocytes, however it enhances the pigmentation of low-melanized melanoma cells and decreases that of highly pigmented normal melanocytes after UV irradiation (37).

*Tazarotene*

This acetylenic topical retinoid improves the irregular hyperpigmentation associated with photoaging and lightening the pigmented spots. Tazarotene 0.1% gel is associated with reduced mottling on the dorsal aspects of forearms. The Fontana stain showed a moderate to marked depigmenting effect with decreased pigmentation on the tazarotene-treated side. Melanin granules were sparser and less heavily pigmented, probably explaining the bleaching of hyperpigmented spots (38).

*Adapalene*

Adapalene gel 0.1% and 0.3%, a synthetic retinoid, improves solar lentigines as well as other features of photodamaged skin and is well tolerated (39).

#### *Liquorice Extracts*

Liquiritin, a flavonoid glycoside of liquorice, has been found to induce a significant improvement of hypermelanosis in patients with bilateral epidermal melasma (40). The mechanism proposed involved melanin dispersion and increased epidermal turnover. Glabridin, the main component of the hydrophobic fraction of liquorice, extracts decreased tyrosinase activity in melanoma cells. Furthermore, this compound inhibits UVB-induced skin pigmentation (41). Although no clinical trials have evaluated its efficacy as a depigmenting agent, one available glabridin may be found in some cosmetics.

#### *Thioctic Acid ( $\alpha$ -Lipoic Acid)*

This compound is a disulfide derivative of octanoic acid. It acts as ROS scavenger and Redox regulator but also inhibits tyrosinase activity, probably by chelating the copper ions, and prevents UV-induced photoactive damage (42). This product is commercially available.

#### *Unsaturated Fatty Acids*

Oleic acid (C18:1), linoleic acid (C18:2), and  $\alpha$ -linolenic acid (C18:3) suppress pigmentation *in vitro*. Some of these compounds have *in vivo* a lightening effect in UVB-induced pigmentation without toxic effects on melanocytes (43). Fatty acids have recently been shown to regulate pigmentation via proteosomal degradation (44). Linoleic acid accelerates the degradation of tyrosinase, thus inhibiting melanogenesis. In contrast, palmitic acid, a saturated fatty acid, retards the proteolysis of and thus accelerates melanogenesis.

#### *Inhibitory Oligopeptides*

A short sequence oligopeptides (10 aminoacids) (Lumixyl) with inhibitory activity against mushroom and human tyrosinase (Elixir Institute of Regenerative Medicine, San Jose, CA, U.S.). This oligopeptide showed no cytotoxicity to human melanocytes. Only a split-face double-blind, randomized and placebo-controlled evaluation including five female participants with moderate melasma has been performed. The results of this clinical trial were positive. However, this oligopeptide warrants further evaluation (45,46).

### **Combination Therapies**

Combination therapies are widely used for the treatment of hypermelanoses. The purpose of these strategies is to augment efficacy by associating active ingredients with different modes of action in order to obtain a synergic effect, to shorten the duration of therapy, and to reduce the risk of adverse effects.

#### *Kligman's Formula*

The most popular combination treatment for depigmenting skin is Kligman's formula. This polytherapy includes 5% HQ, 0.1% tretinoin, and 0.1% dexamethasone in a hydrophilic ointment. Tretinoin functions as an enhancer of HQ penetration in the epidermis. Furthermore, tretinoin increases epidermal turnover, thus facilitating melanin dispersion within keratinocytes and also melanin removal from corneocyte shedding. Dexamethasone decreases the irritation and inflammation caused by HQ and/or tretinoin and melanin synthesis by inhibiting metabolic activity. This formula demonstrated efficacy in the treatment of melasma, ephelides, and post-inflammatory hypermelanosis. Depigmentation occurs rapidly, beginning within 3 weeks after twice-daily application.

Unfortunately, the efficacy of this formula depends upon its stability. Extemporaneous formulation is useful but bears a strong risk of instability. Recently, a stabilized formulation containing 4% HQ, 0.05% tretinoin, and 0.01% fluocinolone acetonide has been launched. Two multicenter, randomized double-blind controlled trials demonstrated the safety and efficacy of this combination treatment in patients with moderate to severe melasma (47). After 8 weeks of treatment, a 75% reduction of melasma was found in more than 70% of the patients. Furthermore, superiority of the formulation over its three components (HQ, tretinoin, fluocinolone acetonide) was demonstrated. There are already many variants of the extemporaneous Kligman's formula. The suggestion that topical steroids are not necessary for achieving depigmentation led to a modification of this formula by removing topical steroids. Clinical trials demonstrated that 2% HQ combined to 0.05%–1% tretinoin cream and lotions are also effective.

#### *4-Hydroxyanisole + Tretinoin (Mequinol®)*

A solution containing monomethyl ether of hydroquinone (2%) and tretinoin 0.01% has been launched in North America for the treatment of actinic lentigo. This combination treatment has been shown to improve the appearance of these lesions in several controlled and non-controlled studies (48,49).

#### *Azelaic Acid + Tretinoin*

A combination regimen of 20% AA with topical tretinoin 0.05% produces an earlier and more pronounced lightening pigmentation during the early phase of the treatment. However, an equivalent efficacy of the combination 20% AA-topical tretinoin 0.05% versus AA monotherapy was obtained after 6 months of treatment (50). 20% AA has also been associated with 15%–20% glycolic acid lotion. This combination regimen was as effective as 4% HQ cream for the treatment of facial hyperpigmentation in dark-skinned individuals (51).

#### *HQ + Kojic Acid*

Kojic acid has also been included in combination regimens. 2% Kojic acid in a gel containing 10% glycolic acid and 2% HQ improves epidermal melasma after 12 weeks of treatment, 1–4 kojic acid combined with tretinoin, HQ, and/or corticosteroid or glycolic acid appears to act synergically (52).

#### *Westerhof Formula*

The combination of 4.7% N-acetylcystein (NAC), 2% HQ, and 0.1% triamcinolone acetonide has been demonstrated to be effective in the treatment of melasma. The mechanism of action of NAC is not fully characterized. NAC exerts an inhibitory effect on tyrosinase. However, it is likely the NAC stimulates pheomelanogenesis rather than eumelanogenesis, clinically producing lighter color. Thus caution again has to be taken with such a drug stimulating pheomelanogenesis.

#### *Hydroquinone-Salicylic Acid Conjugates*

Recently, new conjugates were obtained by joining HQ and salicylic acid: monoester (4-hydroxyphenyl 2-hydroxybenzoate, HPH) and diester (1,4-phenylene bis(2-hydroxybenzoate), PBH) (53). These conjugate compounds inhibit tyrosinase and have efficient skin absorption. Clinical studies on pigmentary disorders are now required to evaluate their tolerance and efficiency.

#### *Cosmetic use of Bleaching Products*

This is a common practice in dark-skinned women from sub-Saharan Africa and a few other parts of the world. The

products used include HQ, potent or super-potent topical glucocorticoids, mercury, salts, and caustic agents such as liquid soaps, hydrogen peroxide, and salicylic preparations. Most users (>90%) apply the products once or twice daily to the whole body, for months or years. Side effects, often very severe, include skin atrophy, delayed cicatrisation, infectious dermatoses (*Bacteriae*, mycoses, parasites), acne, dyschromia with a typical pattern, irritant and allergic contact dermatitis, prominent striae, ochronosis, poikiloderma of the neck, and perioricular hyperchromia (Figure 36.2). Nephrotic syndrome can be observed after the use of mercurial derivatives. The daily use of potent topical steroids over years can lead to Cushing syndrome. This practice is a real health problem, not only in Africa, but in Northern countries receiving large immigrant communities. A careful dermatological examination of patients is helpful to detect the skin symptoms resulting from this practice (54).

#### Chemical Peels

Chemabrasion and peels using various chemicals is another treatment modality for removal of freckles, actinic lentigines and other pigmented spots, melisma, and post-inflammatory hypermelanosis. Deep peels are avoided in patients with hypermelanosis because of the high risk of post-inflammatory hyper- or hypomelanosis, scarring, and keloid formation. Mainly superficial and medium-depth chemical peels have been used for the treatment of hypermelanosis, mainly in fair-skinned individuals.

Glycolic acid is an alpha-hydroxy acid that has an epidermal dis cohesive effect at low concentrations. Removal of corneocytes and epidermal upper layer keratinocytes by chemical peeling reduces the epidermal melanin content and improves hypermelanoses. Glycolic acid peels (50%–70%) are becoming increasingly popular in the treatment of melasma. They can be safely used in dark-skinned patients due to a quite low risk of hyperpigmentation (55).

A few studies have demonstrated the efficacy of chemical peels with other depigmenting agents in patients with hypermelanosis. Complete bleaching of diffuse melasma was observed in patients (30%) treated with glycolic acid 50% plus kojic acid 10% and partial blanching in 60% of patients. Serial glycolic acid peels (30%–40%) combined with a modified

Kligman formula (2% hydroquinone + 0.05% tretinoin + 1% hydrocortisone) provided an additional effect to the standard topical treatment in dark-skinned patients with melasma. Another study suggested that daily application of 10% glycolic acid lotion and 2% hydroquinone combined with 70% glycolic acid peels every 3 weeks showed some improvement of pigmented spots of photoaging in Asian women (56). In contrast, a split-face prospective study in 21 Hispanic women within melasma showed no differences in the bleaching effect of 4% hydroquinone + glycolic peels 20%–30% versus hydroquinone 4% alone.

Five peelings with salicylic acid 20%–30% at 20-week intervals in dark-skinned patients (phototypes V to VI), after initial treatment with hydroquinone 5% for 2 weeks, gave good results for melasma and other types of pigmentation (57).

The use of trichloroacetic 20%–35% followed by hydroquinone hydro-alcoholic 4% solution or tretinoin 0.05% plus hydrocortisone acetate 1% cream has produced excellent results for hypermelanosis in white patients with higher complexion (55).

Resorcinol is used as Jessner's solution (14 g resorcinol, 14 g salicylic acid, 14 g lactic acid 85%, and enough ethanol to make up 10 mL) or in Unna's paste (up to 10% resorcinol plus zinc oxide and ceisatle) have also been demonstrated to be effective in hypermelanosis with an acceptable rate of adverse effects.

Peels that combine kojic acid, salicylic acid, and alpha-hydroxy preparations with or without hydroquinone or resorcinol are commercially available (55). Applied every 3 weeks they do not require neutralization.

#### Dermabrasion

Dermabrasion using rotary diamond fraises has been used for the treatment of melasma. Patients were followed for about 5 years (58). According to the authors, most patients (97%) obtained persistent clearance of melasma and only 12 out of 410 have a partial recurrence. Only two patients developed hypertrophic scars and one patient had permanent hypomelanosis.

Dermabrasion-induced post-inflammatory hyperpigmentation (PIH), common in Asian and dark-skinned individuals, limits considerably the use of this strategy in these groups of patients. Even the more superficial microdermabrasion using a device emitting aluminium oxide crystals bears an important risk of post-inflammatory dyspigmentation. Due to the important risk of PIH along with the risk of relapse, dermabrasion is no longer recommended for treating melasma. It has to be used with great care for treating other pigmentary disorders.

#### Liquid Nitrogen Cryotherapy

Melanocytes are particularly susceptible to freezing, and hence they should be avoided in dark-skinned people because of the risk of permanent depigmentation. The freezing agent must be applied gently to avoid blistering and skin necrosis. Cryotherapy with liquid nitrogen is commonly used successfully to treat individual pigmented lesions. Although satisfactory results are common, cryotherapy for benign epidermal lesions is problematic because of hypopigmentation, hyperpigmentation, atrophy, scarring, and/or frequent recurrence.

Liquid nitrogen cryotherapy has also been proposed for the treatment of nevus of Ota, delayed nevus spilus, and blue nevus. Nowadays laser approaches provide clearly better results and cryotherapy should no longer be used for removing these hypermelanocytoses. Liquid nitrogen can be proposed



**Figure 36.2** Exogenous ochronosis due to chronic application of hydroquinone.



for the treatment of hypermelanoses (actinic lentigos and other pigmented spots of photodamaged skin) and hypomelanosis (idiopathic guttate hypomelanosis).

## LASERS AND PHOTOTHERAPY

The treatment of pigmentary disorders by lasers is based on selective photothermolysis (59). In order to have selective action, the length of the laser impulsion has to be at least 10 times shorter than the relaxation time of the target. This relaxation time is proportional to the size of the target (but also depends on the shape and the diffusivity of the target). For pigmentary disorders due to melanin defects the target is the melanosome. It is a lysosome-related organelle specific to the melanocytes within which the melanin is produced. With their maturation, the melanosomes will be progressively filled with melanin and be transferred to the surrounding keratinocytes (60). The size of a melanosome is about 1  $\mu\text{m}$ . Its relaxation time varies from 1 to 10  $\mu\text{s}$ . Thus, the impulsion time of the laser has to be inferior to 100 ns. The lasers used for pigmentary disorders are Q-switched and their impulsion length is from 10 to 100 ns, allowing them to target the melanosomes and most of the exogenous pigments.

The location of the pigment in the dermis or the epidermis guides in part the choice of wavelength. Thus, dermal pigmentation will be better treated with 1064 nm Nd:YAG lasers which wavelength could penetrate deeper in the skin tissue. Those lasers are also preferred for dark-skinned people as they interact less with the melanin of the superficial layers of the skin.

The type of pigment has also to be taken into consideration. Pheomelanin is a good target for 532 nm Nd:YAG lasers when it is a less interesting chromophore for 694, 755, and 1064 nm lasers (61). The choice of the laser wavelength is even more important for the treatment of tattoos.

All lasers can induce side effects and patients have to be clearly informed about the potential risks. If scars are exceptional and are due to excessive fluencies, PIH is the most common side effect. PIH is mostly observed in dark skin types. Photoprotection is required before and after the laser sessions to decrease this risk. PIH usually regresses in a couple of weeks or months. Topical steroid eventually combined with hydroquinone could be helpful in the early stages. Leukodermas are less frequently observed. They are usually transient, but permanent leukodermas have been reported mainly with 694 nm ruby lasers. The treatment of tattoos leads to more side effects. Cicatricial scars and leukoderma are more frequently observed. Patients and physicians have to be aware of the risk of paradoxical darkening of tattoos after a first session. Finally, allergic or granulomatous reactions, koebner phenomenon, and pseudolymphomas have been also reported.

Ultraviolet (UV) A and B phototherapies are both widely used in dermatology. UVA radiation includes electromagnetic waves with wavelengths between 320 and 400 nm. UVB wavelengths are between 290 and 320 nm. It is used with systemic or topical psoralens, which selectively absorb the radiation (PUVA therapy). The main action of PUVA on biological systems is the inhibition of DNA synthesis due to photo-adducts formed between psoralen and pyrimidine bases in the nucleic acid. PUVA was for a long time considered to be the phototherapy of choice, including for hypochromic disorders such as vitiligo. Because of potential side effects, including cutaneous cancers, many authors now recommend the use of UVB therapy instead (62–65). Prospective studies and meta-analysis have now showed that narrowband UVB

(NB-UVB) therapy (around 311 nm) is superior to PUVA for treating vitiligo (66,67).

The 308 nm excimer lasers have been used in dermatology since 1997 (68). The development of 308 nm excimer lamps is more recent. At the difference of the lasers, the wavelength is not strictly monochromatic and the beam of light is not coherent but those systems are much less expensive than lasers. Those devices emit a wavelength in the UVB spectrum. The wavelength at 308 nm provides photobiological effects theoretically superior for those devices as compared to NB-UVB, especially for their immunologic effects. Indeed, 308 nm is the most effective wavelength to induce lesions to the lymphocyte DNA, and the dose required to induce the apoptosis of lymphocytes is clearly lower with 308 nm as compared with NB-UVB (69,70). However, in vitiligo and more clearly for the other hypopigmentary disorders, the stimulation of the migration and the proliferation of melanocytes appear to have a key role, but fundamental data to compare the respective photobiological propigmenting properties of the 308 nm and NB-UVB wavelengths are still not available. The use of the 308 nm excimer laser and lamps is approved by the FDA (Food and Drug Administration) for the treatment of vitiligo.

The 632.8 nm helium neon laser is the first device that does not use the UV spectrum to repigment hypochromic lesions, especially vitiligo. Indeed, this laser, which emits a wavelength in the red visible light, has been proven to enhance the proliferation and the differentiation of melanoblasts to mature melanocytes in vitro (71). It has also been demonstrated that this laser acts on mitochondria to increase the proliferation rate of the cells (72). These photobiological effects could explain, at least in part, the action of the 632.8 nm helium neon laser in repigmenting vitiligo. However, clinical data remain poor concerning the efficacy of this device in hypochromic disorders (73).

## SURGICAL APPROACHES

Surgical approaches (74) aim to reconstitute the epidermal (and perhaps follicular) compartment of the melanocyte population of the skin by bringing to the hypochromic lesion, after dermabrasion, a new pigmented skin or a suspension of melanocytes, isolated or associated with other epidermal cells such as keratinocytes. For vitiligo such methods are usually considered for stable and localized lesions after medical treatment has failed. However, for piebaldism or to a lesser extent for nevus depigmentosus, they are almost the only treatment available.

Several methods are available including punch grafts, blister grafts, split-thickness grafts, and autologous transplantation of melanocyte suspensions, cultured melanocytes, or cultured epidermal grafts including melanocytes. Grafting of follicular melanocytes to repigment vitiligo leukotrichia has also been performed successfully (75).

Punch grafting (1.2–3 mm punch biopsies) is the simplest technique, and grafts are implanted into perforations prepared at the recipient sites by different techniques (punch biopsy, ablative lasers). Minigrafting using small grafts (1.2 mm) is the best technique. The potential side effects include spotted pigmentation, polka dot appearance, color mismatch, a cobblestone effect, sinking pits, and scarring. Furthermore, this technique is time consuming.

Split-thickness grafting is obtained by a standard or an electrical dermatome. The main advantage of this treatment is to allow treatment of large areas. Esthetical results are often

satisfactory with a homogenous repigmentation. However, this approach may be associated with esthetically unacceptable results at the donor site (dyspigmentation, scarring). Adverse events include miliae-like cyst formation at the recipient site, partial loss of the grafts, hematoma formation, and thickening of the graft margins.

In flip-top transplantation, the epidermis at the recipient site is used to form multiple hinged flaps, each covering an ultra-thin 1.2 mm graft harvested from the donor site by a razor blade (76).

Autologous blisters can be induced in different ways, i.e., vacuum, liquid nitrogen. The mechanical split occurs at the dermo-epidermal junction. The recipient site is prepared by dermabrasion, laser ablation (erbium:YAG or carbon dioxide laser), liquid nitrogen, or PUVA-induced blisters, dermatome. The graft (top of the blister) is applied and secured on the recipient site. The only adverse event is transient hyperpigmentation at both the donor and recipient sites. The advantages of this technique are the absence of scarring and the possibility of reusing the donor site. Split-thickness grafting and blister grafts have a better success rate than punch grafting (77). These techniques are not expensive and can be done in private practice.

The use of epidermal suspension (melanocytes alone or combined with keratinocytes) is more recent. Non-cultured keratinocyte/melanocyte suspensions can be obtained after trypsinization of a shave biopsy of the buttock or full thickness biopsy of the scalp. Melanocytes obtained from the hair follicles and interfollicular epidermis as well as keratinocytes, are put into a suspension with the patient's serum for direct application to the recipient site without expansion in culture (78). The recipient site is prepared with dermabrasion using a dermatome or an ablative CO<sub>2</sub> or Erbium laser. This technique allows treating large lesional surfaces with a small piece of skin, as a ratio of 1:5 to 1:10 is commonly used. A prospective randomized double-blind study has demonstrated that epidermal suspension followed by UV (PUVA or Nb-UVB) was more effective than UV combined with only dermabrasion and serum (79). This technique used to require a specialized laboratory but the development of ready-to-use kits now allow it to be used by any trained physician.

The number of melanocytes (combined or not with keratinocytes) can be expended in culture before grafting. Applied on vitiligo lesions, it gives satisfactory results in 30%–44% of patients. Improvement of melanocyte culture conditions and grafting devices have made possible the transplantation of autologous cultured melanocytes on large areas (up to 500 cm<sup>2</sup> during one session) of vitiligo involved skin (80,81). A 95% repigmentation is obtained in approximately 40% of the treated areas. However, while the results are usually excellent for stable vitiligos, active forms lead to failure of the procedure, and careful selection of patients remains crucial (82). All the techniques involving melanocyte culture and epidermal reconstruction require specialized laboratory expertise and are very expensive. For these reasons, they are not widely used.

## CAMOUFLAGING

Camouflaging has been shown to increase the quality of life (DLQI) of people using it (83,84). The use of medical makeup has become progressively more popular and is now integrated in many dermatological centers. Cosmetic products with high concentration in pigments allow very interesting and esthetical results but require training of the technique (85,86).

A siliconated spray could be used to cover the makeup and increase its waterproofing properties, allowing the practice of water activities (87).

Dihydroxyacetone (DHA) can also be very useful to decrease the contrast of hypopigmented lesions with the surrounding skin. The brown color is due to the chemical combination of the DHA with the amino acids of the skin leading to the formation of polymeric pigments called melanoidins. Those pigments remain in the stratum corneum until desquamation of the corneocytes. The coloration appears a few hours after the application of the DHA and progressively fades after 5–7 days (88–90). The main advantages of this are to resist to water and to not stain the dressings. It is used mainly for the hands and the feet. Concentrations between 2.5%–10% can be used depending on the intensity of the desired color. As DHA combines with the amino acids of the stratum corneum, the intensity of the reaction depends on the thickness of this skin layer. Thus patients should be advised to apply less quantity on locations with thick stratum corneum such as palms, soles, knees, or ankles. The patient would also have to be informed that the pigmentation produced is not photoprotective against UV.

Micropigmentation could also be helpful, especially for areas such as lips or nipples in dark skin phototypes (91,92).

## TREATMENT OF PIGMENTARY LESIONS

### Melasma

The gold standard treatment for melasma is topical bleaching agents (Figure 36.3). Kligman's formula is the most effective treatment, especially in its stabilized form (47,93–96). Peeling and dermabrasion can be also proposed but their efficacy is inconsistent and these strategies frequently induce PIH (60,97).

Ablative and non-ablative fractional lasers have been reported to improve melasma (98,99). However, the risk of relapse is important and up to 10% worsening has been reported with such approaches (100). Moreover, non-ablative fractional laser has been shown to not be superior to Kligman's trio (101). Interesting results have been reported with the 1927 nm thulium laser (102,103). However some PIH were reported along with relatively frequent relapses. Prospective randomized comparative trials are still required to determine the usefulness of this new device for treating melasma.

Pigmentary lasers such as Q-switched ruby, alexandrite, or Nd:YAG lasers induce almost constant PIH and relapses. More recently, Q-switched laser used with low fluencies and repetitive sessions has been reported to be effective for treating melasma. However, when long-term follow-up is performed a constant relapse is observed along with up to 20% of worsening of the hyperpigmentation due to PIH (104). Thus, such approaches can't be recommended for treating melasma.

Intense pulsed light (IPL) has shown some efficacy in the treatment of melasma (105–108). The risk of PIH remains important but appears to be lower than the one observed with Q-switched lasers. Topical bleaching preparations, frequently containing hydroquinone, have been used with IPL in order to prevent PIH (109,110). Although potentially useful, this association approach has never been compared to bleaching cream used in monotherapy.

Increasing data shows that in addition to the increase in pigmentation, melasma lesions have more elastosis and vascularization compared to the perilesional skin (111–113). The association of a fixed triple combination cream and a pulsed dye laser (PDL) with vascular and pigmentary parameters



**Figure 36.3** Melasma before treatment in direct light (a) and UV light (b), and after 3 months of Kligman's preparation in direct light (c) and UV light (d).

showed significantly better decrease of the hyperpigmentation of melasma and reduced the relapses observed after the summer (114,115). Following the same aim of targeting the vascular component of melasma tranexamic acid, an antifibrinolytic used to prevent and to treat some hemorrhagic events was also proposed for treating melasma. The combined use of this agent topically and orally for 8 weeks led to a decrease of the hyperpigmentation in melasma lesions. Histological examinations showed a decrease in melanin content and in vascularization (116,20). Those pilot studies clearly need to be confirmed, but they underline the potential interest of targeting the vascular component for treating melasma.

To conclude, Kligman's trio remains the gold standard for treating melasma and should be used as the first treatment option. Other options can be proposed if the trio fails to depigment the melasma. In all cases, patients have to be advised about the limitations and potential side effect of the treatments. However, all the therapeutic approaches do not prevent relapses of melasma. After the initial treatment (in most cases 3–4 months of Kligman's trio), a maintenance treatment has to be prescribed. Cosmetic depigmenting agents are very useful for maintenance due to their good safety profile in most cases. Last but not least, preventing triggering factors is crucial. Discontinuation of estroprogestative medications should be discussed but not proposed in all cases, as the impact of their discontinuation on the evolution of melasma has been shown to be weak (117). Repetitive friction should be avoided. More importantly, strict photoprotection with clothing, sunglasses, sun avoidance, and sunscreens with very good UVB and UVA protection are mandatory. Recent data showed that the shorter wavelengths of visible light plays can trigger hyperpigmentation of the skin and that protection against these wavelengths in addition to strong UVA and UVB protection is effective for preventing melasma relapses (118,119).

### Vitiligo

Many medical or surgical treatments are available for vitiligo, but only a few have clearly demonstrated their efficacy in treating vitiligo.

Phototherapy (PUVA or Nb-UVB) is the gold standard for generalized forms (67,120,121). If available, Nb-UVB is

preferable to PUVA, as has greater efficacy with lesser side effects and better tolerance (66).

Once-daily applications of topical steroids or twice-daily application of 0.1% tacrolimus or 1% pimecrolimus should be used first for localized forms of vitiligo (67,120,121).

Targeted phototherapy with 308 nm excimer lamps and lasers is also effective for localized forms, but are more expensive (Figure 36.4) (122–127). However, bony prominences and extremities remains extremely difficult to treat (127).

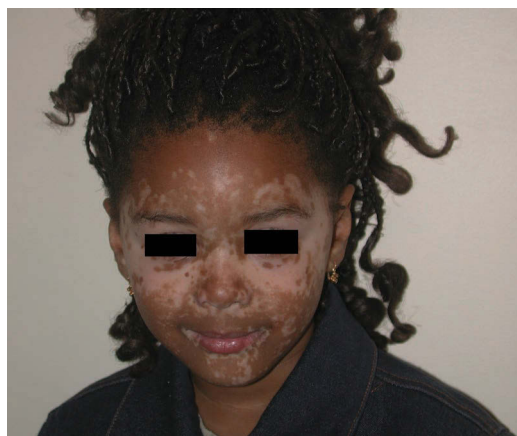
The data concerning the other therapeutic approaches including antioxidants or topical vitamin D are more controversial (128–138).

Combination approaches associating tacrolimus ointment or pimecrolimus cream with phototherapy (308 nm excimer light or Nb-UVB) have shown their superiority to monotherapy (139–144). Such a synergic effect has been also reported with the association of topical steroids and excimer laser (145). Those associations should be proposed for difficult to treat areas such as bony prominences.

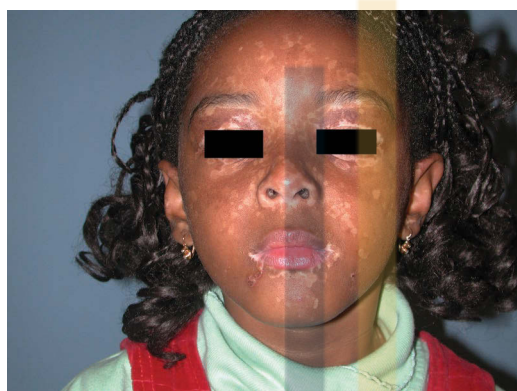
Surgical treatment is a good option for localized or segmental forms that have been stable for at least 3 years (Figure 36.5) (67,120,121).

When treatments have failed corrective cosmetics, use of DHA (dihydroxyacetone 1,3-dihydroxydimethylcetone) or dermopigmentation (especially for nipples and mucosal areas) can be useful (146,60). Finally, depigmentation that we would like as permanent can be proposed usually for people more than 40 years old, after detailed information is given to the patient. Psychological evaluation is also useful. The monobenzylether of hydroquinone (MBEH) causes a permanent depigmentation of the skin that has been used for generalized vitiligo. However, the side effects that include irritant and allergic contact dermatitis, post-inflammatory hypermelanosis, leukoderma en confetti at treated sites, and hypomelanosis at sites distant from the application areas strongly limit the use of this compound and have prompted clinicians to explore therapeutic alternatives. Q-switched lasers are as effective as MBEH with fewer side effects, and should now be preferred (Figure 36.6) (147–150). Better results seem to be obtained if the vitiligo is active (151). Depigmentation therapies should be reserved for limited surfaces and they should not be proposed

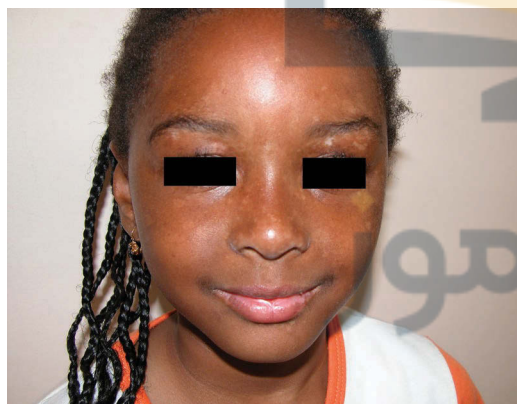




(a)



(b)



(c)

**Figure 36.4** Vitiligo of the face (a). Clinical aspect after 40 sessions of 308 nm excimer laser (b) and 18 months after the end of the treatment (c).

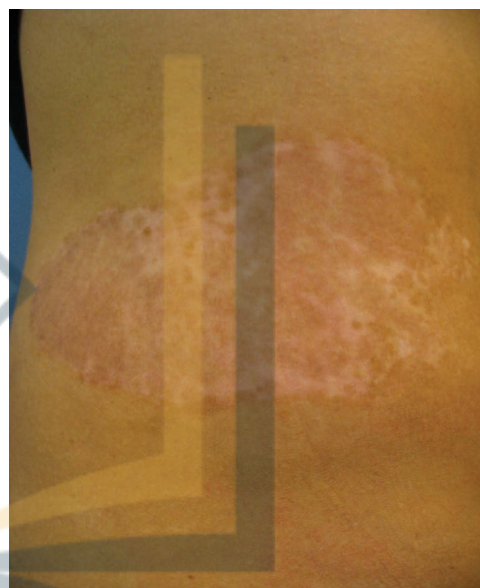
if depigmentation involves less than 50% of the affected area. In all cases, patients must be clearly informed of the potential risk of later repigmentation, and photoprotection of the treated areas should be systematically prescribed.

### Halo Nevus

Halo nevi usually do not require any treatment. However, some patients seek treatment. Some successes have been reported with the 308 nm excimer laser (152).



(a)



(b)

**Figure 36.5** Localized and stable vitiligo lesion (a). Clinical aspect 2 months after epidermal cell suspension graft (b). (Courtesy of Dr. P. Bahadoran).

### Piebaldism

Piebaldism is a rare autosomal dominant disorder with congenital hypomelanosis. Most patients have a mutation in the KIT gene (153). The pigmentary disorder is limited to hair and skin without neurological, ocular, or hearing defects. The topographical distribution of the lesions spreading to the anterior part of the trunk, abdomen, extremities, and the frontal part of the scalp is characteristic of the disease (154,155). Unlike vitiligo, these patches are congenital, stable with time, and do not repigment. Nice results can be obtained with surgical grafting procedures (156–159).

### Nevus Depigmentosus

Nevus depigmentosus can be effectively treated with grafting (160,161). Late recurrences are possible and the patient should be informed about this potential risk (162). The 308 nm excimer laser has been proposed but data are still limited (163).

### Solar Lentigines

Solar lentigines are effectively treated with topical bleaching cream, liquid nitrogen, Q-switched lasers, and IPL.



(a)



(b)

**Figure 36.6** Extensive vitiligo of the face that did not respond to repigmenting therapies (a). Depigmentation of the remaining pigmented areas 2 months after one session of 755 nm alexandrite Q-switched laser (b).



(a)



(b)

**Figure 36.7** Actinic lentigos of the face (a). Clinical aspect 1 month after one session of 755 nm alexandrite Q-switched laser (b).

(Figure 36.7). Treatment with Q-switched laser has proved to be the most effective approach, especially if the lesions are numerous, but it remains more expensive (164). One or two laser sessions are sufficient (165). While 694 and 755 nm are usually preferred in most cases, light lentigos are better treated with 532 nm Q-switched lasers (166). The use of sunscreens and cosmetic depigmenting cream will be advised in all patients to decrease recurrences. If the lesion

is atypical, a skin biopsy has to be performed to detect a lentigo maligna.

### Pigmented Seborrheic Keratosis

Thin pigmented seborrheic dermatosis can be treated with Q-switched lasers and IPL, but shaving, liquid nitrogen, or ablative lasers are the most frequently used treatments.



### Congenital and Acquired Dermal Hypermelanocytosis

Ota nevi are dermal hypermelanocytosis of the periorbital region. Such dermal hypermelanocytosis can also affect the shoulder (Ito nevus) or rarely other parts of the body. Acquired dermal hypermelanocytosis has also been reported (Figure 36.8). Although more frequently observed in Asian people, it affects all races. Ruby, alexandrite, and Nd:YAG Q-switched lasers have shown their efficacy. The depth of the target pigment leads to the use of longer wavelength. The 1064 nm Q-switched Nd:YAG has shown its superiority over the 755 nm alexandrite (167). Early treatment at a young age and brown rather than blue lesions are good predictive factors of response to treatment (168,169). Relapses can be observed and patients should be made aware of this (170).

Although the literature is poor, Ito nevus and acquired dermal hypermelanocytosis can also be effectively treated with the above lasers.

### Poikiloderma of Civatte

Laser and IPL approaches are the best treatments for the poikiloderma of Civatte. Both PDL and IPL have shown their efficacy (171–174). However, persistent depigmentation has been reported as a late adverse event, and high fluencies should be avoided in this fragile location (175). Some authors have suggested the use of fractional photothermolysis with interesting results, but the data remain limited (176,177).

### Café-au-Lait Macules

Café-au-lait macules can be treated with lasers and IPL. The response is variable and recurrences are very frequent. No clinical or histological markers have been determined to predict the response to treatment (178). Thus, the patient should be clearly informed of those risks. For large lesions, we advise a test session on a small area and seeing the patient after one summer to evaluate the response and the stability after treatment before treating the entire lesion.

### Nevus Spilus

Q-switched lasers, including ruby and alexandrite, have demonstrated their efficacy to treat nevus spilus (179,180). As for café-au-lait macules, relapses have been observed. Moreover, the risk of melanoma, though rare, is real, and laser treatment should be proposed with caution and a biopsy must be done if the lesion is atypical.



**Figure 36.8** Acquired Ota nevus (Hori nevus).

### Lentigines and Freckles

Q-switched lasers are effective for treating lentigines, including those associated with a genetic disorder such as Peutz-Jeghers-Touraine syndrome (181).

Freckles can also be treated with laser. As they contain mainly pheomelanin, the optimal wavelength will be the 532 nm (182,61). However, due to the constant relapses we do not recommend the treatment of ephelides.

### Becker Nevus

The hair component of Becker nevus responds well to laser hair removal (Figure 36.9). The hyperpigmentation can also be treated with Q-switched lasers but the response is more inconstant and recurrences are observed. A test session is required before treating large lesions. Most of the authors advise to first treat the hair component, but the sequence of treatment does not change the final result and the choice mainly depends on the type of lesions and the preference of the patient.

### Pigmentary Mosaicism (Linear and Whorled Hypermelanosis)

There is almost no data concerning the treatment of pigmentary mosaicism. Blanching products are ineffective, and only Q-switched lasers have provided some interesting results (183) (Figure 36.10). However, pigmentary mosaicism is a heterogeneous group and response to treatment is highly variable. A test session using several wavelengths is required to determine the optimal laser approach and the risk of recurrences.

### Dark Rings

Dark rings under the eyes are a heterogeneous group with multifactorial etiologies. Superficial location of vasculature and constitutional hyperpigmentation are the most frequent causes. However, dark rings may have other origins such as periorbital edema, PIH, shadowing, loss of subcutaneous fat, xerosis, and skin laxity (184). A vascular origin is noted mainly in fair-skinned individuals, while constitutional hyperpigmentation occurs most frequently in dark-skinned patients. Dark rings due to constitutional hyperpigmentation can be treated topically with tretinoin cream, peeling, CO2 laser, or

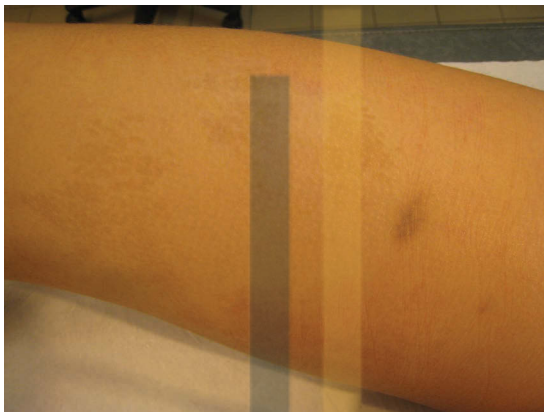


**Figure 36.9** Becker nevus.





(a)



(b)

**Figure 36.10** Pigmentary mosaicism of the thigh. A part of the lesion was chosen for the test session with 532, 755, and 1064 nm Q-switched lasers (a). Two months after the test session only the area treated with the 755 nm laser showed a nice improvement, in this case emphasizing the importance of testing several wavelengths in such pigmentary disorders.

surgical treatment (185–188). However, best results are usually obtained with Q-switched lasers and IPL (189,190). Only cosmetic approaches with makeup can be proposed when the origin is vascular. Skin laxity can be treated surgically and autologous fat injection can be proposed if the cause is loss of subcutaneous fat.

### Pigmentation due to Hemosiderosis and Siderosis

One of the most common manifestations of this is stasis dermatitis, but other causes can trigger such hyperpigmentation (vascular or thrombopenic purpura, post-Kaposi sequelae, iron extravasation ...). Although the data are still limited, treatment with Q-switched lasers and IPL appear to be effective (191–193).

### Drug-Induced Pigmentation

Pigmentation induced by drugs (such as cyclines or amiodarone) can be removed with Q-switched lasers (194–196). The pigmentation is usually in the dermis, so 694, 755, and 1064 nm wavelength should be preferred to Nd:YAG 532 nm.

### Postinflammatory Pigmentation

Postinflammatory pigmentation is frequent and can be observed after a surgical or cosmetical procedure. Laser treatments are not a good option for such hyperpigmentation as they can worsen the lesions. Photoprotection and treatment of the underlying dermatosis, if there is one, is mandatory and could be effective by themselves. If needed, class 3 topical steroids alone or combined with 4%–10% hydroquinone can be added.

### Idiopathic Guttate Hypomelanosis

Idiopathic guttate hypomelanosis is associated with chronic sun exposure (Figure 36.11). Any phototherapy, even focused treatment with the 308 nm excimer laser or lamp, should be avoided. Some isolated successes have been reported with topical tretinoin, liquid nitrogen, or localized superficial dermabrasion treatments (197–199). In our practice, superficial Erbium-assisted dermabrasion provides good results. Recently, interesting results have been reported using ablative and non-ablative fractional lasers (200,201).

### Hypopigmented Striae and Iatrogenic Leukoderma

Although quite frequent, those two conditions have no effective treatment. Isolated cases have reported some success with the 308 nm excimer laser (202–204). The absence of a group of control, the weaknesses in the evaluation of the results, and the need for maintenance sessions should moderate those results.

### Progressive Macular Hypomelanosis

Progressive macular hypomelanosis (PMH) is a common skin disorder that is often misdiagnosed (mostly for pityriasis versicolor) (Figures 36.12 and 36.13). PMH is characterized by ill-defined nummular, non-scaly hypopigmented spots on the trunk, often confluent in and around the midline, and rarely extending to the proximal extremities and neck/head region. There is no itch, pain, or preceding inflammation. Westerhof et al. suggested the causative role of *Propionibacterium acnes* bacteria and thus proposed as a treatment the application of 1% clindamycin lotion during the day, 5% benzoyl peroxide gel at night, and UVA light irradiation three times a week for a period of 12 weeks (205). However, phototherapy alone (PUVA or Nb-UVB) appears also to be effective (206).

### Ochronosis

Alcaptonuria is a rare genetic disorder which leads to endogenous ochronosis. Exogenous ochronosis is much more frequent and is due to the chronic application of hydroquinone. Most cases are seen in dark-skinned people who seek to blanch their skin. Discontinuing hydroquinone application is of course required. Little data are available for the treatment; dermabrasion, CO<sub>2</sub> laser, and more recently Q-switched laser have been proposed (207–209).

### Dyskeratosis

Dyskeratosis, including ictyosis, seborrheic keratosis, dermatosis papulosa nigra, and confluent and reticulated papillomatosis of Gougerot et Carteau can lead to hyperpigmentation.

Ictyosis is effectively treated by the daily use of emollients. The treatment of dermatosis papulosa nigra treatment is similar to seborrheic keratosis (cf. upper), curettage, electrodesiccation, Q-switched lasers, fractional or continuous



**Figure 36.11** Idiopathic guttate hypomelanosis.



**Figure 36.12** Progressive macular hypomelanosis.

ablative lasers have been reported (210–212). The confluent and reticulated papillomatosis of Gougerot et Carteau can be treated with topical agents such as tretinoin or vitamin D but most authors agree on the use of minocycline (213–216).

### Chromhidrosis and Pseudochromhidrosis

Chromhidrosis and pseudochromhidrosis are rare skin disorders. Chromhidrosis refers to the excretion by the apocrine glands of sweat containing lipofuscin pigments, while the terms pseudochromhidrosis or extrinsic chromhidrosis are used when the eccrine sweat is colored on the surface of the skin as a result of the deposit of extrinsic dyes or paints, or by transformation by chromogenic bacteria. Only few fungi and bacteria are known to induce pseudochromhidrosis. *Corynebacteria* are responsible for red pseudochromhidrosis and *Malassezia furfur* and *Bacillus* sp. are the agents involved in



**Figure 36.13** Achromic tinea versicolor.

blue pseudochromhidrosis (217). Treatment of pseudochromhidrosis consists of removing clothes or tissues responsible for the deposit of the extrinsic dyes or treating the chromogenic bacteria or fungi proliferation.

### TATTOOS

Tattoos are inclusions of pigments in the dermis or the hypodermis. Their cause can be traumatic, esthetic, or medical (marks for radiotherapy). The variety of the color sometimes leads to the necessity of using several wavelengths. Black pigments can be easily removed by almost all kinds of Q-switched lasers. Blue and green tattoos will be treated with 755 nm alexandrite lasers and red color will be better targeted with 532 nm Nd:YAG lasers. The quantity, quality, and depth of the pigments in the skin and the individual responses of the patients account for the variability of results and the potential side effects sometimes observed.

Many Q-switched have demonstrated their efficacy to remove tattoos (218). Due to their side effects, the non-fractional ablative lasers are no longer used. The choice of the laser will be guided by the color of the tattoos. Amateur tattoos, permanent makeup, blue-black color, and monochromatic tattoos are good predictive factors of response to treatment (Figure 36.14) (219). For traumatic tattoos, metal and asphalt origin and large-size pigments appear to be more resistant to laser treatment. A 2-month delay between each session is usually recommended to allow elimination of the fragmented pigments and decrease the risk of cicatricial scars. Gunpowder tattoos should not be treated with Q-switched lasers as the high energy pulses of laser on the powder particles creates microexplosions of these fragments, resulting in cavitation and provoking transepidermal holes and subsequent scars (220). Recently, picosecond lasers have been commercialized for removing tattoos. For now they are still relatively close to the nanosecond as their pulse duration time is 500–800 picosec. They aim to induced better fragmentation of the pigments and thus allow better elimination compared to Q-switched lasers. Published data provide encouraging results but prospective comparative randomized intra-individual studies are still missing, preventing us to draw a definitive conclusion about the real contribution of this technique (221,222).





(a)



(b)

**Figure 36.14** Blue ritual tattoos of the face (a) almost completely removed after only 2 sessions of Q-switched 755 nm alexandrite Q-switched laser (b).

Some tattoos (especially cosmetic and yellow tattoos) can darken or change color after a first session. A test session on a limited area is recommended. If the tattoo changes color, an additional treatment eventually, changing the

wavelength, usually allows removing the remaining pigments. However, ablative lasers or fractional non-ablative can also be proposed (223).

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# Treatment of Keloids

Joshua E. Lane

## INTRODUCTION

Keloids and hypertrophic scars represent abnormal wound responses. These occur most commonly in predisposed individuals. Keloids are benign tumors that arise from scar tissue and grow beyond the borders of the original scar (Figure 37.1). The formation of keloids and hypertrophic scars typically occurs following some form of trauma, whether intentional or not. This may include surgery, burns, trauma, inflammation, and/or infection. However, some keloids may form spontaneously, without any apparent predisposing trauma. Keloids are characterized by scar tissue that extends beyond the original dimensions of a wound, while hypertrophic scars maintain size within predictable dimensions of the original wound.

Keloids can thus be differentiated from normal scars and hypertrophic scars, the latter being confined to the original dimensions of a wound. A normal scar heals within the confines of the inciting injury. Hypertrophic scars increase in size as an outward growth while keloids are capable of both inward and outward extension.

Both keloids and hypertrophic scars present a clinical challenge in prevention and treatment. Multiple methods of treatment have been reported; however, no single modality is optimal.

## HYPERTROPHIC SCARS VERSUS KELOIDS

Keloids and hypertrophic scars can occur in individuals of all ages and races; however, they are more common with darker-pigmented skin types. They represent examples of exuberant scarring. The incidence of keloids has been reported to be as high as 16% in African-American individuals. Keloids tend to be less prevalent in the young and the elderly. The highest incidence was reported to range from ages 10–30. Other reports suggest the average age of patients with keloids at the time of initial treatment to be about 26.

Recommendations for ear piercing to avoid keloid formation have been made based on these trends. In one study, individuals formed fewer keloids following ear piercing if performed before the age of 11. In fact, women with multiple ear piercings often demonstrate keloid formation from piercing during puberty or within teenage years, while piercings performed as an infant did not keloid. Genetic inheritance patterns have also been reported.

Keloids tend to demonstrate a predilection for certain anatomic regions, including the presternal area, chest, back, shoulder, anterior and posterior neck, and earlobes. Many summarize this predilection as the “cape area.” Areas of greater skin tension such as the back and chest are common areas for keloid formation. In addition, keloids continue to evolve while hypertrophic scars typically subside. Just as

there are clinical differences between keloids and hypertrophic scars, so too are there characteristic histologic differences which separate the two entities.

## ETIOLOGY

A genetic predisposition combined with some form of external injury may lead to the formation of keloids and/or hypertrophic scarring in certain individuals. An abnormal response of the connective tissue following skin trauma occurs. Injury to the skin may occur in a variety of intentional or unintentional means. Spontaneous keloids develop without a clear-cut history of trauma and often favor the chest, upper back, shoulders, and arms (Figures 37.1 and 37.2).

Another important aspect of keloid and hypertrophic scar formation is that of wound tension, which has been implicated as an instigating factor. Surgical incisions should be performed to minimize these forces of tension whenever possible. Additionally, the use of dermal sutures placed appropriately can assist in the reduction of wound tension and in this way help minimize the chance of keloid formation.

A combination of factors including the nature of injury, severity, depth, anatomic location, tensional stress, infection, environmental factors, and genetic predisposition all contribute to the potential for and severity of hypertrophic scar and/or keloid formation. Evidence of a genetic predisposition for keloid formation is demonstrated by its increased frequency in different ethnic populations, a family history of keloid formation, and its occurrence in twins.

## CLINICAL ASPECTS

An understanding of both hypertrophic scars and keloids is essential for accurate diagnosis of keloids. Clinical examination can typically differentiate between these two entities. Hypertrophic scars are confined to the traumatized region while keloids extend beyond the initial confines of trauma (Figure 37.2). This is the primary visual means of differentiating hypertrophic scars and keloids. In areas where stretch-back scarring can be predicted, hypertrophic scar formation is often mistaken for keloid formation because of the increased width of the scar. Occasionally, removal of a keloid results in a hypertrophic scar which, again, is often confused with a recurrence of the keloid. In reality, the distinction may not be as important, as treatment methods are similar.

Hypertrophic scars typically develop quickly after an inciting surgery and subside gradually over time. In contrast, keloids often develop more slowly and do not resolve (Figure 37.3). Hypertrophic scars may be treated with surgical revision while this may result in additional keloid formation and/or worsening of the treated keloid.





**Figure 37.1** Keloids on the chest with restriction of movement due to extensive involvement. These are spontaneous keloids and occurred without any known trauma.



**Figure 37.2** Spontaneous keloids on the chest wall. No known surgical or trauma occurred to form these keloids.

The size of a hypertrophic scar is usually reflective of the inciting injury; however, even a small injury can yield a large keloid (Figures 37.4–37.8). Keloids in areas such as the neck and chest can grow to massive size and cause restriction of movement (Figure 37.1). Treatment of keloids of this size and body restriction prove especially difficult. Keloids may form secondary to hair cut short with resultant inflammation of the skin from the hair with keloid formation (acne keloidalis nuchae, Figure 37.9). Hidradenitis suppurativa can include keloid formation, most commonly involving the axillae and inguinal region of the groin (Figure 37.10). Keloids can form after severe acne as well (Figure 37.11). It is important to remember that intentional therapeutic measures such as re-excision, injection, or laser treatments can themselves cause keloid formation.

## HISTOLOGY

Histologic examination of a keloid (and hypertrophic scars) demonstrates a random array of thick, hyalinized, eosinophilic collagen bundle deposition (Figure 37.12). This is in contrast to that of normal skin, in which the collagen bundles are seen in parallel to the skin surface. Differentiation between hypertrophic



(a)



(b)

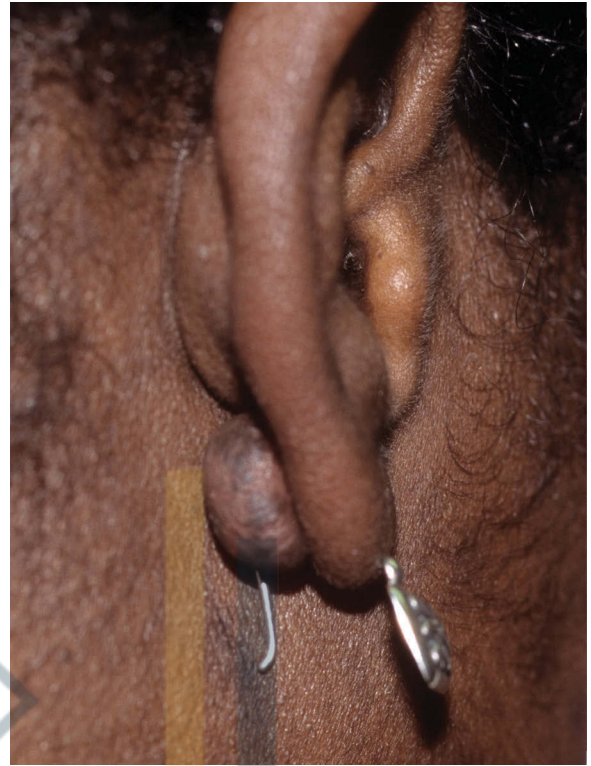
**Figure 37.3** Hypertrophic scar (a) following a surgical procedure and keloid (b) resulting from trauma to the left arm.

scars and keloids is also possible. The collagen bundles seen in hypertrophic scars are flatter and less demarcated than those in normal skin. Collagen fibers are seen in a wavy pattern. These features are more pronounced in keloids. Occlusion of microvessels is reported to occur in both hypertrophic scars and keloids. Differentiation is typically possible via clinical examination.

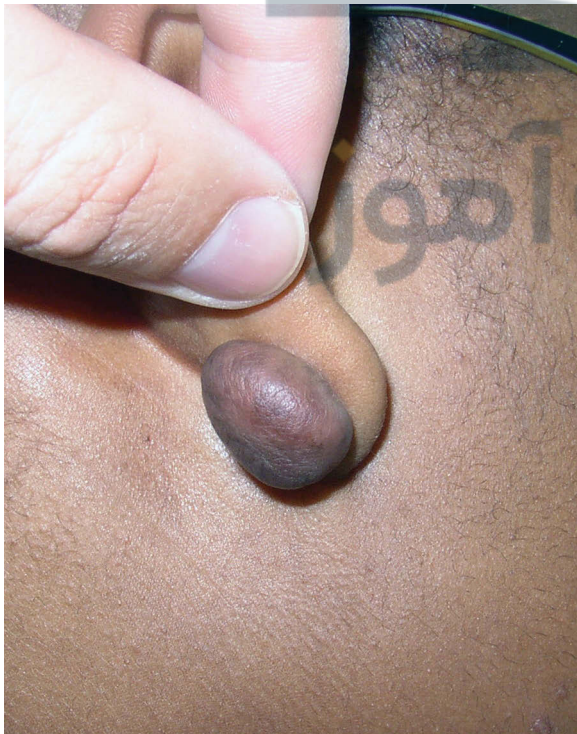




**Figure 37.4** Keloid formation secondary to piercing of the ear in an adolescent. Piercing was performed at the age of 13.



**Figure 37.6** Keloid on the right earlobe secondary to piercing of the ear in adolescence.

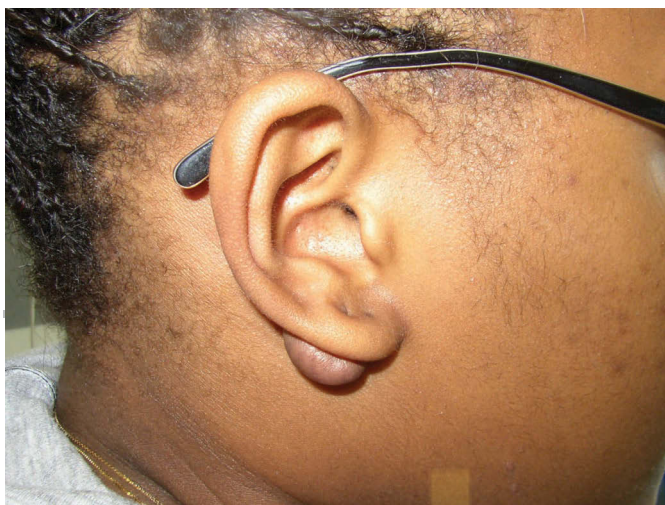


**Figure 37.5** Keloid on the posterior ear secondary to piercing of the ear.



**Figure 37.7** Large pedunculated keloid on the right earlobe. Patient had piercing performed in adolescence.

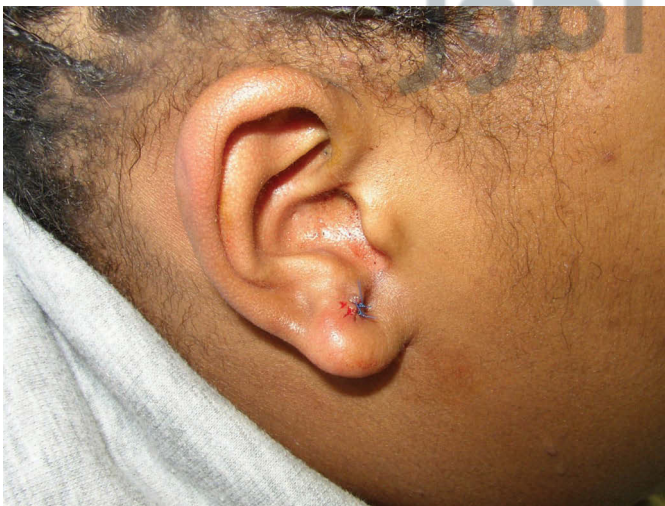




(a)



(b)



(c)

**Figure 37.8** (a, b, c) Excision of keloid on right earlobe with transmural excision (dumbbell technique).



**Figure 37.9** Acne keloidalis nuchae in the scalp secondary to short haircut.



**Figure 37.10** Keloid formation in the setting of axillary hidradenitis suppurativa. Treatment is typically difficult and may consist of topical and oral antibiotics. The use of CO<sub>2</sub> laser as well as some biologics may offer some benefit.

## PATHOGENESIS

The pathogenesis of keloid formation still remains largely unknown. Recent advances have implicated the role of transforming growth factor- $\beta$  (TGF- $\beta$ ) and platelet-derived growth factor (PDGF). These play a role in modulating contractile forces in skin fibroblasts. A brief overview of wound healing assists in the discussion of pathogenesis.

There are three primary phases of normal wound healing: inflammatory, proliferative/fibroblastic, and maturation/remodeling. The inflammatory phase consists of an immediate influx of inflammatory mediators into the site involved. A fibrin clot is initiated during this phase. This occurs by



capillary dilation and subsequent delivery of these mediators. The fibroblastic phase consists of fibroblast advancement into the fibrin clot with production of new collagen. The maturation phase occurs as the wound matures via collagen synthesis and degradation. A variety of signaling molecules (TGF- $\beta$ ,

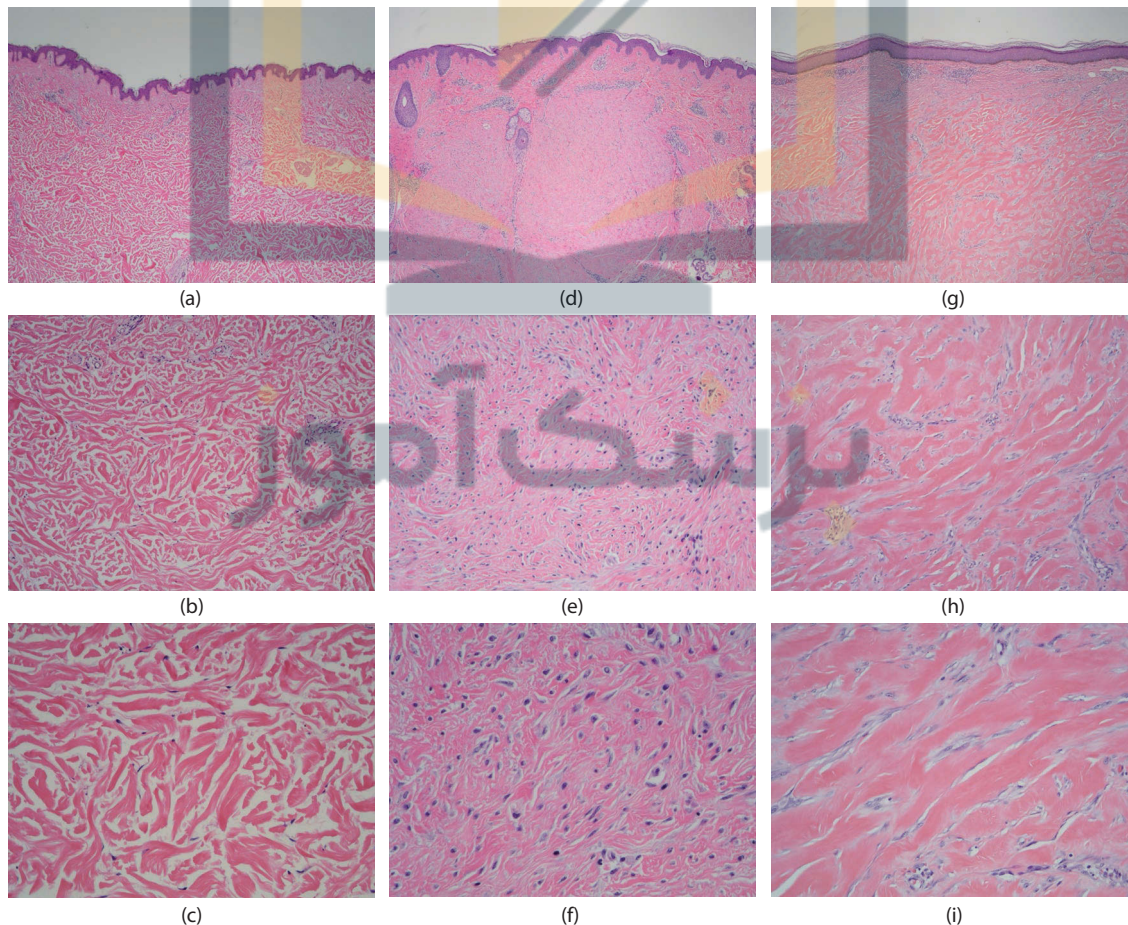


**Figure 37.11** Keloid formation secondary to severe acne on the back.

PDGF, matrix metalloproteinases [MMPs], tissue inhibitors of metalloproteinases [TIMPs]) regulate this process.

With this basic template of the wound healing process in place, the faults by which keloids and hypertrophic scars occur can be demonstrated. Early forms of fibroblasts have been shown to persist longer in keloids than in normal skin. This persistence of early fibroblasts likely results in increased collagen production. This collagen synthesis is 20 times greater in keloids than in normal skin. While the dominant type of collagen in normal skin is type I, keloids have both types I and III.

Growth factors have shown the most promise in the quest for keloid pathogenesis. TGF- $\beta$  promotes fibroblasts to localize to sites of inflammation to begin extracellular matrix protein synthesis. While this activity is normally turned off when repair is complete, dysregulation of TGF- $\beta$  activity is likely a key factor in keloid production. Decreased synthesis of molecules that promote collagen matrix breakdown (MMPs) has also been shown to be a factor in keloid pathogenesis. Other studies have shown that infrared light can inhibit fibroblast proliferation and activity. How this happens is unknown, but it may be why some success has been reported with CO<sub>2</sub> laser excision of keloids.



**Figure 37.12** Histopathology of normal skin (a–c), hypertrophic scars (d–f), and keloids (g–i). These are shown at 4× magnification (top row), 10× magnification (middle row), and 20× magnification (bottom row). Normal skin has distinct collagen bundles that are predominantly arranged parallel to the epidermal surface. Evaluation of hypertrophic scars demonstrates less order of the collagen bundles, and keloids are marked by haphazard arrangement of collagen fibers with random orientation.

TREATMENT

Treatment of keloids and hypertrophic scars presents a clinical challenge (Table 37.1). As the two entities are similar, treatments are also similar. The fact that traumatic injury is the typical cause highlights the difficulty of any type of surgical treatment. Treatment options include a multitude of possibilities ranging from noninvasive to invasive. The choice of treatment depends on a variety of factors, including the patient (age, health), location, size, depth, and previous treatments.

A number of topical treatment modalities have been successfully used to treat keloids and hypertrophic scars. A common technique used at the initial time of diagnosis is gentle massage of the site. Instructions to the patient include a gentle rocking massage for several minutes to be performed several times per day. This can be useful for smaller scars and especially in sites where web formation is a possibility. The use of topical “keloid medications” has not been shown to be better than just massage alone.

The use of mechanical pressure is used as both treatment and prophylaxis. This is commonly seen with pressure earrings used to both treat and prevent keloids secondary to ear piercing. Treatment with mechanical pressure takes time and may require as long as 6 to 12 months or longer for acceptable results. Additionally, pressure garments should be used 23 to 24 hours/day. Success in treatment is largely dependent on patient compliance.

Silicon gel sheeting is believed to act by scar hydration, resulting in decreased capillary flow with subsequent reduction in collagen deposition from a decrease in circulating proinflammatory cytokines. Some authors have demonstrated excellent success rates with silicon gel sheeting, while others attribute its success to the occlusive wound effects. Silicone sheeting should be worn for a minimum of 12 hours per day and for a minimum of 2 months. The true “gel” types of sheeting have been shown to give more consistent results than the dry “card-like” sheeting.

The use of cryosurgery in the treatment of keloids and hypertrophic scars is much like that in treatment of other dermatologic conditions. Cryosurgery utilizes a cryogenic agent (liquid nitrogen) to induce direct cellular and microcirculatory damage. This leads to tissue necrosis and hopeful flattening of the lesion. The primary risk of this method is

hypopigmentation of the treatment site. Success rates as high as 74% have been claimed with the use of cryosurgery to treat keloids. It has also been noted that younger keloids tend to respond better to cryosurgery than older keloids. It can also be used as an adjunct to initial corticosteroid intralesional injection. The edema which results from cryosurgery loosens the scar-like fibers and facilitates easier injection.

Topical corticosteroids remain a common first-line treatment of both keloids and hypertrophic scars. The known side effect of skin atrophy is harnessed and used to the clinician’s advantage. Similar medications such as tacrolimus and pimecrolimus demonstrate some potential; however, these are weaker than most corticosteroids utilized and thus may be most efficacious in anatomic locales where stronger corticosteroids are not possible.

Intralesional injection remains a mainstay of treatment of keloids and hypertrophic scars. The most common and perhaps most useful medication used is triamcinolone acetonide. Triamcinolone acetonide as injected is available in multiple concentrations. Stock concentrations of 10 mg/mL and 40 mg/mL are most often used, while alternate dilutions can be prepared easily by diluting these stock concentrations. A common diluent is lidocaine, which offers the added benefit of local anesthesia at the injection site. The dose and concentration varies based on the size and location of keloid; however, this may range from 5 to 40 mg/mL. Injecting a medication into a keloid often represents a physical challenge due to the density and firmness of a keloid. A basic understanding of Poiseuille’s law is important in the delivery of medication. This law defines the volume flow-rate by the pressure difference divided by the viscous resistance. More succinctly, it determines the resistance to flow. The important point for the clinician is that a smaller diameter syringe allows a greater mechanical advantage for injection. A 1-cc tuberculin syringe with a lure lock tip and 27-gauge or larger needle is recommended. Lack of a lure lock system frequently results in propulsion of the needle from the syringe. The N-tralig injector utilizes a ratchet-type mechanism to allow injection of medication into keloids and hypertrophic scars (Figures 37.13 and 37.14). Use of this device proves especially helpful for firm keloids and initial treatments, where the mechanical force needed to inject is great. Another technique with keloid injection is the derma-jet (Figure 37.15). This specialized syringe allows one to inject medication without using a needle.

In all cases, it is important to avoid injecting into the subcutaneous fat, as the atrophy that follows is difficult to

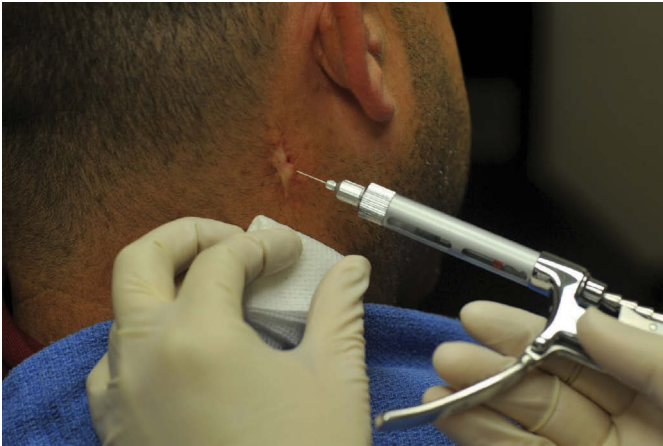
Table 37.1 Treatment of Keloids and Hypertrophic Scars

Topical
Massage
Pressure
Silicon gel
Cryotherapy
Tacrolimus
Retinoids
Intralesional injection
Corticosteroid (Triamcinolone acetonide)
Interferon- $\alpha$ -2b
5-fluorouracil
Mitomycin C
Verapamil
Laser
Pulsed dye
Argon laser
Nd:YAG laser
CO <sub>2</sub> laser
Oral
Surgical
Radiation therapy
Other

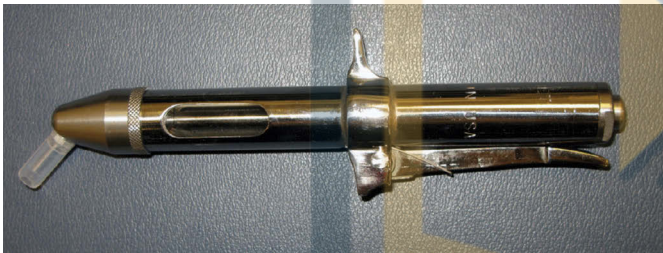


Figure 37.13 N-tralig mechanical injector.





**Figure 37.14** Intralesional treatment of a hypertrophic scar with the N-tralig mechanical injector.



**Figure 37.15** Dermajet injector.

correct. Injection around or near the eye should also be carried out with caution, as particles from the suspension have been shown to cause amaurosis through embolization.

The use of retinoids for the treatment of keloids and hypertrophic scars has been suggested topically; however, they have also been cited as a potential causative agent when taken orally. Decrease in size of keloids treated with tretinoin 0.05% topically for 12 weeks has been described. The risk of scarring is well documented following recent treatment with isotretinoin and acitretin. Typical recommendations suggest waiting 6 months after cessation of accutane prior to elective surgery when keloid formation may be of concern.

The use of imiquimod 5% cream has been met with mixed reviews in the prevention and treatment of keloids. Imiquimod is a topical immune response modifier that stimulates interferon- $\alpha$  (INF- $\alpha$ ). This is a proinflammatory cytokine that increases collagen breakdown. Although there are conflicting results in the literature, most of these studies consist of small study groups. Given the favorable results in many studies and the overall safety profile of imiquimod, it is a simple and potentially useful adjunctive therapy for treatment of keloids.

Reports of using calcium antagonists to retard extracellular matrix production in connective tissue equivalents and intralesional verapamil to treat keloids have also appeared in the literature.

The use of lasers to treat keloids and hypertrophic scars has been met with some success (Figures 37.13–37.19).



(a)



(b)

**Figure 37.16** (a) Treatment of keloids with CO<sub>2</sub> laser. Multiple treatment sessions were required with (b) resultant improvement and decreased thickness. Of note, the resultant scars represent hypertrophic scars, an improvement over the previous keloids.



(a)

**Figure 37.17** (a, b) Use of CO<sub>2</sub> laser to ablate large keloid on the pre- and postauricular cheek. (Continued)





(b)

**Figure 37.17 (Continued)** (a, b) Use of CO<sub>2</sub> laser to ablate large keloid on the pre- and postauricular cheek.



(c)

**Figure 37.18 (Continued)** Postoperative result after CO<sub>2</sub> ablation of keloid (from Figure 37.17) at 2.5 months (a) and 4.5 months (b and c). The resultant surgical scar represents a hypertrophic scar but an improvement over the previous keloid



(a)



(b)

**Figure 37.18** Postoperative result after CO<sub>2</sub> ablation of keloid (from Figure 37.17) at 2.5 months (a) and 4.5 months (b and c). The resultant surgical scar represents a hypertrophic scar but an improvement over the previous keloid. (Continued)



(a)



(b)

**Figure 37.19** Large keloid on right cheek of a young man (a) and after CO<sub>2</sub> resurfacing (b).



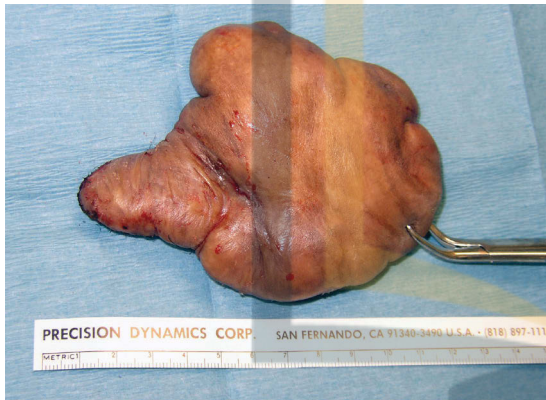
A number of different lasers have been tried but the main two that remain in use are the pulsed dye laser and the carbon dioxide (CO<sub>2</sub>) laser. The pulsed dye laser is a 585 or 595 nm system. Frequency-doubled Nd:YAG systems have also demonstrated benefit in the treatment of keloids and hypertrophic scars. Pulsed dye lasers have the benefit of reduction in size and redness of keloids and hypertrophic scars as well as normalizing the surface texture of keloids. The CO<sub>2</sub> laser is an ablative laser that vaporizes tissue and can be used to excise keloids (Figures 37.13–37.23). It is thought to have an inhibitory effect on fibroblasts.

Surgery is commonly used to treat keloids and hypertrophic scars; however, it must be used with caution as this may result in keloid formation itself, sometimes worse than the initial lesion. The risk of anticipated keloid formation from surgical intervention often warrants adjuvant therapy such as postoperative corticosteroid injections. Recurrence of keloids following surgical intervention varies from 50% to 80%.

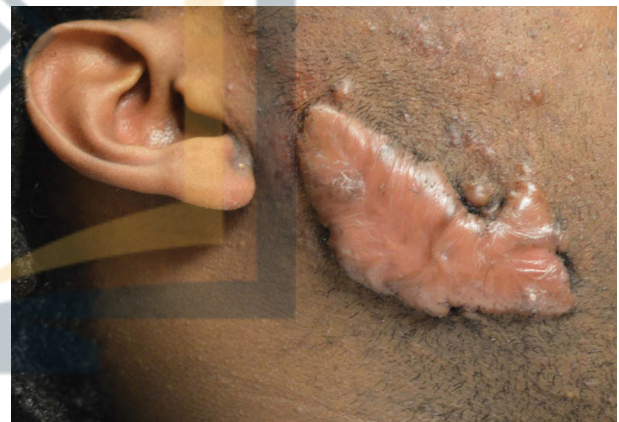
Radiation therapy may be utilized for reduction of keloid size on account of its ability to destroy fibroblasts and



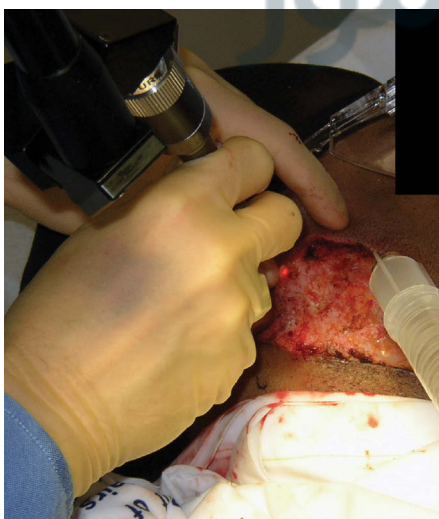
**Figure 37.22** Use of CO<sub>2</sub> ablation to treat a keloid secondary to ear piercing.



**Figure 37.20** Surgical excision of large keloid.



(a)



**Figure 37.21** Surgical excision and CO<sub>2</sub> resurfacing of large keloid on cheek.



(b)

**Figure 37.23** Use of CO<sub>2</sub> ablation to remove and recontour keloid on right cheek. The initial keloid (a) was first surgically excised (b) in an effort to debulk the lesion. Despite the use of intralesional corticosteroids, silicon sheeting, and topical imiquimod, the keloid recurred. (Continued)



(c)



(d)

**Figure 37.23 (Continued)** Use of CO<sub>2</sub> ablation to remove and recontour keloid on right cheek. The initial keloid (a) was first surgically excised (b) in an effort to debulk the lesion. Despite the use of intralesional corticosteroids, silicon sheeting, and topical imiquimod, the keloid recurred.

neovascular buds via ionizing radiation. Radiation is typically used in conjunction with surgery. The risks of ionizing radiation must certainly be weighed against the possible benefits.

A number of less common but reported treatments include the use of onion extract, cultured epithelial autografts, pentoxifylline, colchicine, calcium antagonists, tranilast, and vitamin E.

The use of combination therapy to treat keloids is not new but has become a focus in more recent literature. This has classically included excision with either immediate or subsequent injection of intralesional corticosteroids. More recently, reports of combinations including excision, mitomycin C, and radiotherapy have demonstrated some success. The use of additional lasers such as the 1064 nm Nd:YAG has also been reported to have some benefit.

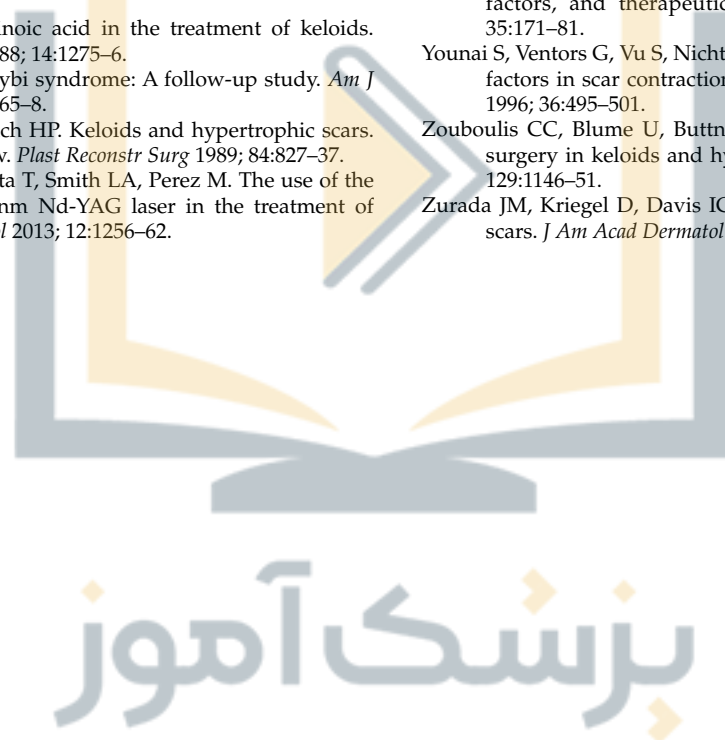
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# Keratolytic Treatment of Acne

Brigitte Dréno

## INTRODUCTION

Topical keratolytics are agents that dissolve or break down the outer layer of skin. They loosen and assist exfoliation of the skin cells. In acne, their target is the comedo and the most powerful keratolytics are used **topically**. These topical keratolytic agents have long been employed for acne treatment and have always played a **central** role in the treatment of retentional lesions. Shalita et al. state that “the first histologically visible change in acne is a disruption in the normal pattern of keratinization, resulting in dense, coherent squamæ of keratinous material that accumulate to form a plug in the orifice of the follicle, leading to formation of the microcomedo.” Microcomedo is the precursor of acne lesion, histologically but not clinically visible. Furthermore, these aberrancies in proliferation, adhesion, and differentiation of the keratinocytes obstruct the infundibulum and the sebaceous duct, paving the way for excessive sebum secretion, bacterial overgrowth (*Propionibacterium acnes*), and inflammatory response due to **activation** of cutaneous innate immunity. Under light microscopy, microcomedones are visualized as layers of horny cells surrounding a sebum and bacteria core (1). Keratolytic agents are thought to function by relaxing the cohesiveness of the stratum corneum (SC) layer, which serves as a crucial, life-sustaining barrier, keeping hydration “in” and harmful foreign agents “out.” The mechanism of action does not involve keratin lysis as the name implies, but rather disintegration of desmosomes and corneodesmosomes that link keratinocytes of the follicle and bind them to the extracellular matrix, respectively (2). In this manner, these agents can secondly modulate and correct abnormal follicular keratinization.

Currently many classes of keratolytics exist (Table 38.1). Available in varying concentrations and vehicles, they may be specifically indicated depending on the type, duration, and severity of acne and the sensitivity of the skin and the part of the body (face or trunk). Their targets are the microcomedo and the comedo. This chapter covers widely available topical and oral keratolytics, controlled trials comparing keratolytic agents, and in vivo keratolytic protein assays. Uncontrolled trials and older acne treatments are discussed briefly.

This overview was based on PubMed, Embase, and Science Citation Index utilizing the following words: keratolytics, benzoyl peroxide, azelaic acid, retinoids, sulfur, resorcinol, glycolic acid, salicylic acid, combined therapy, peeling, acne vulgaris. For clinical trials, we firstly considered double-blind controlled trials and if not, randomized controlled trials with more than 80 included patients. Only studies that assessed subjects with acne vulgaris on the face were included.

## BENZOYL PEROXIDE

Benzoyl peroxide (BPO), a mainstay treatment of mild to moderate acne for decades, has mainly antimicrobial, anti-inflammatory effect and only mild anticomedogenic effects. Acting through oxidation and **formation** of free radicals, its bacteriostatic activity is **superior even** to that of topical antibiotics (3). It decreases inflammation by killing polymorphonuclear leukocytes (PMNs), preventing the release of reactive oxygen species (4). The mild keratolytic activity is probably linked to the destruction of *P. acnes*. Indeed recently it has been shown that *P. acnes* is able to increase the proliferation and modulate the differentiation of keratinocytes, thus playing a role in the formation of the comedo (5). Unfortunately, oxidative destruction of the SC may deplete cutaneous vitamin E, resulting in oxidation of surface lipids and proteins; this may predispose to skin dryness and desquamation (6). BPO is absorbed effectively into the epidermis, particularly by pilosebaceous units, and converted to benzoic acid, with approximately 2% entering the systemic circulation (7,8). Its lipophilicity allows it to enter and accumulate in the lipid-rich pilosebaceous units and subcutaneous fat (4). It is an FDA Pregnancy Category C agent, with little known about potential fetal harm or breast milk excretion, and positive in the rodent photocarcinogenicity assay. In Europe, it can be prescribed to pregnant women. It is widely available both over the counter (OTC) and by prescription, and comes in different concentrations ranging from 2.5% to 10%. Adverse effects include dryness, peeling, burning, and redness of skin, with contact allergy only in 1% to 2% of patients (3). To that end, patients should avoid excessive UV radiation, which can exacerbate irritation. Additionally, the water-based formulations may exert **less** drying, scaling, burning, and erythema than the alcohol-based formulations (3,9). Of note, BPO, an oxidizing agent, can bleach hair, clothing, and colored fabrics. It may also inactivate tretinoin if both are applied concurrently (10); in contrast, adapalene and tazarotene remain stable in the presence of BPO (7).

The 2.5% formulation may be as effective as the 5% and 10% formulations in reducing retentional and inflammatory lesions, while causing fewer adverse reactions than the 10% solution (11).

Benzoyl peroxide has been combined with other molecules to increase its keratolytic efficacy. In a split-face, double-blind trial, a combination of BPO 5% and urea 8% lotion was not more efficacious in diminishing acne than BPO 5% lotion alone; the combination took longer to dry and was stickier, according to subjects (12). Combination therapy with topical antibiotics and BPO may be more effective than BPO alone. Both the clindamycin/BPO and the erythromycin/BPO formulations have shown superior efficacy when compared with either the antibiotic or BPO

**Table 38.1** Keratolytics Currently Used in the United States and Europe in Acne

Name	Class	First Introduced	Usual Concentration(s) (%)	Vehicle(s)
Salicylic acid	$\beta$ -Hydroxy acid	1887	No more than 2.0	Bar, foam, cream
Glycolic acid	$\alpha$ -Hydroxy acid	1900	<10%	Superficial peeling
Benzoyl peroxide	Organic peroxide	1920s	2.5, 5.0, 10	Gel, bar
Tretinoin	Retinoid	1962	0.025, 0.05, 0.1	Cream, gel
Isotretinoin	Retinoid	1979	0.05	Gel
Tazarotene	Retinoid	1997	0.1, 0.05, 0.5	Gel, cream
Adapalene	Retinoid-like	1996	0.1	Gel, cream
Azelaic acid	Dicarboxylic acid	2002	15.0, 20.0	Cream, gel
Sulfur	Sulfur	1998	10.0	Bar
Urea	Urea	1828	<10%	Cream
Resorcinol	Phenol	1866	—	Peeling
Clindamycin/benzoyl peroxide	Antibiotic combination	2009	1.0/5.0	Gel
Erythromycin/benzoyl peroxide	Antibiotic combination	2006	3.0/5.0	Gel
Adapalene/benzoyl peroxide	Retinoid-like/BPO	2009	0.1/2.5	Gel
Tretinoin/clindamycin	Retinoid/Antibiotic	2010	0.025/1.2%	Gel

alone (13). Three well-designed, randomized, double-blind, vehicle-controlled, multicenter clinical trials comparing the clindamycin/BPO gel with each individual agent and vehicle demonstrated significantly superior efficacy both in retentional and inflammatory lesion reduction after 10 to 16 weeks. Furthermore, the side effect profile (dry skin, peeling, and erythema) of combination therapy is comparable to that of BPO alone (4). Two other multicenter, double-blind randomized studies of 2813 acne patients with moderate to severe acne compared the efficacy and safety of a fixed combination clindamycin phosphate 1.2% and BPO 2.5% with each drug alone and a vehicle arm, in moderate or severe acne subpopulations. Clindamycin-BP 2.5% gel significantly reduced inflammatory and non-inflammatory lesions compared with each active ingredient and vehicle only in moderate acne at week 12. Rates of adverse events were low and similar between treatment groups and baseline acne severity. This study confirms that the target of this combined therapy is moderate and not severe acne (14).

Concerning the combination BPO/erythromycin, no double-blind study has been performed. Leyden et al. compared clindamycin/BPO and erythromycin/BPO demonstrating statistically equivalent lesion reduction and global improvement, with similar tolerability (15).

In vivo data suggest that the increased efficacy of a BPO/antibiotic combination may have an immunological basis as demonstrated by decreased antioxidant enzyme activities in leukocytes after month-long combination treatment (16). Additionally, this combinatory approach may prevent the evolution of resistant *P. acnes* strains (17).

## RETINOIDS: TRETINOIN, TAZAROTENE, ADAPALENE

Topical retinoids encompass a group of powerful comedolytic, anticomedogenic, and anti-inflammatory agents. They are powerful keratolytics, targeting both primary and secondary prevention of comedones. There are two generations of topical retinoids—first-generation, represented by the tretinoin, and second-generation, represented by adapalene and tazarotene—that are specific of retinoic acid receptors (RARs). Retinoids exert their effects through nuclear receptor families RARs and RXRs (retinoic X receptors), subsequently inducing retinoic acid-responsive target gene expression (18),(19). Both

receptor families are ligand-dependent transcription factors and consist of three receptor subtypes (a, b, and  $\gamma$ ), encoded by three separate genes (20).

Although RAR-a is ubiquitous in embryonic skin, RAR- $\gamma$  is the most abundant RAR in human epidermis, cultured keratinocytes, and dermal fibroblasts (20,21). Retinoids also inhibit expression of certain genes by down regulating other transcription factors, notably activator protein 1 (AP-1) and nuclear factor-interleukin 6 (NF-IL6) (18). This inhibitory action may be partly responsible for the antiproliferative and anti-inflammatory actions of retinoids (20).

Prior to binding with nuclear RARs, retinoids must first bind to intracellular proteins. Cellular retinoic acid proteins (CRABP I and II) are present in the skin. Intracellular retinoid concentrations are dependent on CRABP, primarily type II (18). However, CRABP II is not essential for biological retinoid activity as adapalene does not bind to it; interestingly, it may play a role in retinoid-induced epidermal irritation (20). Through this genetic regulation, retinoids are thought to affect cellular differentiation and proliferation (22). Experimental studies, some using primary neonatal mouse epidermal keratinocyte cultures, have confirmed this concordant decrease in keratinocyte differentiation and proliferation (23). Retinoids also regulate activity of keratinocyte adhesion and cohesion molecules (integrins), resulting in breakdown and obliteration of the horny plug (21). Mechanisms of action are numerous and include an anti-inflammatory activity with an inhibition of neutrophil chemotaxis, expression of toll-like receptors involved in innate immunity, inhibition of prostaglandins, leukotrienes, and interferon- $\gamma$  release. They also inhibit the release of proinflammatory cytokines (interleukins 12 and 8 and tumor necrosis factor) via down regulation of monocyte TLRs (24,25). Interestingly, *P. acnes* acts through TLR-2 to stimulate proinflammatory cytokine production (26). The major drawback to topical retinoids is local skin irritation and acne exacerbation, also termed “retinoid flare,” which may occur during the first month of treatment and last several weeks (7). This flareup may be secondary to release of follicular inflammatory factors after topical retinoid treatment (27,28). Another limiting factor of topical retinoids is the contraindication to their use during pregnancy. Limb-reduction defects and ear malformations have been reported with maternal use of topical retinoids in two papers (29,30). However, Jick et al.,



in a retrospective study, did not substantiate this suggestion, and the clinical issue remains to be confirmed (31).

## Tretinoin

Tretinoin, the first topical retinoid to be studied, binds with high activity to all three RAR subtypes and to CRABP, and with low activity to RXRs. It is both a comedolytic and anticomedogenic, preventing formation of microcomedones (20). Employing the technique of skin surface biopsy, microscopic examination of comedones showed progressive loss of cohesiveness and significant alterations in epithelial structure; thick keratinous plugs infested with bacteria were transformed into a few wispy layers of keratin with few bacteria. Using transmission electron microscopy, it was possible to track microcomedones with compact, adherent SC morphing into spongy, loosely adherent layers of corneocytes (1). Mills and Kligman, using a cyanoacrylate follicular biopsy technique, demonstrated a profound microcomedone reduction in 8 and 12 weeks (32). From an immunological perspective, *in vitro* studies have demonstrated that tretinoin down regulates and decreases surface expression of TLR-2 and CD14 mRNA, preventing secretion of tumor necrosis factor and IFN- $\gamma$ , as well as production of free radicals (21,23). Tretinoin cream 0.025% significantly reduced inflammatory but mainly non-inflammatory acne lesions compared with vehicle by 12 weeks. Numerous trials have also demonstrated the efficacy of 0.05% and 0.1% gel tretinoin in mild to moderate acne with a decrease of retentional lesions between 30 and 50% according to the studies (1). Additionally, tretinoin may bring out the postinflammatory darkening that occurs in healing acne of darker-skinned patients (27). Surprisingly, topical tretinoin has poor percutaneous absorption and does not alter systemic retinoid levels, which stay constant despite application (7). Side effects include peeling, erythema, dryness, burning, exfoliation, and itching (7,21,33). Side effects included erythema, dry skin, and exfoliation. Despite some evidence to the contrary, topical tretinoin is not advised during pregnancy and lactation. Potential for systemic exposure and excretion in breast milk have not been adequately studied.

Addition of some chemical substances or medical devices has been proposed to increase efficacy and decrease irritation. Thus, addition of polyolprepolymer-2 (PP-2), localizes drug molecules in upper skin layers, preventing deep penetration (33). PP-2 forms a liquid reservoir of polymer and solubilized drug on the skin surface, slowing percutaneous absorption and transcellular cutaneous diffusion, potentially targeting folliculo-infundibular delivery in the process. Clinical trials have demonstrated reduced irritation as less drug penetrates the skin (34). The Microsponge Delivery System found in 0.1% microsphere gel also helps reduce drug release rate and increase drug retention in the SC, inhibiting deeper penetration (7). Tretinoin is trapped within porous copolymer microspheres which selectively localize to the follicle, releasing tretinoin over time and producing less irritation (than the standard 0.025% cream) due to reduced concentration on the skin (21,35).

A recent study compared the efficacy and safety profile of tretinoin 0.05% with adapalene 0.1 and 0.3% and placebo in Mexican subjects with acne vulgaris. Tretinoin 0.05% and adapalene 0.3% were more effective than adapalene 0.1% and placebo in the reduction of both inflammatory and non-inflammatory lesions, but the adverse events (topical irritation) were also more important (36).

Two other studies have compared micronized tretinoin gel 0.05% versus tretinoin gel microsphere 0.1%, with similar efficacy in both. Concerning tolerance, the results were contradictory between the two studies, not permitting any conclusions (37).

**Combination:** The alteration in SC integrity incurred during tretinoin treatment may enhance penetration of other agents such as topical antibiotics (38). Topical retinoid therapy, by weakening the horny layer barrier, may increase skin permeability, enhancing penetration of antimicrobial agents. Increased cell turnover of follicular epithelium enables greater access of antibiotic into the canal that houses *P. acnes*. A hydrogel containing 1% clindamycin and 0.025% tretinoin was found to be more efficacious in treating both inflammatory and non-inflammatory acne lesions than either agent alone or vehicle (39). Three other double-blind randomized studies have been performed confirming that the combination clindamycin phosphate 1.2% and tretinoin 0.025% decreased significantly more both retentional and inflammatory lesions in mild to moderate acne compared with each of the drug used alone (40–42). The inflammatory flareup of the first days of treatments was also less important.

Finally, a BPO 6% cleanser–tretinoin 0.1% microsphere gel demonstrated significantly greater inflammatory lesion reduction than tretinoin alone (43). However, tretinoin should not be used with BPO (an oxidizing agent), which can result in degradation and deactivation.

## Topical Isotretinoin

One multicentric double-blind randomized study (44) has compared isotretinoin 0.05% gel with its vehicle. Patients were treated twice daily for up to 14 weeks. Efficacy of Isotretinoin was significantly better than vehicle both for inflammatory and retentional lesions with low irritation. But in a general manner topical Isotretinoin is considered as less efficacious than other topical retinoids with a low keratolytic effect. The efficacy cannot be compared with systemic Isotretinoin.

## Tazarotene

Tazarotene, a second generation of topical retinoids, is a topical acetylenic retinoid indicated in both psoriasis and acne vulgaris. Currently, only the 0.1% formulation of tazarotene is approved by the FDA for acne and tazarotene is not approved in Europe for acne. It is primarily used in cases of acne refractory to tretinoin and adapalene treatment (7).

Tazarotene is hydrolyzed by keratinocyte esterases to tazarotenic acid, its active metabolite (18). It binds all three RARs but not RXR, activates gene expression only in RAR- $\gamma$  and  $\gamma$ . It down regulates AP-1 (18,20,45). As tretinoin, it normalizes the keratinization pattern and decreases coherence of follicular keratinocytes, manifesting both comedolytic and anticomedogenic properties (22). Tazarotene also has anti-inflammatory properties (22). In the systemic circulation, tazarotenic acid is rapidly converted to inactive sulfur-oxidized forms, resulting in limited exposure (45). Nonetheless, animal studies have demonstrated that tazarotene has low systemic absorption with no toxic effects even at high topical doses (45). Additionally, after 12 weeks of normal tazarotene application, serum samples from 22 subjects demonstrated limited systemic exposure with most below the quantifiable limit (<0.05 ng/mL) (22). Despite little evidence of fetal malformations or spontaneous abortions, topical tazarotene is an FDA

Pregnancy Category X drug; little is known about its excretion in breast milk. Of all topical retinoids in acne treatment, it is the only one requiring sufficient contraception in women of childbearing age (21).

At the clinical level, a randomized, double-blind, vehicle-controlled study demonstrated that 0.05% and 0.1% tazarotene gels significantly decrease retentional acne lesions and produce a higher success rate than vehicle at 12 weeks (22). Moreover, 0.1% gel was significantly more efficacious than 0.05% gel, mainly on inflammatory acne (22). Two randomized trials comparing tazarotene 0.1% cream to adapalene 0.1% cream demonstrated tazarotene to be significantly and rapidly more effective in reducing comedone and inflammatory lesions with no significant difference in side effects at 12 weeks in one study (22,46). But another more recent study showed that daily therapy with adapalene 0.1% gel was not inferior to tazarotene 0.1% cream in total acne lesion reductions and during initial stages of treatment and demonstrated better tolerability with respect to erythema and scaling. Finally, one trial compared once-daily tazarotene 0.1% cream and adapalene 0.3% gel in patients with moderate to severe acne (47).

Tazarotene 0.1% cream appeared to be more effective and nearly as well tolerated as adapalene 0.3% gel in reducing acne lesions and was more effective than adapalene 0.3% gel in reducing PIH. Furthermore, a large clinical trial suggests that even short-contact application (<5 minutes), once daily for 12 weeks, produces significant reduction in both inflammatory and noninflammatory acne lesions (48). A multicenter, double-blind, randomized trial found a daily 5% BPO/1% clindamycin gel-tazarotene 0.1% cream regimen to be more effective than daily tazarotene monotherapy in reducing comedo count and inflammatory lesion count, with a similar, if not slightly improved, tolerability profile (49). Recently, two randomized study determined the efficacy of tazarotene foam, 0.1% once daily compared to vehicle for 12 weeks (50). The weakness of this study is the evaluation of adverse events, not very clearly explained.

Local side effects include itching, burning, irritation, and erythema (7,22). In fact, tazarotene is thought to be the most irritating of the topical retinoids. In the Bershad study cited above, half of the patients applying tazarotene for only 2 to 10 minutes daily reported local skin irritation. These side effects are most common during the first 2 weeks of therapy; cream formulations, alternate-day application, and short-contact therapy can curtail side effects (21).

## Adapalene

Adapalene 0.1% or 0.3%, as tazarotene, is a second generation of topical retinoids. It is a derivative of retinoic acid that binds selectively to RAR- $\beta$  and - $\gamma$  in vitro but can activate gene expression through all three RARs; it does not bind CRABP II but increases CRABP II mRNA (18,20). It has comedolytic, antiproliferative, and anti-inflammatory properties which are more important than the first generation of topical retinoids (20). Its anti-inflammatory action stems from inhibitory effects on PMN chemotactic response, free radical production, and toll-like R2 receptors expressed by perifollicular monocytes (21). It also inhibits production of leukotrienes by 5- and 15-lipoxygenase pathways (20,21). Furthermore, adapalene may have a dose-dependent response, with 0.3% statistically superior to 0.1% in several different measures, while demonstrating equivalent tolerability (51).

Adapalene's particle size (diameter between 3 and 10  $\mu$ m) and its lipophilic properties result in optimal follicular

duct penetration (20); furthermore, after 5 minutes of exposure, 14-C labeled adapalene applied to human skin in vitro demonstrates radio sensitivity in the pilosebaceous units, with sparse activity in the SC and epidermis. Adapalene demonstrates higher stability than tretinoin in the presence of light, in the dark, and with BPO (10). In a study comparing the chemical stability of 0.1% adapalene gel/10% BPO and 0.025% tretinoin gel/10% BPO after 24 hours of light exposure, approximately 100% of adapalene remained intact versus only 20% of tretinoin (52).

At the clinical level, a meta-analysis of five large randomized trials (900 patients) demonstrated equivalent acne reduction, quicker onset of action (significant at 1 week), and fewer side effects in 0.1% adapalene gel compared with that in 0.025% tretinoin gel (53). Recently it has been confirmed that adapalene 0.1% is more keratolytic than benzoyl peroxide.

**Maintenance therapy:** Cyanoacrylate strip data suggests that application of adapalene 0.1% gel every other day may be effective maintenance therapy in microcomedone reduction, resulting in decreased exposure (62).

Side effect profile was significantly better in regard to scaling, erythema, dryness, immediate and persistent burning, and immediate pruritus (53). With its three aromatic rings, in a study examining two 21-day-long trials, adapalene 0.1% gel demonstrated greater tolerability and significantly less irritation than tretinoin 0.1% cream, tretinoin 0.05% cream, tretinoin 0.025% cream, tretinoin 0.01% gel, tretinoin 0.025% gel, and tretinoin 0.1% gel microsphere (54). Furthermore, in both studies, adapalene 0.1% gel was no more irritating than the petrolatum control (54). Favorable tolerability to adapalene may be explained by its receptor specificity, neutral molecular structure, and lack of breakdown products.

**Combination:** A study testing 0.1% adapalene/2.5% BPO combination gel against vehicle and individual monotherapies demonstrated combination therapy to have faster onset of action, significantly greater reductions in all lesion types, and no increase in adverse effects compared with monotherapy (26). This efficacy has been confirmed in a double-blind randomized trial in 1670 patients (55). Focusing on subgroups of patients, 0.1% adapalene/2.5% BPO is also well tolerated in the black subjects with similar results to Caucasians. No cases of treatment-related PIH were observed (56) in young preadolescent patients with moderate acne (57). The combination associated with systemic cyclines (doxycycline 100 mg/D, lymecycline 300 mg/D) can be efficient in severe acne and thus can be an alternative to a contraindication to isotretinoin (58,59). In addition of 0.1% adapalene, the combination 0.1% adapalene/2.5% BPO is also able to prevent the occurrence of relapse among patients with severe acne, and reduces acne lesions during 6 months (58).

## AZELAIC ACID

Azelaic acid, a naturally occurring, saturated C9-dicarboxylic acid, modifies epidermal keratinization (cytostatic), combats both aerobic and anaerobic bacteria (reducing *P. acnes* proliferation), and exhibits anti-inflammatory activity (18,60). This anti-inflammatory activity may potentially be mediated through inhibition of hydroxyl and superoxide radical production by neutrophils (61). Contributing to its anti-inflammatory properties, in vitro, azelaic acid is an oxygen free radical scavenger, inhibiting hydroxylation of aromatic compounds and arachidonic acid peroxidation (60,62). In their review article, Fitton and Goa describe that azelaic acid in vivo affects

differentiation of human keratinocytes by decreasing synthesis of filaggrin (keratin filament aggregating protein) (62). This results in alterations of epidermal keratinization, including reductions in the number and size of keratohyaline granules and tonofilament bundles in the SC, abnormal tonofilament arrangements, intercellular edema, swollen mitochondria, enlargement of rough endoplasmic reticulum (RER), and reductions in the thickness of the horny layer in infundibular areas. Azelaic acid functions in a cytostatic, antiproliferative manner on keratinocytes, affecting both early and terminal phases of keratinocyte differentiation, with primary effects on mitochondria and RER (63).

After application of azelaic acid, 3% to 5% remains on the SC, up to 10% penetrates into the epidermis and dermis, and 4% is absorbed systemically (although this can double with gel formulations). Nevertheless, baseline serum and urine levels are not altered by topical usage and are primarily dependent on dietary intake of whole grain cereals and animal products (7). It is an FDA Pregnancy Category B drug, as animal studies have shown favorable results; meaningful human studies are lacking.

In 2 weeks of topical treatment, 200 mL of 20% azelaic acid attenuated tetradecane-induced comedo formation in the rabbit ear, a model of follicular epithelial hyperplasia (62,64). These microscopic and experimental findings indicate keratolytic and anticomedogenic properties for azelaic acid via normalization of disordered keratinization of the follicular infundibulum. Cyanoacrylate skin surface biopsies have demonstrated significant reductions (>50%) in comedo count after 4 months of twice-daily 20% azelaic acid treatment when compared with vehicle (65). Azelaic acid has demonstrated significant inflammatory and non-inflammatory acne reduction in numerous studies (60,61). Comparing 20% azelaic acid to 0.05% tretinoin over 6 months, one group found statistically equivalent comedone and total lesion reduction and similar overall improvement. However, tretinoin use led to increased erythema, scaling, and irritation-induced discontinuation over azelaic acid (66). Another trial comparing 20% azelaic acid with 5.0% BPO demonstrated a more rapid initial effect with BPO but similar results for global response, and inflammatory lesion reduction by 4 months. Keeping with the theme, azelaic acid demonstrated milder, more transient adverse events than BPO (67). Transient side effects lasting 2 to 4 weeks have been described. These include burning, erythema, dryness, scaling, pruritus, and hypopigmentation (60).

Despite efficacy as a monotherapy, a large randomized trial demonstrated that azelaic acid functions better in combination with one of the following drugs: 4% BPO gel twice daily, 1% clindamycin gel twice daily, 0.025% tretinoin cream once daily, or 3% erythromycin/5% BPO gel twice daily (61).

Azelaic acid 15% is indicated in rosacea but has no labeling in acne.

## SALICYLIC ACID

A core component in many OTC acne treatments, salicylic acid (SA) is a widely available topical keratolytic agent. It may have a profound structural effect on the SC, resulting in disruption of intercorneocyte cohesion and subsequent desquamation (68). Dissolution of intercellular cement is further supported by scanning electron microscopy, which has demonstrated marked squamous cell separation in SA-treated human skin (69). Mills and Kligman, using cyanoacrylate follicular biopsy, demonstrated significant decreases in microcomedone count. Although

various concentrations exist (0.5%–10%), 2% is the maximum strength allowed by the FDA and European Medicines Agency (EMA) in OTC products. In five human subjects, microcomedo formation was induced via 10% coal tar distillate ointment at four sites on the back; formation was confirmed by cyanoacrylate biopsy. At each site, subjects were treated with one of three different concentrations of SA (0.5%, 1%, and 2%), twice daily for 2 weeks, and one site was left untreated (70). Ultimately, lesions were rebiopsied and examined microscopically; all three concentrations displayed tremendous comedolytic activity, with the 2% preparation superior to the lower concentrations (73).

SA is well absorbed as evidenced by numerous studies; its bioavailability in topical application varies according to duration of contact (71,72). One study estimated bioavailabilities for topically applied SA at 57.6% and 44.0% for hydro-alcoholic and cream delivery vehicles, respectively (73). The same study also demonstrated the hydro-alcohol vehicle to have superior peak plasma SA concentrations and earlier time to peak when compared with a cream vehicle.

In a related study, absorption was enhanced significantly in a mineral oil/petrolatum ointment compared with an ointment containing polyethylene glycol, glycerol, petrolatum, 10% urea (Kerasal) (74).

Two 12-week studies comparing 0.5% and 2% SA pads to placebo pads demonstrated significant efficacy in reducing inflammatory acne, non-inflammatory acne, and total lesions, while producing significantly higher proportions of good-to-excellent overall treatment assessments (70). In both studies, side effects were minimal and well tolerated (with 73). In a study comparing medicated pads 0.5% SA in an alcoholic detergent (Stridex) to placebo (pads soaked in buffered water), the treatment group experienced a 54% reduction in inflammatory acne compared with 29% in the placebo group. Reductions of open comedones and total lesions were also significant compared with placebo.

A 4-week crossover study comparing a 2% SA acne cleanser to a 10% BPO wash demonstrated that only patients treated with the SA cleanser had a significant decrease in comedonal lesions (75). A small study comparing a 2% SA/1% clindamycin combination with placebo demonstrated a significant reduction in inflammatory and non-inflammatory lesions, with 71% of subjects reporting improvement after 8 weeks (compared with 11% of placebo group) (76).

Although local skin irritation (e.g., peeling) at concentrations greater than 2% is common, systemic toxicity is rare (7). However, if applied to large areas of the body for prolonged periods of time, salicylate toxicity, toxic inner ear damage, and hypersensitivity reactions are plausible (7). To that end, these manifestations are uncommon in appropriate acne therapy. Like several other keratolytic agents, SA is an FDA Pregnancy Category C agent, with unknown effects on breast-feeding.

As a member of this family, the SA derivative known in the literature as 2-hydroxy-5-octanoyl benzoic acid or beta-lipohydroxy acid has also been proposed as an exfoliant and as a treatment of acne. The lipophilic nature of C8-LHA and its relatively slow penetration in the skin afford it an exfoliating effect that is efficient at low concentrations. It appears to have antimicrobial, anti-inflammatory, and anticomedogenic properties. It targets more specifically the coneodesmosome (77).

New chemical peels using 30% SA in polyethylene glycol vehicle have demonstrated efficacy and safety, with marked reductions in comedones and papules (78). Polyethylene glycol may be a more tolerable vehicle than the commonly used ethyl alcohol in SA chemical peels.



## SULFUR

Sulfur, a yellow nonmetallic element, is an old ingredient that has many dermatological indications, including but not limited to acne vulgaris, rosacea, seborrheic dermatitis, and dandruff (79). Once a very common ingredient in acne treatments, sulfur has fallen out of favor, partly due to its pungent odor (80). In the acne area, sulfur is thought to be keratolytic and bacteriostatic. After application to skin, sulfur reacts with cysteine in the SC, resulting in reduction to hydrogen sulfide. Hydrogen sulfide is thought to break down keratin and inhibit growth of *P. acnes* (79). Sulfur penetrates skin; it is detectable in the epidermis at 2 hours, throughout the skin in 8 hours, and completely undetectable by 24 hours. There is no evidence of systemic absorption in intact skin (79). Appearing in a variety of vehicles (lotions, creams, soaps, ointments), it appears to be more efficacious when used in combination with other drugs, namely BPO and sodium sulfacetamide (7). Clinical trials have demonstrated that lotions containing sulfur 5% with sodium sulfacetamide 10% have resulted in reduction of inflammatory lesions, comedones, and seborrhea (79).

Rare, transient side effects include dryness, itching, and malodorous skin. Nonetheless, given a lack of knowledge, it is an FDA Pregnancy Class C drug with nothing known regarding breast milk excretion.

## GLYCOLIC ACID AND SUPERFICIAL PEELINGS

Glycolic acid, a naturally hydrophilic organic acid (hydroxy acid), has keratolytic properties targeting SC and is present in many peel formulations due to its desquamating efficacy. In the context of acne, research has been conducted examining glycolic acid chemical peels. Various chemical preparations have been employed, all of which result in a partial thickness skin injury, or peel (81); The exact mechanism of action may be due to inhibition of ionic bond-forming enzymes involved in creating sulfated and phosphorylated mucopolysaccharides, glycoproteins, sterols, and lipid phosphatides. This results in fewer electronegative groups on the outer walls of keratinocytes and corneocytes, effectively diminishing cohesion forces (82). This diminished cohesion loosens keratinocytes in the follicular epithelium, resulting in breakdown of comedones, inhibition of comedone formation, and unroofing of pustules (83). Comedones are removed after only two or three peels, and the procedure may be repeated every 2 or 3 weeks. Between peels, low concentrations of glycolic acid may be used as a daily cleanser to prevent occlusion of follicles (83). A randomized split-face prospective clinical trial comparing glycolic acid to Jessner's solution (salicylic acid, lactic acid, and resorcinol) demonstrated significant acne improvement in both after three treatment sessions. Furthermore, glycolic acid was associated with significantly less exfoliation than Jessner's solution, resulting in more facile makeup application and suggesting a more favorable side effect profile (84). In a similar study comparing glycolic acid and SA peels, both were equally effective by the second treatment; however, SA demonstrated greater sustained effectiveness and a more favorable side effect profile (81). A study examining 35% and 50% glycolic acid peels on Asian patients found significant resolution of comedones, papules, and pustules; decrease in follicular pore size; improvement in acne scarring; and few side effects (85). One recent article examines the evidence base that supports the widespread use of superficial peels in acne. The conclusions were that search of the literature

revealed very few clinical trials of peels in acne. A majority of these trials included small numbers of patients, were not controlled, and were open label. Notably, no studies of chemical peels have used an acne drug as a comparator (86).

## RESORCINOL

We have very limited and old information about resorcinol and keratolytic activity in acne; all are extracted from a review article by Karam in 1993 (87). No longer significantly used in the United States and Europe, resorcinol, an isomer of hydroquinone and a relative of phenol, is soluble in water, ether, and alcohol. It is a reducing agent with antibacterial and keratolytic properties. Even at low concentrations, it can disrupt hydrogen bonds of keratin. A 50% resorcinol paste is used in some countries for chemical peels. It is used to treat the post inflammatory hyperpigmentation, erythema, and shallow scars resulting from facial, chest, upper back, and buttocks acne. One facial peel, typically 30 minutes in duration, may be sufficient for treatment. Additional peels can be done a few hours to 2 days later. Patients typically receive pretreatment with 0.05% retinoic acid cream for a period of 2 weeks to 3 months before the facial peel. This pretreatment may help assist in resorcinol absorption, resulting in a deeper peel. In addition to acne, resorcinol peels can be used to treat melasma, sun-damaged skin, and freckling. Contraindications include pregnancy and skin type VI, due to inadequate data regarding complications. Acne surgery prior to the peel is recommended to prevent aggravation of deep comedones and subsequent pustular development. Side effects include burning sensation and paresthesia, which can be felt anywhere from 2 to 30 minutes after application. Dizziness immediately after the peel may last 10 to 15 minutes and is probably secondary to flushing related to resorcinol application. Burning intensity increases initially, stopping after 1 hour; despite discomfort, pain is usually tolerable. Subsequent resorcinol applications cause more intense burning sensation, prompting shorter exposure. Corticoid creams and cold compresses may provide some relief. Histologically, shortly after resorcinol application, splitting occurs at the granular cell layer along with vasodilatation. One week later, prominent basal cell layer mitosis, fibroblast proliferation, vasodilatation, and formation of a thickened dermal band are visible. Although the vasodilation resolves, the other changes are present even 4 months later. Recently, the FDA give rules of new warnings of labeling for OTC topical acne drug products containing resorcinol, resorcinol monoacetate, salicylic acid, and/or sulfur (88).

## CONCLUSION

Taken together, a century of clinical trials and clinical use support the efficacy of topical keratolytics in acne. But we also cruelly lack new keratolytic—no new original molecule has been found for more than 30 years. In addition, taking into account the mechanism of action of a keratolytic—that is, it dissolves or breaks down the outer layer of skin—they are all topical. We strongly hope that the near future will provide more rapid advances, based on the power and ease of interpretation of the newly devised in vivo human keratolytic assay.

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# Hidradenitis Suppurativa

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Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease which characteristically presents with recurrent inflamed and scarring lesions restricted to axillae, submammary or perigenital areas (1) (Figures 39.1 and 39.2). The prevalence of the disease has not been accurately described, but population surveys in Europe indicate a prevalence of 1%–2%, whereas a much lower figure is suggested by studies relying on the general patient records and predominantly published from U.S. centers (2–5). The discrepancies have not been fully elucidated but reviews of patients' records show an apparently rapidly increasing incidence, indicating the possibility of reporting or diagnosing bias (2,6).

Underreporting is in good agreement with the cumulative life course of these patients, which is often described as a disheartening experience. Because of the seemingly banal symptomatology, most patients are treated as simple infections by physicians using antistaphylococcal antibiotics combined with incision and drainage (7). These treatments are rarely if ever effective, and therefore patients who are ultimately diagnosed at specialist centers often experience a significant delay in both diagnosis and effective treatment. For some patients physician contact stops after a few unsuccessful attempts at therapy and they are consequently not diagnosed correctly.

## AGE AND SEX DISTRIBUTION

HS typically appears when the patient is past puberty, although cases in prepubertal and pubertal children are well described. The age at onset clearly differentiates HS from acne vulgaris.

Numerous studies have noticed the sex difference seen in HS. Typically the F/M ratio is described as approximately 3/1, implying that either female factors or the absence of male factors play a role in the pathogenesis of HS (8). In prepubertal children HS appears associated with adrenarche or hyperandrogenism, which has led researchers to look for signs of cutaneous virilization in the many women suffering from HS (9–12). Hitherto the search has been unrewarding, although some women are prescribed oral antiandrogens in an effort to control the disease, with good effect (13).

The real-life prognosis is described in a long-term follow-up study (14). Although the data was based on self-reported presence of HS, the authors speculated that when dealing with a painful suppurating recurrent chronic disease, the validity of self-reported disease activity will be high. In a 22 year follow-up of 212 patients in the Netherlands and Denmark, a self-reported remission rate was 39.4%, indicating that the clinical impression that the disease becomes progressively more rare as the patients age may be true.

## IMPACT

HS is a high-impact disease. It causes acute symptoms such as smell, scars, itching, and pain during active phases, and scar-

ring and soreness during more quiet periods (8). It furthermore affects private intimate areas of the skin, including the genitalia and perigenital skin, linking it directly to personal relationships with significant others. Finally, the sociocultural aspects of the symptomatology should not be underestimated. The plight of Job describes the prototypical psychosocial understanding of recurrent abscesses in many cultures and is widely maintained today in spite of the secularization of many societies (15).

It is therefore easily understood that HS has a significant emotional impact on patients. Smell, appearance, and a strong feeling of lack of control affect interpersonal contacts, leading to significant psychological reactions (16). These affect many aspects of patients' lives negatively. In addition to obvious consequences such as negatively affecting patients' sex lives, the emotions include irritation with the unpredictable course of the disease, frequent itching, and significant (17–19) pain (8,16,20). Several studies have described an increased prevalence of depressive thoughts as well as clinically diagnosed depression in patients suffering from HS (21–26). These reactions have a significant correlation with disease severity, underlining the importance of the individual patient's coping abilities. Similarly, several studies have described the negative impact of HS on the patients' quality of life (QoL) (15,18,27–30). Using different general and specific questionnaires to describe HS patients' QoL, the negative effects of the disease have consistently been described as very significant across the studies, e.g. as reflected by DLQI scores from 8.4 to 12.7. It is speculated that the strength of the symptoms, the chronic recurrent nature of the disease, as well as the location and the psychosocial connotations of the disease all contribute to this high negative impact on QoL.

## TREATMENT

A limited number of randomized controlled trials (RCTs) has been published on the treatment of HS, and guidelines for the treatment of HS have recently been published (1). These suggest that all patients should be approached in a multimodal way, i.e. using adjuvant, medical, and surgical treatments combined, tailored to the severity of the disease.

Adjuvant treatment includes the judicious use of analgesics to ameliorate the pain, treatment of superinfections following identification of pathogens and their susceptibility to antibiotics, and support aimed at weight reduction and cessation of tobacco smoking. Although these adjuvant measures are not supported by high-grade RCTs, there is a high degree of consensus that they are necessary to reduce both the short-term morbidity as well as improve the overall prognosis (1).

Medical treatment is based on RCTs as well as substantial observational studies, and should be appropriate to disease severity. For milder cases the use of topical clindamycin 0.1% b.i.d. applied to inflamed lesions for up to for 10 weeks is supported by an RCT (31), as is the use of systemic tetracycline



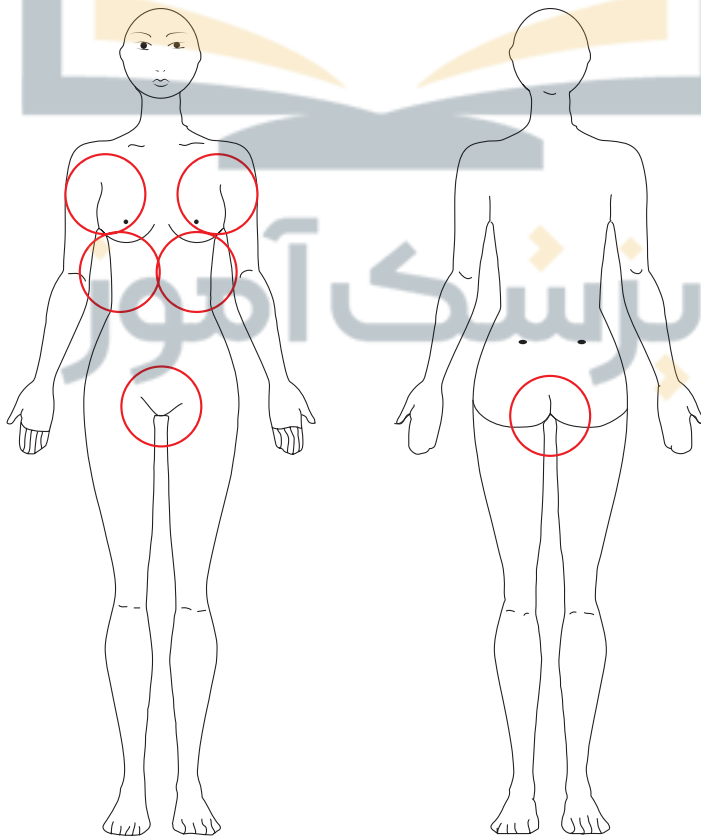
**Figure 39.1** An example of a hidradenitis lesion. Inflammation of the right axillary with multiple nodules. Scarring and sinus tract formation from earlier activity is evident.

500 mg b.i.d. for up to 12 weeks (32), the former being more suitable for individual lesions, the latter for more scattered lesions.

For more advanced disease, observational studies support the use of a combination of clindamycin 300 mg b.i.d. and rifampicin 300 mg b.i.d. for 10 weeks, but the treatment is untested in an RCT (33–37). In yet more advanced cases, the use of TNF $\alpha$  antibodies is based on RCTs. Infliximab (5 mg/kg) has been tested and found effective, although only in a post-hoc analysis (38). The beneficial effect is, however, supported by numerous reported case series. In contrast, adalimumab has been tested in three RCTs and found effective based on an intention-to-treat analysis of each of the trials (39,40). The dosage of adalimumab is identical to that used to treat Crohn disease (baseline: 160 mg, week 2: 80 mg, and then 40 mg every week).

Surgical treatment is similarly graded, ranging from minor surgery to major excisions of all diseased skin (1). The traditional surgical experience of most patients is that of repeated but generally unsuccessful attempts at incision and drainage. It is usually unsuccessful if inflamed nodules are incised, carries 100% recurrence rate, and may only add scarring to an already scarring disease. Incision and drainage should only be used if soft, fluctuating, large abscesses are present.

The formation of sinus tracts predisposes to recurrences. Sinus tracts can be surgically treated with simple excisions if few and small, but are generally better managed using so-called “deroofing.” In this simple office procedure the “roof”



**Figure 39.2** Areas most often affected by hidradenitis (circles): axillary, submammary, groin and perianal area.



of the sinus is removed after the tracts have been probed using a blunt narrow steel probe. By only removing the “roof” and leaving the already epithelialized floor of the sinus in place, healing is usually rapid and recurrences few (41).

Larger lesions can be excised or evaporated using a CO<sub>2</sub> laser with a scanner head, and the wounds left to heal by secondary intention (42–46). Even larger wounds generally heal within 8–12 weeks, and although secondary intention healing of larger wounds is in contradiction with the general surgical paradigms, patients mostly take well to the procedure, which has a low recurrence rate. Primary closure appears to have a higher recurrence rate, possibly because the excisions are subconsciously made more conservative to ease closure (47). A trial comparing split-skin grafting with foam dressings of postsurgical wounds indicated that patients preferred foam dressings over skin grafting (48). Advanced disease may require major excisions usually carried out in cooperation with surgeons and sometimes requiring the creation of a temporary ostomy to ensure optimal healing. If the resources are available even very large wounds have been left successfully for secondary intention healing.

Randomized trials have been used to document the effect of depilatory treatment of HS lesions and HS-prone skin, using either a Nd:YAG laser or an IPL device (49–52). Using repeated monthly treatment of HS lesions with a Nd:YAG laser, the investigators achieved a clinically significant reduction of the disease severity compared to contralateral lesions treated with topical clindamycin. Similar results were achieved independently using IPL. These trials are in agreement with current understanding of HS as a disease of the hair follicle, and although the exact mechanism of action on the pathogenesis of HS is not fully understood, the use of these methods provides a stepping stone between the purely medical aspects of the disease and the cosmetic.

## COSMETICS OF HS

Like any skin disease, HS has a cosmetic aspect, although the disease is primarily a medical condition causing scarring and pain of the affected regions. One important factor appears to be that while the lesions are also easily hidden to others, they are clearly visible to the patient and thereby affects their sense of self-worth and QoL negatively.

Very little attention has been paid to the cosmetic aspects of HS, although the affected regions are often a focus of other cosmetic products and procedures such as depilatories, deodorants, or mammoplasties.

## SMELL

Smell is a particularly unpleasant adverse consequence of active HS. Patients are disturbed by it as it may appear suddenly and unexpectedly, and is noticeable even though the lesion that causes it remains hidden (16). Smell was addressed in the trial of infliximab, but is generally not included as an outcome variable in studies (38). Foul smell may derive from bacterial decomposition of necrotic tissue, indicating that in addition to overall disease control, reduction of the surface bacteria population may be a useful approach (53). Topical disinfectants are often recommended for this, although no formal evidence of their efficacy exists. If a disinfectant is chosen, the sensitizing potential must be considered as it is applied to naturally occluded and diseased areas of the skin, which may aid sensitization. Topical clindamycin may achieve a similar

effect, but the published RCT contains no description of any effect on odor.

If better control of the disease and the surface bacteria cannot achieve the desired control for the patient, it is an option to seal the area with an occlusive absorptive bandage that can also absorb suppuration (54).

## SUPPURATION

Episodes of increased suppuration and pain characterize superinfections but can also be seen in flares of the disease not associated with superinfection. If recognized pathogens such as *Staphylococcus aureus* are identified in the suppurating lesions, it may be possible to reduce the suppuration with targeted antibiotic therapy, whereas flares of HS itself may require a more immunosuppressive approach. In either case suppuration can be treated with some effect. Many patients however find the effects of treatment unsatisfactory, and have to resort to adjuvant therapies such as bandages. The bandages preferred by patients cover a wide range, from simple rags or sanitary pads for simple absorption to state-of-the-art occlusive bandages that are capable of absorbing drainage and containing odor. Most modern bandages are however designed to be applied to convex surfaces, and therefore require some experience in application. Modifying modern bandages breaks the seals that prevent leakage of pus and odor and is therefore generally not recommended. Most patients find square to oblong bandages with a maximum width of 4–7 cm most useful. In addition to good sealing and absorptive qualities, the bandages should be soft and the adhesive not too strong, to avoid peeling of the skin when changing the bandage. There have been no specific investigations to guide the choice of bandage, and the collaboration of a nurse trained in wound management and bandaging is therefore often very useful in the management of HS patients.

## SCARS

Considerable efforts are made toward creating less noticeable scars following surgery, or improving the cosmesis of existing scars (55). The areas are affected by HS are generally invisible during normal social intercourse. The axilla may even be used as a place to hide scars in cosmetic surgery. Nevertheless, the appearance of such private areas is of considerable cosmetic relevance, as reflected by e.g. the apparently growing demand for genital cosmetic surgery (56). The perception of the human body may therefore be affected by scars irrespective of their location, and scars are generally perceived as unsightly by most patients. In addition, contractures due to disease activity or surgical intervention may have functional consequences for HS patients by restricting the movement of e.g. an arm, adding to the morbidity caused by the disease. Scarring of the intimate areas of skin involved in HS is therefore of importance to patients on many levels.

Disease-associated scarring is best dealt with through improved disease control. The judicious use of excisional surgery and intralesional corticosteroid injections in combination with medical treatment can often reduce disease activity sufficiently to make the ensuing scarring acceptable to the patient both functionally and cosmetically. Atrophic scarring rarely appears to be a problem, while hypertrophic scarring or frank keloid formation may occasionally pose therapeutic challenges. The treatment of hypertrophic scars or keloids follows the general guidelines for management of these complications (57).

## CONCLUSION

HS is a disease that may alter the life course of patients. There are no pathognomonic tests for HS, but a clear clinical definition based on characteristic constellations of lesions, affected regions of the skin, and a chronic recurrent pattern of easily recognizable flares. It appears to be more common than hitherto reflected in hospital records, possibly due to underdiagnosing and underreporting of cases. International guidelines for the management of cases have been published and provide a simple approach to treatment, which should include adjuvant therapies and surgical and medical elements.

The impairment caused by HS generally overshadows the cosmetic consequences of the disease; nevertheless, improved cosmesis can be seen as a natural part of the necessary adjuvant therapy. In particular, management of smell, suppuration, and scars have a cosmetic aspect which is of the utmost importance to patients.

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پزىشک آموز

# Section V

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Specific Groups



بزنسک آموز



پزسک آموز



## Age-Related Changes in Male Skin

Stefanie Lübberding and Nils Krüger

### BACKGROUND

At first glance, the physiological properties of the human's largest organ seem equal in both genders. However, with the beginning of hormonal production in puberty, skin differences become conceivable. Moreover, it is known that the susceptibility to several skin diseases, such as acne, rosacea, and seborrheic eczema, vary between the sexes, and that therapeutic needs of male and female skin are often diverse (1,2).

As in modern society attractiveness and beauty play an important role, the market for cosmetic and aesthetic treatments is also booming (3). However, men and women likewise try to enhance their personal imperfections, but the use of the men's cosmetics and aesthetic procedures has been widely ignored in the past (4). As women have been the marketing target over many years, dermatological research and cosmetic science have mainly been focused on female skin. Men were the forgotten customers when it came to cosmetics and skin care, but as modern men have recently changed their beauty and grooming habits there is an increasing demand for cosmetic products for men (5). This burgeoning trend is also attracting the attention of the traditional cosmetics manufacturers, who launch and implement cosmetics series for male skin. As a response to the demands of men, this development is extremely gratifying, however very little information is known about the dermatological needs and physical properties of male skin. Most published data including male skin deal with gender differences (6,7) or evaluate skin care products related to shaving procedures (8) and androgenetic alopecia (1).

Due to the fact that male skin is still an uninvestigated area, a unique research project was conducted with the objective to carry out the first systematic assessment of the skin physiology in men. A large cohort of 150 Caucasian subjects, evenly distributed among five age groups, was included following very strict inclusion and exclusion

criteria. Well-accepted biophysical measuring methods and clinical scores were used for the assessment of skin barrier parameters (9), including sebum excretion (SE), stratum corneum (SC) hydration, transepidermal water loss (TEWL) and skin surface pH, as well as wrinkle severity (10). The aim of the project was to get a better understanding of the skin physiological properties in males and how they change with aging.

### MATERIAL AND METHODS

A carefully selected cohort of 150 healthy Caucasian men aged 20 to 70 years was included in the large clinical trial following very strict inclusion and exclusion criteria, involving age, sun behavior, smoking habits or minimally-invasive treatments. To guarantee a balanced age structure the subjects were distributed evenly into five age groups (group I: 20–29 years, group II: 30–39 years, group III: 40–49 years, group IV: 50–59 years, group V: 60–70 years) (Table 40.1).

The male subjects were invited to the study site for a one-time skin examination. Worldwide-accepted biophysical measuring methods and clinical scores were used in the assessment of the skin physiological properties, including the skin barrier parameters SE (Sebumeter® SM 815, Courage & Khazaka), SC hydration (Corneometer® CM 825, Courage & Khazaka), TEWL (Tewameter® TM 300, Courage & Khazaka) and skin surface pH (Skin-pH-Meter® PH 905, Courage & Khazaka). Wrinkle severity was assessed with 3D fringe projection method (PRIMOS<sup>premium</sup>, GF Messtechnik) and 5-point photonic rating scale validated assessment scales (VAS). To consider physiological cutaneous variations, the measurements were performed at the forehead, cheek, neck, volar forearm, and dorsum of the hand. Measurements were done under standardized room conditions (20°C and 50% relative humidity), after an acclimatization period of 30 minutes.

**Table 40.1** Group Assignment According to Subject's Age

Age Group	Range	Age ± SD	n
<b>Group I</b>	20 to 29 years	25.70 ± 2.48	30
<b>Group II</b>	30 to 39 years	33.23 ± 2.90	30
<b>Group III</b>	40 to 49 years	44.23 ± 3.09	30
<b>Group IV</b>	50 to 59 years	54.20 ± 3.13	30
<b>Group V</b>	60 to 74 years	66.63 ± 2.61	30

## SKIN BARRIER FUNCTION

Cutaneous health and functionality is best represented by assessment of the skin barrier which is primarily formed by the epidermis (11). This physiological barrier is a selectively permeable squamous epithelium protecting against desiccation and preventing the penetration of foreign molecules into the body (12). This two-way protection is based on the physical barrier, formed by the unique structure of intercellular lipids and corneocytes in the SC (11), and a chemical/biochemical barrier, built by the hydro-lipid film of the skin surface (13,14). Although the skin barrier cannot be directly measured, TEWL, SC hydration, SE, and pH value are considered to be the standard surrogate markers for the assessment of the skin barrier function and permit an objective evaluation of the skin physiological properties in vivo (15–17).

## TRANSEPIDERMAL WATER LOSS

TEWL is regarded as one of the most important parameters for assessment of skin physiology, as it shows the quantity of water that passes through the skin's protective barrier. Therefore, the ability of the SC to retard evaporative water loss is a surrogate marker for the functionality of the skin barrier in vivo. Data analysis indicates that TEWL in men varies by localization (Figure 40.1). Average values for the forehead, cheek, and hand range from 9 to 11 g/h/m<sup>2</sup>, while TEWL is lower at neck and forearm with mean values between 5.5 and 7 g/h/m<sup>2</sup>. The higher water evaporation in the face and the dorsum of the hand is probably caused by the higher number of sweat glands at these localizations, as it is known that the TEWL is particularly influenced by water evaporation from eccrine acrosyringium (18,19). Data analysis also indicates that TEWL is not subjected to greater fluctuations over a lifetime. However, TEWL at the neck significantly increases with age, which is contrary to other studies that showed a decreasing TEWL in non-facial areas with aging (17,20–22). Further, men between the ages of 50 through 60 years show significantly higher water loss (with the exception of the hand) in comparison to the data of other age groups. It can be assumed that the higher water evaporation is due to hot flushes caused by progressive hypoandrogenism (23–25). It was previously assumed that hot

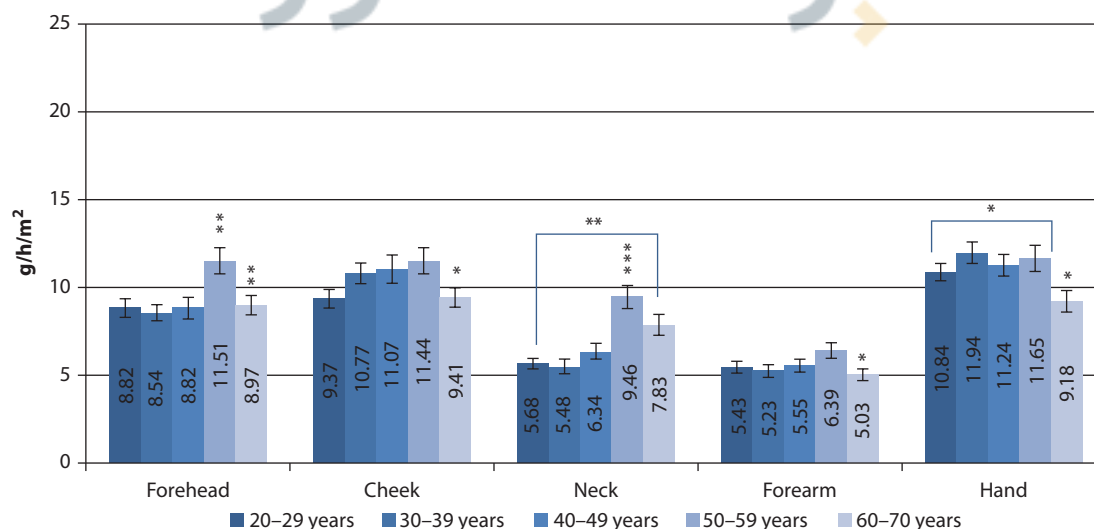
flushes commonly occur in castrated men and cancer patients (26,27), but current studies indicate that even “normal” aged men suffer from flushes. Therefore, the prevalence seems to be highest in men's mid-50s (23), which can be an explanation for the sudden increase in TEWL in the age group IV.

## STRATUM CORNEUM HYDRATION

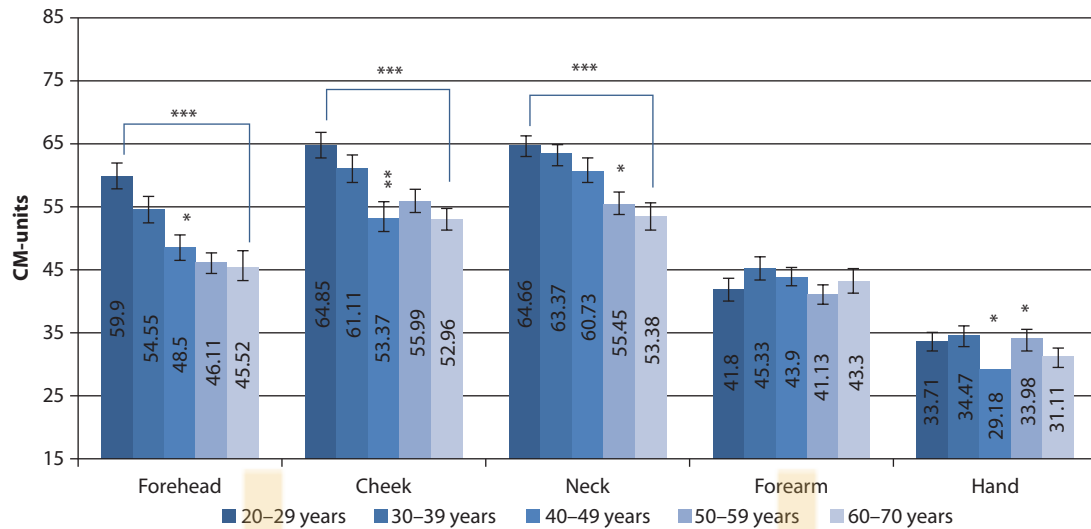
While TEWL is an indicator for the ability of the epidermis to hold water, it is closely related to the hydration of the SC which keeps the skin surface smooth and prevents the penetration of hydrophobic substances (28). Whereas TEWL generally does not seem to be affected by the subject's age, man's skin partly loses its ability to maintain water in the SC (Figure 40.2). At the forehead, cheek, and neck, SC hydration significantly decreases with aging. Therefore, the greatest decrease in hydration was assessed at the forehead with a decline of about 25% from the youngest to the oldest subjects. Also, Man et al. (6) demonstrated a decrease in epidermal hydration level at the forehead with age, which confirms the generally accepted assumption that skin gets drier in elderly people (29,30). Several factors may contribute to the lower SC hydration mainly influenced by a progressive reduction of natural moisturizers in the SC, such as epidermal lipids and lactate (31,32). Whereas the age related decrease is significant at the face and neck, SC hydration at the forearm and hand remains unchanged. These localization-dependent differences are probably influenced by the higher sun exposure of the face and neck. This presumption was confirmed by Liu et al. (33) who showed that cumulative sun exposure does reduce SC hydration, probably due to a UVB-induced down-regulation of epidermal ceramides, fillaggrins, and aquaporins (34–37).

## PH VALUE

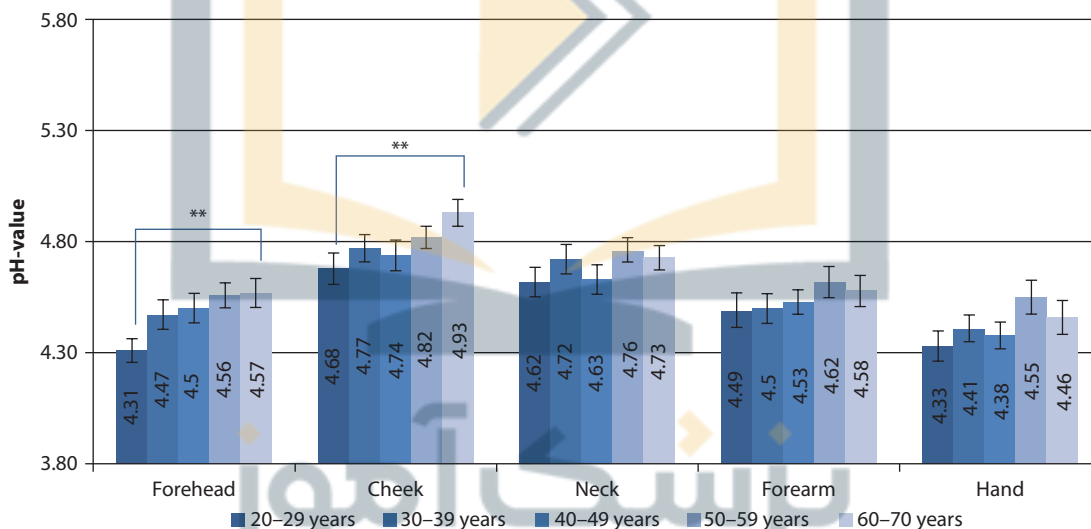
The pH value on the skin surface is of great importance for cutaneous antimicrobial defense and regulates epidermal enzyme activity and expression (13). The pH also influences the buffering capacity of the skin, with respect to both acidifying and alkalinizing effects from internal and external influences (38). The skin surface pH in men was in physiological range



**Figure 40.1** Mean values (SE) of transepidermal water loss arranged by age group and location.



**Figure 40.2** Mean values (SE) of stratum corneum hydration arranged by age group and location.



**Figure 40.3** Mean values (SE) of pH value arranged by age group and location.

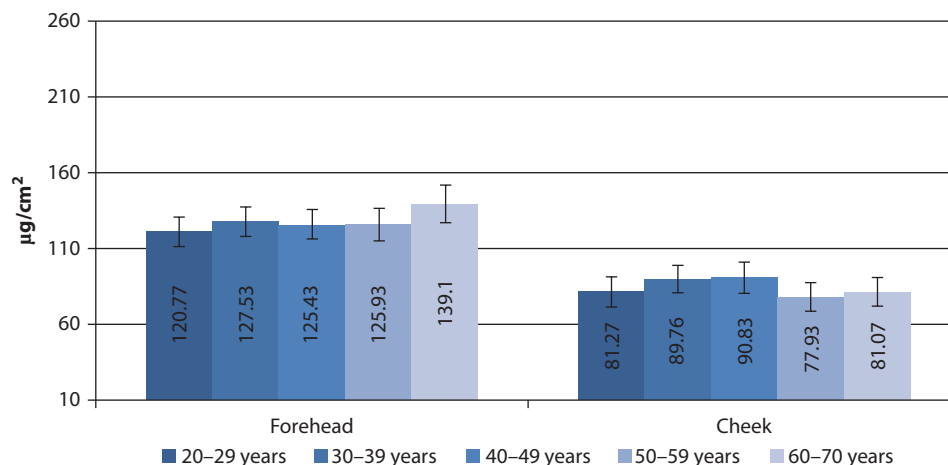
for all age groups. Overall, lowest pH value was measured at the hand and highest at the cheek (Figure 40.3). The results of the present study indicate that skin surface pH increases at all assessed localizations, but significantly at the forehead and cheek. This result is in agreement with other studies (6,39) which found an increase in skin surface pH in age compared with younger men. Man et al. (6) assume that the elevated skin surface pH with aging is linked to the simultaneous decrease in epidermal expression of sodium-hydrogen antiporter ( $\text{Na}^+/\text{H}^+$ ), as  $\text{Na}^+/\text{H}^+$  is known as one of the key factors regulating skin surface pH (40). Further, the increased SC pH negatively influences cutaneous permeability barrier homeostasis and lipid processing which may have a negative influence on water holding capacity of the SC resulting in lower hydration levels (39,40). This connection is probable

as age-related changes occur simultaneously in the present study.

### SEBUM EXCRETION

The influence of androgens, such as testosterone and dehydroepiandrosterone, on the sebum production has been shown in several studies (41–43): the higher the level of sexual steroids, the higher is the SE rate (43). As with increasing age men experience a gradual decrease of circulating bioavailable androgens (17,25,44), it can be assumed that sebum production decreases accordingly, resulting in drier skin. While this hypothesis was confirmed by prior studies (6,45), the results of this large study show no age-related decrease in SE in males (Figure 40.4). A possible explanation for this





**Figure 40.4** Mean values (SE) of sebum production arranged by age group and location.

result can be the wide distribution of measured values found in all age groups, indicating that inter-individual differences in SE are huge and probably overlay the relatively small influence of aging. Therefore, a larger cohort or an assessment of individual subjects over a longer timeframe would be necessary to confirm the decrease of sebum production with age. However, it should be noted that previous studies, which found a distinct influence of aging onto SE, had a preponderance of young men in their cohorts, which may result in a right-skewed distribution and a potential statistical bias. (6,45).

## WRINKLES AND MECHANICAL PROPERTIES

The most obvious and probably the most unloved sign of the aging process is the development of facial wrinkles and the loss of skin firmness and elasticity. Despite the different clinical manifestations, skin laxity and wrinkles share the same underlying mechanisms and are most likely due to complex structural and molecular alterations in the dermal connective tissue (46). Therefore, the progressive breakdown of the collagen and elastin fiber network throughout the lifetime results in less tensile strength and a decreased elastic ability of the skin to recover after stretching (47–49). These alterations, especially the loss of elasticity, significantly correlate with the clinical visibility of facial wrinkles (50,51). Even though the reasons for wrinkle formation with aging are not yet fully understood, it can be assumed that lines and furrows are the visible results of deep dermal creasing, caused by repeated facial muscle contraction, combined with dermal elastosis (52); since the elastic fibers are altered with aging they do not permit the skin to snap back to its initial shape after deformation, resulting in the development of permanent facial wrinkles (53).

## WRINKLE DEVELOPMENT

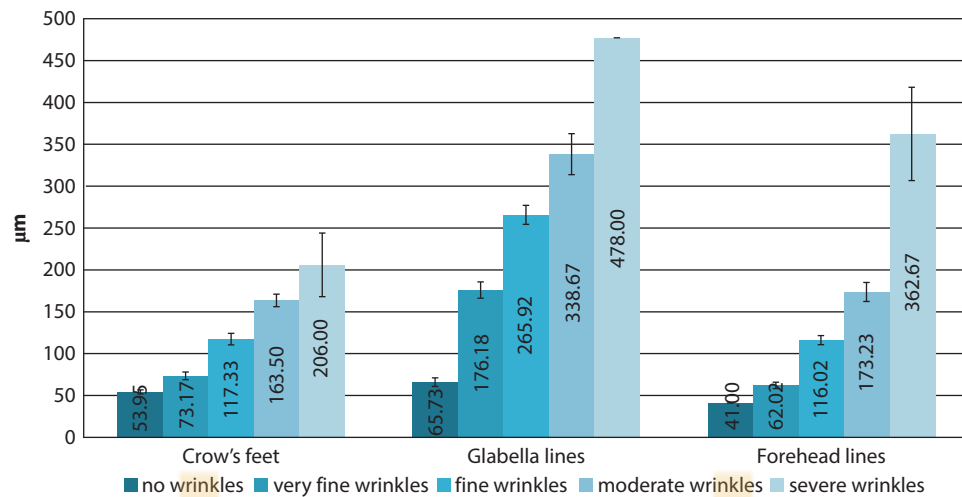
According to expectation, clinical rating and 3D measuring indicate that wrinkle severity in male skin increases with aging at all assessed localizations. This age-related increase of skin wrinkling is largely linear. Therefore, facial wrinkles manifest clinically first at the forehead and latest at the

glabella area. Whereas wrinkles at crow's feet constantly increase about 0.5 grades of the VAS every 10 years, wrinkle severity at glabella lines increase very slowly until the age of 40 but sharply increase in the fifth decade of life (Figure 40.5).

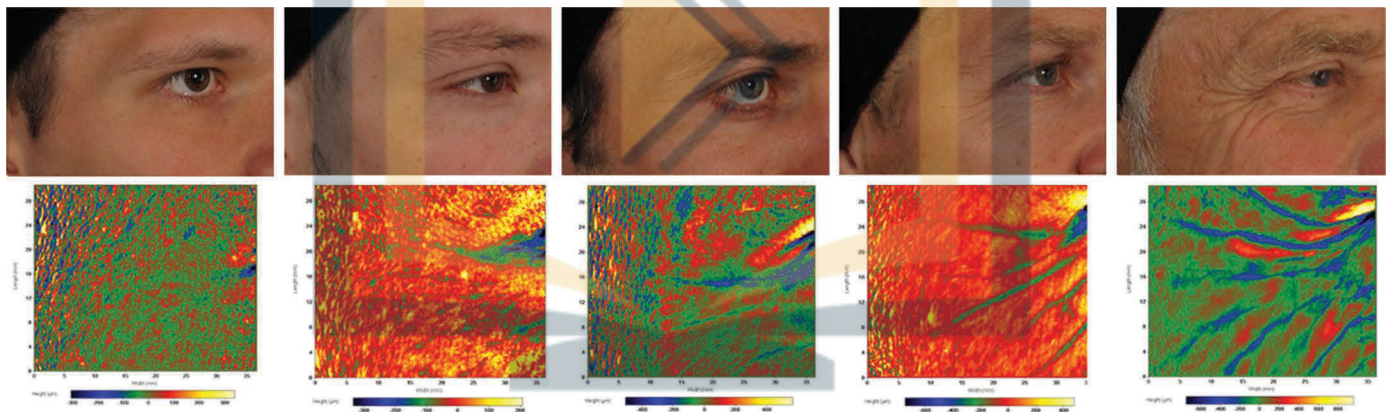
The study results show that the visibility of wrinkles in clinical rating also vary by location. While forehead lines in men can be recognized as *very fine wrinkles* already by an average wrinkle depth of 60  $\mu\text{m}$  and periorbital lines by a depth of 70  $\mu\text{m}$ , glabella frown lines are first recognized at 180  $\mu\text{m}$ . It can be assumed that the surrounding macrostructure of the craniofacial bones, including Orbita and Os nasale, and the eyebrows probably deflect from the very fine lines of the glabella. Furthermore, the relatively small area of only 2–3  $\text{cm}^2$  makes it even more challenging to recognize early wrinkles.

The results also indicate that wrinkles do not develop simultaneously on all areas of the face, but vary by location. Facial rhytides in male skin manifest clinically first at the forehead, then at the crow's feet, and latest at the glabella area. Therefore, forehead lines and crow's feet are pronounced in males in their end-20s/mid-30s, whereas glabella frown lines develop in subject's mid-40s. The wrinkle severity further increases at all locations every 10 years of age by one level of the VAS. Thus exemplary, at crows' feet the average age for *no wrinkles* is  $\leq 28$  years, 37 years for *very fine wrinkles*, 49 years for *fine wrinkles*, 59 years for *moderate wrinkles*, and 66 years for *severe wrinkles* (Figure 40.6).

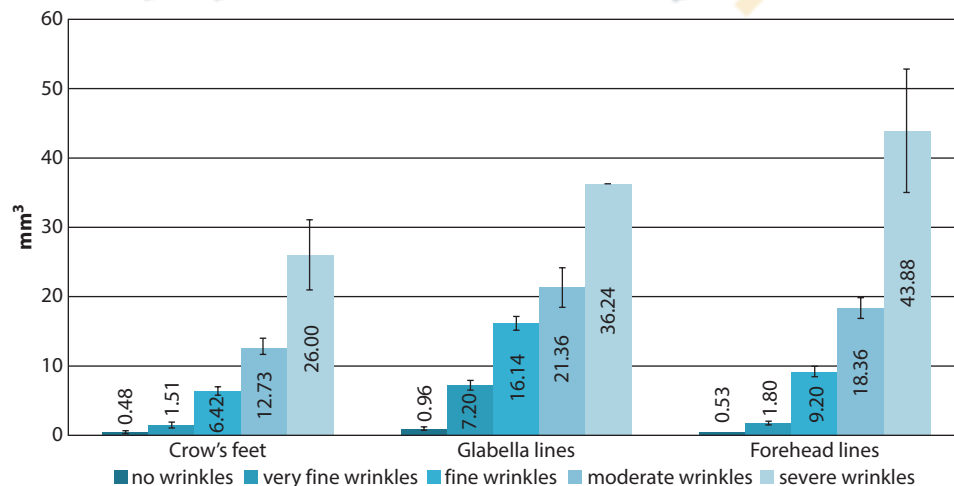
The same progressive increase with each level of the VAS can be seen for the parameters of the 3D fringe projection method. As a broad rule of thumb, the increase of *wrinkle depth* ( $W_d$ ) per VAS level is about 100  $\mu\text{m}$  at glabella and forehead lines and about 50  $\mu\text{m}$  at crow's feet. *Wrinkle volume* ( $W_v$ ) is relatively low at VAS level *no wrinkles* and *very fine wrinkles*, but doubles with every additional VAS level (Figure 40.7). The explanation for this effect is probably the strong influence of the number of wrinkles on the *wrinkle volume* ( $W_v$ ) parameter, while the *wrinkle depth* ( $W_d$ ) is independent from the number of wrinkles: the more wrinkles in the area of interest, the higher the total wrinkle volume. Thereby, it can be assumed that wrinkles not only become more and deeper over the years, but also wider, resulting in an additional increase in wrinkle volume (54).



**Figure 40.5** Matching of VAS with 3D imaging arranged by the parameter overall average wrinkle depth in men.



**Figure 40.6** Matching of VAS with 3D fringe projection method (a: VAS=0, no wrinkles; b: VAS=1, very fine wrinkles; c: VAS=2, fine wrinkles; d: VAS=3, moderate wrinkles; e: VAS=4, severe wrinkles).



**Figure 40.7** Matching of VAS with 3D imaging arranged by the parameter total wrinkle volume (Wv) in men.

## CONCLUSION

Based on the statistical analysis of the data of 150 male subjects, this study assigns systematic reference values for standardized biophysical measuring methods reflecting men's skin physiology in relationship to age for the very first time. The results show that the physiology of male skin partly changes with aging. Therefore, the ability of the SC to retard evaporative water loss does not decline with aging in general, but TEWL is significantly higher in men's 50s. This temporary increase is probably due to hot flushes caused by progressive hypoandrogenism. An expected negative impact of hypoandrogenism onto sebum production cannot be confirmed in this cohort.

The present study further shows age-related changes in the lifetime development of skin wrinkles in men. The increase in wrinkle formation is largely linear in men's lifetime, however, varies strongly by localization. Therefore, the first pronounced wrinkles in men, the forehead lines, are already marked in their 20s, while periorbital and glabella lines manifest in their fourth and fifth decade of life.

The results of the present study are a first step toward a systematic understanding of male skin and the lifetime development of skin physical properties, including skin barrier parameters, wrinkle severity, and mechanical properties. This new knowledge is an important step toward a better understanding of age- and gender-related influences in male skin and can be used as a base to develop gender-specific skin care solutions as well as age-appropriate treatment concepts for skin rejuvenation. This is of particular importance to address the increasing demand for skin care and antiaging approaches in our constantly aging society.

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## Ethnic Cosmetics

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### INTRODUCTION

Ethnic differences in skin physiology and reaction to environmental stimuli are more and more described (1), but notwithstanding the increasing number of studies, data are often conflicting. In fact, it is difficult to define and interpret the cutaneous pathophysiologic phenomena that are not only anatomical and functional characteristics of ethnic groups but also the result of socioeconomic, hygienic, and nutritional factors. Furthermore, skin status may be influenced by climate, circadian rhythms, and changes in circulating sex hormones or stress hormones. Indeed, even though it is well established that all humans belong to the same species, many physical differences exist among human population. This chapter reviews the more consistent differences reported between racial groups and their implications in determining different responses after use of topical products and in treating skin aging.

### SKIN COLOR

According to the classification of Fitzpatrick, Caucasians are generally included in phototypes I to III, having fair complexion, red to light brown hair, tendency to sunburn, and tanning difficultly, whereas colored skin belongs to skin type IV, V, or VI, rarely burning, tanning easily. Phenotypically ethnic skin ranges from brown to black-brown. Clearly, differences in skin color are the most striking characteristic in ethnic skin. The color of the skin is due to the combination of four chromophores: hemoglobin, oxygenated hemoglobin, exogenously produced carotenoids, and melanin. Melanin is the most important pigment for the determination of skin color. The increased quantity of melanin correlates with the activity of tyrosinase and probably with the level of protease-activated receptor-2 (PAR-2) that is involved in melanosome transfer from melanocyte to keratinocyte (2–4). No interracial variation concerning the number of melanocytes has been reported.

### STRATUM CORNEUM STRUCTURE, WATER CONTENT, AND pH

Stratum corneum is equally thick in black and white skin (5,6). However, the number of cell layers and resistance to stripping and other physicochemical insults is still debated (7–18). TEWL, skin conductance, and skin mechanical properties have been measured under basal conditions in whites, Hispanics, and blacks to assess whether skin color (melanin content) could induce changes in skin biophysical properties (19). Differences appear in skin conductance are more marked in biomechanical features such as skin extensibility, skin elastic modulus, and skin recovery. They differ in dorsal and ventral sites according to race and highlight the influence of solar irradiation on skin

and the role of melanin in maintaining it unaltered. Wilson et al. (15) demonstrated higher in vitro TEWL values in black compared to white skin taken from cadavers. They also found differences in black and white skin physiology: the TEWL of both races increased with skin temperature. In their own study, they concluded that black skin would have a greater rise to achieve the same temperature and therefore a higher TEWL. Since TEWL depends on passive water loss that is theoretically directly related to the ambient relative humidity and temperature (20), then the increased TEWL in black skin could be associated with an increase in temperature because it is well established that a difference in black and Caucasian temperature exists. Most studies using the forearm, back, and inner thigh (13,16,21–23) show a greater TEWL in blacks compared to whites; however, other studies don't confirm these findings (11,12). Skin lipids may play a role in modulating the relation between stratum corneum water content and TEWL resulting in higher conductance values in blacks and Hispanics. Ethnic differences in skin conductance are difficult to interpret in terms of stratum corneum water content because other physical factors, such as the skin surface or the presence of hair, can modify the quality of the skin-electrode contact. In all races, significant differences exist between the volar and dorsal forearms (19). These results are in apparent contrast with TEWL recordings. Indeed, increased stratum corneum water content correlates with a higher TEWL (24). The data may be explained on the basis of the different intercellular cohesion or lipid composition. A greater cell cohesion with a normal TEWL could result in increased skin water content. The acidity of the skin mostly derives from the fatty free acid content of the skin surface and the buffer capacity of the skin is due to several mechanisms—one of the most important is the lactic-acid-lactate system. According to Berardesca et al. (22), pH values in skin surface, measured on the volar forearm, are similar in black and in Caucasian women, but it decreases after tape stripping only in black subjects. Warrier et al. (11), who measured the pH on the cheek, reported a significantly lower pH in black women than in the Caucasian ones. The results of Fotoh et al. (12) are quite the reverse: a significantly higher cutaneous pH in black women compared to the other groups.

### CUTANEOUS APPENDAGES

Although the amount of sweat is variable between racial groups, with more sweat secretion found in black subjects, the phenomenon does not derive from differences in gland number but more likely from differences in density of actively sweating glands (25). Concerning apocrine glands, studies highlight that, compared to Caucasians and Chinese, blacks present apocrine glands greater in number and size (25). Evaluating

the sebum quantity present on skin surface, measured on the forehead using Sebumeter<sup>1</sup>, Fotoh et al. (12) showed similar results in all groups in contrast to previous studies that reported significantly higher level of sebum secretion in black people in comparison with white subjects.

## SKIN DISEASE AND COSMETIC PROBLEMS

### Irritation and Stinging

Dark skin is generally believed to be more resistant to irritation (26–28). These differences seem to be modulated by stratum corneum, since its removal by stripping induces similar responses in different ethnic groups. Stinging may occur in the nasolabial folds and on cheeks after an irritant (i.e. lactic acid) is applied. Frosch and Kligman reported that the most “stingers” were light-complexioned persons of Celtic ancestry who sunburned easily and tanned poorly (29). Later, however, Grove et al. found no skin type propensity to stinging; they applied 10% lactic acid to the nasolabial folds and cheek of volunteers and noted that increased stinging was related mainly to the person’s history of sensitivity to soaps, cosmetics, and drugs (30). Jourdain et al. (31) have performed an epidemiological survey aimed to assess ethnic variations in self-perceived sensitive skin. They included four ethnic groups: Afro-Americans, Euro-Americans, Hispanics, and Asians. Fifty-two percent of the women declared to have sensitive facial skin but the prevalence of self-reported sensitive skin was alike in all ethnicities. Among the sensitive skin subpopulation, some slight differences between ethnic groups have been noted concerning the cause of irritation or sensitization. Euro-Americans showed higher reactivity to environmental stimuli such as cold and wind, and less reactivity to cosmetics, whereas Afro-Americans reacted less to environmental factors and Asians tended to react to wind, spices, and alcohol. Kaidbey and Kligman studied race-dependent cutaneous reactivity to topical coal (32). There was a strikingly different response in the two groups: in whites, the response was primarily inflammatory, with development of papules and papulopustules in about 2 or 3 weeks, whereas in blacks the inflammatory response was largely absent and, after about 14 days, an eruption of small open comedones appeared. The follicles of white subjects responded early, with rupture of the wall and outpouring of follicular contents in the dermis, whereas in blacks, the first response was proliferative with production and retention of horny cells. That is, in blacks, the skin reacts to a comedogenic compound with hyperkeratoses rather than with disintegration of follicles, suggesting a greater resistance to irritants. Conflicting findings have been reported on the incidence of allergic contact dermatitis in blacks. Kenney reported a decreased rate (5% in black patients) (33). Marshall and Heyl reported that the incidence of industrial contact dermatitis in South Africa is less in darkly pigmented blacks (34). Dogliotti showed a 74% prevalence among Bantus (35). Scott noted that contact dermatitis was less frequent in Bantus handling detergents, waxes, and fuels (36). Despite a previous report describing an increased sensitization rate in whites, Kligman and Epstein found no significant difference in the two races after testing many topical materials (37). Fisher reported an approximately equal incidence of contact dermatitis in blacks and whites (38). Paraphenylenediamine, nickel, and potassium dichromate appeared to be the most common allergens. In Nigeria, nickel was the most frequent sensitizer, with an incidence of 12.3% (39) compared with 11% in North America. In Lagos, the female-male ratio is 1:1, whereas Fregert

et al. recorded a ratio of 6:1 (40). In North America, the ratio is 3:1 and in Stockholm it is 7:3. Clinically, acute contact dermatitis with exudation, vesiculation, or bullae is more common in whites, whereas blacks more commonly develop disorders of pigmentation and lichenification. Hypopigmentation has been described from contact with phenolic detergents (41), alkyl phenols, and monobenzylether of hydroquinone (42).

### Acne

Acne vulgaris is believed to be one of the most frequent dermatologic disorders in ethnic patients. Although the acne pathophysiology is the same in all ethnicities, the most dramatic difference between black and white skin is the higher incidence of post-inflammatory hyperpigmentation (PIH) and keloidal scarring as a result of inflammatory reaction. Hyperpigmentation occurs as darkly pigmented spots or macules that may persist for months or years after the resolution of acne lesions (43). Therefore, because of the elevated risk of important acne sequelae that influence quality of life, clinicians may have recourse to more aggressive therapies to treat acne in ethnic skin and thereby limit its negative consequences. The high rate of PIH has been confirmed in a recent study including black, Hispanic, and Asian patients (44). PIH can derive from both acne lesions such as inflammatory papule or pustules or comedones and skin irritation due to topical or systemic therapy. Keloidal scarring is also considered more frequent in ethnic skin than in white patients, with an incidence that can be between 5 and 16 times higher (45). Furthermore, in treating acne in ethnic skin, dermatologists must always assess the cosmetic habits and the use of some over-the-counter (OTC) products. In particular, almost half of acne African American patients regularly apply greasy hair moisturizers. This custom leads to a special form of acne, the so-called “pomade-acne” (46). This eruption, consisting mainly of comedones on the forehead and temporal area, seems to be a peculiar response of black skin to topical agents, because this reaction can be detected in black children from 1 to 12 years of age (47). Plewig et al. examined 735 blacks and found that 70% of long-term users of pomades had a form of acne (48). The more elaborate formulations induced pomade acne more frequently and more intensively than simpler preparations such as mineral oil and petroleum jelly. The distribution of the lesions corresponded to the area of contact. Comparable data for whites are lacking. According to the previously outlined problems, acne treatment in dark skin requires a delicate balance between aggressive and nonirritating therapy. Topical retinoids are considered the first choice therapy as they act either on the acne itself or on PIH (49,50). To reduce their irritating side effect, it is advisable to start with a low concentration or with alternate-day dosing and choose a cream rather than a gel formulation. Among retinoids, adapalene 1% cream or gel has been reported to be effective and well tolerated even in patients with dry or sensitive skin (51). Once-daily tazarotene 0.1% cream has also shown to improve acne and PIH in blacks (52). Concerning nonretinoid acne topical, azelaic acid is often prescribed because of its low potential of irritation and bleaching effect (53,54). Severe forms of acne require early employing of systemic isotretinoin (55). Skin dryness is the most common side effect of the treatment and may itself result in PIH but can easily be corrected by regular application of emollients.

### Post-Inflammatory Hyperpigmentation

When acne is under control, therapy can be focused on PIH. Hydroquinone remains the gold standard for PIH treatment.



Its effectiveness is related to its concentration and the stability of the preparation (56). The OTC products available, usually already used by ethnic patients at time of presentation, contain 1% to 2% hydroquinone but are often inefficacious. In clinicians' prescriptions, the concentration varies from 3% to 5%, compounded in cold cream or hydroalcoholic base. It is normally applied once daily and results are appreciable after 4 to 8 weeks of therapy, and optimal effects are observed after 6 to 10 weeks of therapy. Hydroquinone is usually combined with other proximate principles, exploiting the synergistic action of each compound. Hydroquinone 5% with tretinoin 0.1% and dexamethasone 0.1%, known as the Kligman formulation, represents the most famous association. As long-term use of this preparation may determine skin atrophy, telangiectasia, erythema, rosacea-like acneiform eruptions, increased growth of vellus hair, and perioral dermatitis, FDA has approved a modified combination of the Kligman formulation, containing hydroquinone 4%, tretinoin 0.05%, and fluocinolone acetonide 0.01%. This association has proved its efficacy without significant side effects in a multicentric safety study (57). Irritation is the most common acute complication, but hydroquinone may also induce infrequent allergic reactions, PIHs, and transient hypochromia. Chronic adverse events consist of leukomelanoderma en confetti, exogenous ochronosis, and nail discoloration; these are usually related to prolonged use of formulations containing high concentrations of hydroquinone (56). Beside OTC bleaching products, ethnic people still employ traditional drugs, transferred from generation to generation. An interesting recent screening of some of these compounds, used by Nepalese people to treat acquired pigmentation disorders, has been performed by Adhikari et al. (58). They have found 53 crude drugs—52 were plant extract, and one derived from a shell called *Cypraea moneta*. All the products tested for the tyrosinase inhibitory activity showed some efficacy. Extracts of roots of *Glycyrrhiza glabra*, leaves of *Morus alba*, flowering bud of *Syzygium aromaticum*, fresh peel of *Citrus aurantifolia*, shell of *C. moneta*, seed of *Punica granatum*, and fresh peel of *Citrus aurantium* demonstrated the higher activity, some with more than 50% inhibition.

### Exogenous Ochronosis

Exogenous ochronosis is a bluish-black pigmentation of connective tissue in the area treated with hydroquinone. The pathogenesis is unknown, but it has been supposed that it derives from the accumulation and polymerization of homogentisic acid (HGA) resulting from the inhibition of its oxidase by hydroquinone. In particular, pigmentation may be induced by the binding of HGA to fibrillar collagen. Exogenous ochronosis can occur in pigmented skin as a consequence of the use of some topical compounds such as pyric acid, phenol, resorcinol, and hydroquinone. Usually, when induced by hydroquinone (which is a phenolic compound similar to HGA), the discoloration appears within few months of application (59). In the U.S. population, the condition has been described to appear in pigmented skin (blacks and Hispanics) after use of topical hydroquinone at concentrations of 2% or higher for months or years and who have failed to observe sun protection. In these subjects, hydroquinone was applied continuously as a bleaching agent to treat dark pigmentation or dark skin discoloration such as melasma or post-inflammatory pigmentation (60). Nevertheless, exogenous ochronosis is not so frequent in the United States as in some African populations and countries. This appears to be due to the high concentrations of hydroquinone available in skin-lightening products prior to 1984 in

South Africa (average 6% to 8%) (61). Other compounds capable of inducing irreversible depigmentation, such as tert-butyl alcohol and mercury, were included in skin care products in South Africa up to 1986. Resorcinol, used in some African countries in cosmetic products for acne, has been also related to the onset of exogenous ochronosis. Hydroquinone and resorcinol are also used simultaneously to achieve faster depigmentation (61). Furthermore, alcoholic lotions and vehicle used in lightening and acne products can increase the percutaneous absorption of hydroquinone (62). From a clinical point of view exogenous ochronosis can be classified in three stages (63):

- Stage I involves erythema and mild pigmentation of the face and the neck.
- Stage II is characterized by appearance of papules and mottled pigmentation.
- Stage III includes papulo-nodules and inflammation.

While low concentrations of hydroquinone inhibit tyrosinase, higher ones can increase melanin synthesis apparently as consequence of tyrosinase stimulation (64). Melanocytes can be involved in the process of ochronosis since it does not appear in areas affected by vitiligo. The role of sun exposure is still debated as well (65). Indeed, the condition is often limited to sun-exposed areas. The treatment of exogenous ochronosis is difficult. Generally, it tends to resolve slowly after stopping the drug. Avoidance of exposure to the causative agent may improve the condition; chemical peels, cryotherapy, and retinoic acid have been used with poor results.

### SUN PROTECTION

As mentioned previously, different skin colors are due to the adaptation to different intensities of sun irradiation that changes with latitude. In the late 1970s, Kaidbey et al. (66) demonstrated that 5.7% of UVB are transmitted into the dermis in blacks as opposed to the 29.4% in whites. In blacks, 17.5% of UVA reaches the upper dermis whereas in whites 55.5% penetrates. Antoniou et al. (67) have investigated the optical transmission properties of different skin types, demonstrating that, as expected, skin of color is naturally better protected against damage caused by UVA and also against visible and red range of the spectra. They conclude that, because of the different relation between UVA and UVB protection among skin types, specific sunscreens should be developed.

### CHEMICAL PEELING

As underlined by Roberts (68), chemical peeling was first employed precisely by people of color. Cleopatra's habit to bathe in sour milk can be correctly considered an early employment of  $\alpha$ -hydroxy acids as an exfoliating agent. In ethnic patients, chemical peeling is useful especially for the management of pigmentary disorders such as PIH, solar lentigines, mottled dyschromia, and melasma. Acne vulgaris, scarring, and pseudofolliculitis barbae represent other significant indications (68).

### Acne

Among  $\alpha$ -hydroxy acids, glycolic acid is the one most frequently used. Its application on the skin induces epidermolysis in a few minutes. Concentration and vehicle are both important to modulate peeling intensity as well as the amount of acid delivered and the method of application. To reduce side effects, partially buffered glycolic acid is recommended (69).

Concentration varies from 30% to 70%. It has shown to be effective and safe for the treatment of acne in ethnic skin (70). Chemical peeling with a 20% to 30% liquid salicylic acid solution improves acne with a good safety profile (71).

### PIH

PIH may benefit from glycolic or salicylic acid peeling. Trichloroacetic acid (TCA) 25% and Jessner's solution have been used successfully. A full face peeling or alternatively a "spot peel" can be performed. Spot peel is recommended using TCA 25% and Jessner's solution. Improvement is perceptible after three to six peeling sessions. Patients should always have been pretreated with topical skin-lightening agents prior to peeling, and topical therapy should be continued for about 4 to 8 weeks (68).

### Melasma

Melasma is a common complaint of patients with skin of color, especially Asians, fair-complected African Americans, and Hispanic women. Once possible causative factors have been screened, sunscreens, topical hydroquinone, and chemical peels remain the treatments of choice. Among available chemical peels, glycolic acid and salicylic acid represent a good tool to reduce epidermal pigmentation in melasma in ethnic skin. Asians and Asian Americans respond better to glycolic peels (72) than to salicylic ones whereas the opposite occurs in blacks. Pre- and posttreatment with topical bleaching agents is advisable as well as daily UV protection.

### Acne Scarring

Nonhypertrophic acne scarring may be improved with a series of combined peels with 70% glycolic acid gel and 25% TCA. Seventy percent glycolic gel is first applied followed by 25% TCA; at the beginning of the frosting, the peel is neutralized with a sodium bicarbonate solution (68).

### Pseudofolliculitis Barbae

Excellent results are obtained treating pseudofolliculitis barbae with series of glycolic or salicylic peels (68).

### Solar Lentigines and Mottled Pigmentation

As in fair complexions, solar lentigines and mottled pigmentation are a manifestation of aging. Spot peel with a 25% TCA, applied until a white frost is achieved, represents a good therapeutic option for solar lentigines in ethnic skin. Mottling is improved by series of 50% to 70% glycolic acid peels or by 20% to 30% salicylic acid peels (68).

## SKIN AGING AND REJUVENATION

### Skin Aging

Thanks to progress in in vivo imaging modalities such as ultrasound (US) and optical coherence tomography (OCT), differences between ethnicities concerning skin structure have been recently better investigated. With a 25 MHz US imaging system, it is possible to measure skin thickness and the subepidermal nonechogenic band (SENEB) that depends on skin aging. On the other side, OCT imaging supplies a description of epidermis morphology. Querleux et al. (73) have reported interesting data. On the basis of OCT measurements, their study has demonstrated that epidermis thickness, taken at the top of the papillae, does not change with age in all ethnic groups, whereas dermo epidermal junction (DEJ) is influenced by the aging process and

flattens. This phenomenon concerning the DEJ is accentuated in Caucasians compared to blacks. It is therefore deducible that blacks show signs of aging later than whites. This data is confirmed by the measurement of SENEb by means of US imaging at 25 MHz; SENEb is thinner in African Americans than in Caucasians. In blacks, photoaging appears unusually before the sixth decade. Mottled pigmentation, fine wrinkling, laxity, and dermatosis papulosa nigra represent the most common manifestations of photoaging in African Americans (74). In Asians, due to their geographic position, photoaging is frequent, especially in East Asians whose skin color is quite light. Features of photoaging in East Asians are mainly seborrheic keratosis and wrinkling (74).

### Nonablative Photorejuvenation

As ablative resurfacing implies high risk of serious and prolonged side effects in ethnic skin, such as PIH, scarring, and postinflammatory hypopigmentation, the choice of nonablative devices is advisable (75). Wherever using visible and near-infrared lasers or light devices, efficient cooling is fundamental to avoid thermal injury. 532 nm has been used for rejuvenation in ethnic skin with improvement of erythema, texture, pigmentation, and wrinkling (76,77). Low incidences of hyperpigmentation and hypopigmentation have been reported. Successful rejuvenation, without side effects, has been obtained by Trelles et al. (78) using a combination of 595 and 1450 nm lasers. Different authors have described the use of IPL in ethnic skin. Excellent results have been achieved employing a 550 nm cut-off filter (79,80) or a 640 nm cutoff filter. Recently, perioral and periorcular rhytids as well as neck and forehead rhytids have been treated with a 1540 nm erbium:glass laser in patients with type IV skin. Clinical improvement was also confirmed by US imaging and digital profilometry; the procedure has demonstrated an excellent safety profile (81). Light-emitting diodes (LEDs), fractional photothermolysis, and radiofrequency represent new nonablative devices for rejuvenation. LED light therapy has been approved by FDA. It has been established that specific wavelengths of visible red and infrared light emitted by LEDs stimulate fibroblastic activity and the release of adenosine triphosphate (ATP), boost collagen production, diminish hyperpigmentation, induce formation of new capillaries, and increase lymphatic system activity. In skin type IV, clinical results have been excellent, and there were no reported adverse effects (82,83). Fractional photothermolysis is delivered by a nonablative erbium:glass laser (1500 nm) and consists in microscopic columns of thermal damage (84). It is an FDA-approved device for treating pigmented lesions, periorbital rhytides, skin resurfacing, and melasma. As epidermis is protected from injury and melanin is not a target of this type of laser, fractional photothermolysis possesses suitable features to be used in skin of color. Indeed, the procedure has been described as safe and effective for treating photoaging as well as acne scarring in ethnic patients (85). Together with photoaging, skin laxity is an important sign of advanced age and is common in all skin types. The demand for an effective treatment for skin laxity is increasing. Patients with skin of color seek nonsurgical procedures because of their proneness of scarring. Radio frequency (RF) is electromagnetic radiation in the frequency range of 3 kHz to 300 GHz. It works by selectively delivering heat energy to the dermis, thus inducing collagen remodeling and contraction. In their study performed on Asian patients, Kushikata et al. (86) concluded that RF represents an effective and safe tool to achieve skin tightening in skin of color.

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# Ethnic Variation in Hair

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## INTRODUCTION

The diversity of human skin types and its appendages is a result of various stages of evolution, climate changes, and migration. Studying the complexity of ethnic variations in human hair and skin is not only interesting from an anthropological point of view but certainly of interest for the dermatologist as well as for the cosmetic and pharmaceutical industry.

Classifying different ethnic groups is particularly difficult because ancient or more recent migration processes and mixes between ethnic groups or subgroups are usually not taken into account. Many studies have broadly distinguished three ethnic human subgroups: African, Asian, and Caucasian. Such a broad classification cannot account for the great complexity of human biological diversity, resulting from multiple past or recent mixed origins.

The term African refers to people who live in Africa or people who trace their ancestry to indigenous inhabitants of Africa. This includes people who were displaced in the African diaspora resulting from the Atlantic Slave Trade such as African-Americans, African-Canadians, Afro-Latin Americans, Afro-Caribbeans, and Black British. The term *black people* is often used as a synonym for people of African ancestry (in particular sub-Saharan Africa). The term Asian refers most commonly to people of predominantly East Asian and Southeast Asian ancestry. The term Caucasian has been used to characterize the general physical type of some or all of the indigenous populations of Europe, North Africa and western, south and middle Asia (1). The concept of a Caucasian, Asian, or African race is highly controversial today. It is rejected by many academics and political activists who view any system of categorizing humanity based on physical type as obsolete (1,2). Responses to drugs and cosmetics can vary dramatically based on ethnicity. There is a great debate as to whether ethnic categorizations as broad as Caucasian, Asian, and African are medically valid (3,4).

Nevertheless, since most studies on ethnic hair diversity use this broad and unsatisfactory characterization, this chapter retains the terms Caucasian, Asian, and African.

## SCALP HAIR

Scalp hair is probably one of our most distinctive features. It represents health, beauty, sexual attraction, and moreover it reflects our personality and individuality. Depending on its structure and physical properties, scalp hair is subject to limitations that will only permit certain styles without major damage to the hair shaft.

Several features of the scalp hair, such as form, color, thickness, density, maximal length, and tensile strength are genetically determined and show great variation between

different ethnic groups and subgroups. The understanding of the diversity of scalp hair and its different response to physical and chemical treatments is the prerequisite for a proper development and application of hair care and is likewise important for the dermatologist, cosmetic scientist, product formulator, and hair stylist.

## Ethnic Diversity of Human Scalp Hair

### *Hair Shape and Thickness*

The term Caucasian or Indo-European comprised a vast diversity of different ethnic subgroups originating from Europe, North Africa, and western (as well as south and middle) Asia. Therefore this group shows a tremendous variability in hair shaft shape and caliber. Luther et al. found that Caucasians had significantly larger terminal hair follicles than Asians and Africans (5). In general, Caucasian hair has a slightly flattened or oval cross-section with a diameter ranging from 50–90  $\mu\text{m}$  (6,7). In Europeans, hair shaft diameters can range from approximately 50–120  $\mu\text{m}$  (Otberg, unpublished data). Very fine hair with diameters less than 50  $\mu\text{m}$  is most frequently seen in the Scandinavian population and northwestern Europe (6). Straight Caucasian hair is relatively untwisted along the shaft, whereas wavy or curly hair shows a higher degree of twisting proportional to the degree of curling (6,8–11).

Hair of people originating from East Asia (China, Korea, and Japan) is usually referred to as Oriental or Asian hair. It generally shows the greatest diameter, ranging from 100–130  $\mu\text{m}$  (6) (Otberg, unpublished data). Asian hair shafts are straight with no or very few twists along the shaft and with a round cross section (8–10).

Hair from people of sub-Saharan Africa is highly characteristic in shape. African hair is considerably flattened, grooved, and frequently varies in diameter along one single shaft. It tends to be highly twisted, with random reversal in twist direction. Lindelof et al. showed that the hair follicle in African hair is spiral in shape. They found that the shape of the hair shaft conformed to the shape of the follicle in all three major ethnic hair types (Caucasian, Asian, and African) (12). African hair can be quite sharply kinked at the edges and is especially vulnerable to damage at such points. The hair of people from different African countries shows great variability in the degree of curling, with eastern African hair showing the least degree of curling (6,13,14). African hair tends to be more easily harmed by cosmetic procedures than cylindrical hair and grooming usually requires more force, especially when the hair is dry (6,13,15).

Since the conventional classification of three ethnic subgroups hardly accounts for the great diversity of human hair characteristics, De la Mettrie et al. developed a new approach

to classify hair based on physical features. Hair types were defined according to three different hair shape criteria (curve diameter, curl index, and number of waves) without referring to human ethnicity. This method leads to a classification of hair in eight well-defined categories and may be more appropriate and more reliable than conventional standards in both cosmetic sciences and anthropology (16).

#### *Hair Density*

The appearance of thick full hair depends not only on the number of hairs but certainly on the thickness of the hair shaft, on its shape and color, and its contrast to the skin. In general, there is a relationship between the number of hairs (density) and size of the hair shafts (thickness), so that ethnic groups with the thickest caliber hair have the lowest density (i.e., Asians) and those with fine hair have the highest density (i.e., Scandinavians) (17). An average Caucasian brown-haired man is believed to have approximately 100,000 hair follicles on the scalp, while blondes tend to have 20% more and redheads around 20% less hair (18,19).

Surprisingly few studies are published on normal scalp hair density. Whiting, Aslani et al., Sperling, Templeton et al., and Lee et al. utilized horizontal sections of scalp biopsies to determine terminal and vellus hair density as well as anagen:telogen ratio in Caucasian, African-American, and Asian patients. Lee et al. showed the lowest hair counts in Asian patients (35 Korean patients) with 1.2 terminal hairs/mm<sup>2</sup> (120/cm<sup>2</sup>) on average (20). Higher counts, showing 1.5 terminal hairs/mm<sup>2</sup> (150/cm<sup>2</sup>) were found in 22 African-American patients (21). Highest hair counts were found in scalp biopsies of 22 Caucasian men by Whiting 3.1 hairs/mm<sup>2</sup> (310/cm<sup>2</sup>) (22). Aslani et al. counted 2.8 hairs/mm<sup>2</sup> (280/cm<sup>2</sup>,  $n=21$ ) in scalp biopsies of male volunteers and slightly lower numbers in women (2.5 hairs/mm<sup>2</sup>=250/cm<sup>2</sup>,  $n=9$ ) (23), Templeton et al. found 2.7 terminal hairs/mm<sup>2</sup> (270/cm<sup>2</sup>) (24), and Sperling found 2.5 terminal hairs/mm<sup>2</sup> (250/cm<sup>2</sup>) (21) on average in Caucasian patients. Whiting and Aslani et al. obtained specimens from control subjects with no evidence of alopecia (22,23), whereas Sperling, Templeton et al. and Lee et al. evaluated hair density in specimens from clinically normal occipital scalp of patients with androgenetic alopecia (20,21,24).

Surprisingly, great variations were found in the terminal:vellus hair ratio (T:V ratio) among the different studies. Whiting found a T:V ratio of 1.7:1 (22), whereas Sperling, Lee et al. and Aslani et al. found much higher values (Caucasian: 6.0:1 [21], 17.6:1 [23], Asian: 13.5:1 [20], African: 6.1:1 [21]). Different levels of the horizontal section of the punch biopsy may explain these differences. Vellus hair follicles on the scalp seem to be much smaller compared to vellus hair follicles on the rest of the body. Their infundibulum is located in the very upper part of the dermis; therefore vellus hairs can easily be missed if the horizontal section is evaluated at a deeper level (unpublished data, Otberg).

Noninvasive techniques have also been utilized to determine scalp hair density. One of the earliest methods of measuring hair density was devised by Bouhanna, who used camera attachments to create a "phototrichogram," an ultra close-up photograph of hair exiting the scalp (25). van Neste combined the phototrichogram with computer-assisted image analysis (26). A further development is the TrichoScan® technique introduced by Hoffmann, which combines epiluminescence microscopy with digital image analysis for the measurement of hair density and hair growth parameters (27). Loussouran et al. confirmed the results for African and Caucasian hair

density by using the phototrichogram technique. They found 162 terminal hairs/cm<sup>2</sup> in the occipital area of 106 male volunteers of African descent. Hair density was highly variable (from 90 to 396 hairs/cm<sup>2</sup> and significantly higher on the vertex than on occipital and temporal areas. Women showed a slightly higher hair density in the occipital region than men (167/cm<sup>2</sup> on average,  $n=110$ ). Vertex and temporal area showed no statistically significant difference between male and female volunteers; the vertex area showed the highest count (M:188/cm<sup>2</sup>, F:199/cm<sup>2</sup>); the temporal area showed the lowest values (M:128/cm<sup>2</sup>, F:121/cm<sup>2</sup>). Male Caucasian volunteers ( $n=56$ ) showed an average terminal hair density of 217 hairs/cm<sup>2</sup> in the occipital area, 264/cm<sup>2</sup> in the vertex area, and 151/cm<sup>2</sup> in the temporal area. Female Caucasian volunteers showed significantly higher values (occipital: 250/cm<sup>2</sup>, vertex: 308/cm<sup>2</sup>, and temporal: 169/cm<sup>2</sup>) (28). Much higher values compared to Lee et al. were found in the Asian group. Loussouran et al. measured hair densities in 188 Chinese volunteers. Male Chinese volunteers ( $n=92$ ) showed 179/cm<sup>2</sup> in the occipital area, 217/cm<sup>2</sup> in the vertex area, and 122/cm<sup>2</sup> in the temporal area, female Chinese volunteers ( $n=96$ ) showed significantly higher values in the occipital and vertex area (occipital: 185/cm<sup>2</sup>, vertex: 231/cm<sup>2</sup>) and lower values in the temporal area: 169/cm<sup>2</sup> (28). The finding of Loussourian et al. and Lee et al. indicate the diversity of hair characteristics among different Asian subgroups. Furthermore, the results indicate that sexual difference might have different patterns according to the ethnic background (28).

Jiménez and Poblet used phototrichograms to measure follicular unit (FU) density in the occipital area in patients undergoing hair transplantation. Follicular unit density ranged from 60–100 FU/cm<sup>2</sup> with an average hair density of 260 terminal hairs/cm<sup>2</sup>. The authors note that FU density is significantly lower in Asian and African patients. Follicular units tend to maintain a certain distance between each other. This distance correlates with the FU density according to a formula described by Jiménez and Ruiferandez  $L = 10/\sqrt{n}$ , where  $L$  represents the average distance between FUs expressed in millimeters and  $n$  the number of FUs/cm<sup>2</sup> (29,30).

Rassman et al. used a small handheld magnifier called a desitometer to determine hair density and follicular groupings in patients seeking hair restoration surgery. They found that the average hair density and the average follicular unit density vary significantly among different ethnic groups. African-American patients showed an average hair density of 1.6 terminal hairs/mm<sup>2</sup> (160/cm<sup>2</sup>) in contrast to Caucasian patients, showing 2.0 hairs/mm<sup>2</sup> (200/cm<sup>2</sup>). In patients of African descent FU groupings were predominantly found to be in 3s, in Caucasian in 2s and 3s, opposed to Asian patients showing FUs with only 1–2 hairs (17).

A more modern noninvasive technique to measure scalp hair density is videodermoscopy (31–33). Videodermoscopy allows evaluating the scalp in 20- to 100-fold magnification. Hair and scalp disorders can be easily diagnosed, and when combined with digital image analysis it allows measurement of hair density and thickness of the hair shaft without shaving, clipping, or dyeing.

#### *Hair Growth*

Whiting, Aslani et al. and Sperling and Lee et al. found a very similar percentage of terminal anagen and telogen hairs of approximately 94:6 in all three major ethnic groups (Caucasian, Asian, African) (20–23). Lee et al. state that this ratio could be uniformly applied as a normal value in interpreting scalp



biopsy specimens regardless of the ethnic background of the patients (20–23). Loussouran used the phototrichogram technique to measure hair growth parameters in Caucasian and African men and women (28). Increased telogen counts compared to the data obtained from scalp biopsies were found in both the African and Caucasian groups. The percentage of telogen hair in the vertex area was found to be 17% in African volunteers and 16% in Caucasian group; the occipital area showed 19% of telogen hairs (African) versus 11% (Caucasian). Growth rates were highly variable in both groups, with a higher variability and overall lower growth rates in the African group. On average, growth rates of 260  $\mu\text{m}/\text{day}$  (African) versus 397  $\mu\text{m}/\text{day}$  (Caucasian) were found for the vertex area and 252  $\mu\text{m}/\text{day}$  (African) versus 402  $\mu\text{m}/\text{day}$  (Caucasian) for the occipital area (28).

#### *Hair Color*

Hair color is determined mainly by melanin pigmentation within keratinocytes of the hair fiber. Melanin is a complex quinone/indole-quinone-derived mixture of biopolymers produced in melanocytes from tyrosine (34). Melanocytes are dendritic neural crest-derived cells that migrate into the epidermis in the first trimester. Hair and epidermal pigmentation are, insofar as melanocytes are concerned, similar processes: in interfollicular skin, melanin, packed in melanosomes, is passed from the melanocytes to the adjacent keratinocytes; in hair a similar process exists, with pigment being added to the growing keratinocytes (35). Hair melanin is formed by melanocytes situated in the hair bulb epithelium around the upper half of the dermal papilla among cells destined to form the hair cortex. The pigment is donated to cells undergoing early differentiation to form the hair cortex. Melanosomes are also found in the hair medulla, whereas it is unusual to find melanin in the cuticle of human hair shafts (35). Two different types of melanin can be distinguished: eumelanin, which is brown or black, and pheomelanin, which results from the incorporation of cysteine, is yellow or red (36–39). Eumelaninogenic and pheomelaninogenic melanosomes can coexist in the same melanocyte but are produced in different pathways (40–42). The absence or relative absence of both melanin types is associated with white hair; a preponderance of eumelanin with brown or black hair; and a preponderance of pheomelanin with red or blond hair.

Since the term Caucasian comprises such a large number of ethnic subgroups, the entire range of hair colors can be found within this group. People of Celtic descent seem have predominantly pheomelanin and therefore reddish-brown or -blonde hair (6), whereas in people originating from southern Europe, North Africa, or western, south, or middle Asia, eumelanin is the predominant chromophore and therefore leads to all shades of brown to deep black hair. Asian and African hair is usually densely pigmented with eumelanin and is therefore black or dark brown.

Differences in pigmentation between people are not a result of the number of melanocytes but are largely the result of differences in the amount and types of melanin produced and the macromolecular structure and packaging of melanin. Blonde hair appears light because melanosomes are poorly melanized (40). Melanosomes are secreted in different shapes and sizes; such differences change the way of light scattering and thereby the hair color (36).

Furthermore, hair color may vary both in time and site. For example, scalp hair may be blonde in childhood and become brown or black in adolescence, before becoming white

again in middle or old age; beard or pubic hair may be red and the scalp hair black or dark brown in the same individual (36,40,43).

#### *Hair Biochemistry*

Despite the obvious differences in phenotype, hair of different ethnic origin shows relatively little biochemical difference (44). Keratin fiber analysis showed no significant differences in the amino acid composition in Caucasian, Asian, and African hair (10,45). The distribution of cystine-rich proteins in the hair of volunteers of African descent, Caucasians, and Asian volunteers was found to be similar (46). No ethnic difference was found in the composition of low-sulfur S-carboxymethylated fibrous proteins (10,47,48). However, a difference in the ratio of fibrous protein and matrix protein, with African hair showing a lower ratio of low-sulfur fibrous proteins to high-sulfur matrix proteins compared to Asian and Caucasian hair was found (48).

#### *Hair Characteristics*

A cross-section of fully mature and keratinized human terminal hair reveals three major structures. The central layer is the medulla, the intermediate layer is the cortex, and outermost layer is the cuticle. The medulla consists of a vacuolated, trabecular mass formed from aggregated trichohyalin granules. It contains melanosomes and citrulline-red granules (49). The cortex gives the hair shaft its elasticity and tensile strength. It consists of elongated cells containing tonofilaments and interfilamentous matrix material, which is rich in cysteine. Cortex keratin is different from epithelial keratin, primarily in the increased number of disulfide bonds in the hair shaft. The cuticle forms the protective shield of the hair shaft. It consists of flattened highly keratinized cells arranged like shingles. The scale-like cells overlap tightly in a proximal-to-distal direction along the hair shaft. The cuticle mainly protects the hair shaft from an excessive penetration of water and swelling of the cortex. When the cuticle is intact, the scales are flat and smooth, reflect light, and provide a shiny, healthy look to the hair (6,49).

Function and appearance of scalp hair depends on three major characteristics: Porosity, elasticity, and texture.

Porosity of the hair measures the ability to allow moisture to pass through the cuticle into the cortex. In a healthy undamaged hair, only minimal amounts of water or other substances can penetrate the cuticle. In order to allow chemicals to penetrate the hair for permanent waving or hair coloring, which takes place in the hair cortex, the cuticle has to be altered. A higher permeability is achieved by increased temperature or/and by changing the pH of the environment along the shaft. When the processing of the hair is finished, the cuticular scales gradually close again. If processing is too harsh or applied too many times, the cuticle cells cannot return to their original tightness, which results in an imperfect barrier and increased porosity. Increased porosity can also be a result of UV radiation, overheating from flat or curling irons, and close, hot blowdrying as well as from excessive combing and teasing (6,49). Highly porous hair tends to be dry and fragile, appears dull, and often contains split ends.

Asian hair is more resistant to chemicals that open the cuticle. Therefore stronger chemicals are needed if cosmetic alterations are desired such as coloring and permanent waving. Asian hair in general is more fragile and sensitive to chemical alterations. It tends to be drier and since grooming requires more force, especially in longer African hair, the cuticle is more likely to be damaged (6).

Elasticity is a measure of how far the hair can be stretched without losing the ability to spring back to its original length. Healthy hair can stretch approximately 1 1/3 of its original length when wet and return to its regular length when dry (49). The tensile strength of the hair shaft is related to its elasticity and is assessing the weight it can support. One healthy hair shaft can support approximately 100 g weight without breaking. Elasticity and tensile strength depend on the hair cortex. Thick, curly hair shows the highest elasticity and tensile strength, whereas thin, straight Caucasian hair shows the least resistance to stretching and the lowest tensile strength.

The third major characteristic is the hair texture. It depends on the diameter and shape of the hair shaft. As discussed earlier, Asian hair shows the thickest diameter and a round, cylindrical shape. Healthy Asian hair is very well suited for long hairstyles; it may become spiky if worn short (Figure 42.1). African hair is highly twisted with high variability in diameter along the shaft and a flat oval cross-section. Therefore light reflects in different directions, which may result in a dull appearance. African hair is particularly difficult to manage and to remain healthy when worn in longer hairstyles. A tremendous variety in hair texture can be found in Caucasian hair, ranging from very thin cylindrical hair to thick curly hair shafts.

#### *Hair Care and Styling*

Hair care and styling depends on the cultural background and on current trends. As discussed earlier, African hair is very different from Asian and Caucasian hair. African hair tends to be very dry; considerable force is needed to comb the hair and although the hair is susceptible to mechanical damage, African hair can be more easily combed when wet. Too-frequent shampooing will result in excessive dryness and brittleness. It was found that 50% of African-American women shampoo once weekly and another third only once every other week (10,50). This is considerably less compared to people of Caucasian or

Asian descent. African hair is usually easy to care for when worn short. Oils and pomades are often used to assist in grooming or to treat dry, scaly scalp skin. Braiding is a popular way to manage longer African hair. Artificial braids are often attached to natural braids; classical “cornrow” braids are more frequently seen in children. Tight braids or ponytails may result in prolonged tension on the hair follicle; this may result in traction alopecia and ultimately in permanent hair loss (Figure 42.2). Today only very few African-American women are wearing a natural hairstyle. Many women have their hair regularly straightened. African hair with its tight curls can only be straightened properly by using hot comb procedures or chemical relaxers. Hot combing involves the application of oil to the hair shaft, combing the hair straight with a hot comb (150–250 °C) until there are no more curls and then optionally reshaping the hair with large rollers. Chemical relaxers contain either sodium hydroxide or guanidine hydroxide and lithium hydroxide (lye-free straighteners). Chemically relaxed hair can also be gently re-permed to induce light curls (6,10,49). The use of hot combs and straighteners has been associated with central centrifugal cicatricial alopecia (CCCA), an inflammatory hair loss condition that leads to permanent progressive hair loss, especially on the central parietal scalp (51–53).

Very fine straight Caucasian hair is difficult to style, especially if left to grow much beyond shoulder length. A layered haircut as well as styling products such as volumizing shampoo, mousse, and hair spray can help to create the impression of more volume. Setting straight hair can give waves or curls by temporarily changing the position of the weak hydrogen bonds in the hair shaft by wetting, rearranging, and holding with hair spray. Fine Caucasian hair is especially vulnerable to mechanical damage when wet and therefore excessive combing of wet hair should be avoided; less damage is done to the cuticle when the hair is dry. Teasing or backcombing involves combing or brushing the hair toward the root to create small tangles that will hold the hair in place. This procedure was very popular in the 1950s



**Figure 42.1** Spiky appearance of hair in a Chinese man with short haircut. (Courtesy of Dr. Jerry Shapiro.)



**Figure 42.2** 34-year old African-Brazilian patient with cicatricial traction alopecia in her frontotemporal hairline caused by tight hair styles.



and 1960s to create voluminous hairstyles and the popular beehive style. However, backcombing leads to severe damage of the cuticle and will result in increased porosity and hair breakage. A permanent wave chemically alters the strong disulfide bonds in the hair cortex. Since the hair cortex holds the perm, very fine Caucasian hair may be difficult to perm, because the hair cortex forms a lower proportion of the hair fiber. Thick, straight Asian hair is generally difficult to set or perm because it doesn't flex readily in any preferred direction. Higher concentrations of perming solutions are usually required for Asian hair (6).

The cold wave or base permanent was invented in the 1930s. It involves the application of a solution of ammonium thioglycolate to break the disulfide bonds; after that the hair is neutralized and hardens into a new shape by applying hydrogen peroxide, perborate, or sodium or potassium bromate (49). In the 1970s a more gentle acid-based glycerol monothioglycolate (GMTG) permanent wave was introduced. GMTG separates the cuticular scales to a lesser degree with a lower pH and therefore the cuticle is more likely to return to the original stage after treatment. The acid-based permanent requires heat to be activated. This kind of wave is usually not strong enough to perm very coarse Asian or African hair. A high incidence of contact dermatitis caused by GMTG has been described (49,54).

Changing the color of hair that is heavily pigmented with eumelanin, such as Asian, African, or East-Indian hair is particularly difficult, especially if a much lighter hair color is desired. Bleaching destroys eumelanin and tends to produce red-tinged hair as well as leading to increased weathering and porosity.

#### *Ethnic Differences in Hair and Scalp Disorders*

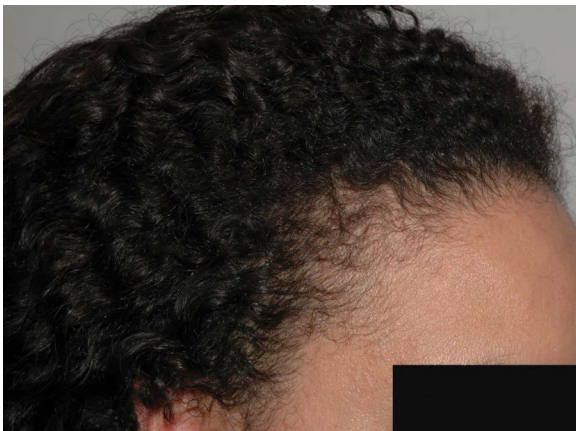
Ethnic variations in the incidence of *androgenetic alopecia* have been reported. In 1951, Hamilton found that most Chinese retained the frontal hairline after puberty and that baldness was less common, less extensive, and started later in life compared to Caucasians (55). In 1981, Takashima et al. found that Japanese men develop male pattern hair loss approximately 10 years later in life and have a 1.4 times lower incidence in each decade compared to Caucasians (56). Tang et al. investigated the scalp hair of 254 men in Singapore. They found a prevalence of 87% in the East Indian population and 61% in the Chinese population (57). Paik et al. investigated 5531 Korean

men. An increase of prevalence with age occurs with an overall prevalence of 14.1% (58). A study carried out on 1124 Chinese and Thai men aged between 18 and 90 years in Bangkok showed a prevalence of 38.5% of male pattern hair loss type III or higher (59). Setty reported that androgenetic alopecia is four times less frequent in men of African origin (60) (Figure 42.3).

Certain inflammatory hair disorders are more frequently seen in people of African ancestry. *Pseudofolliculitis barbae* is found in 45%–83% of African-American men who regularly shave (10). *Pseudofolliculitis barbae* is characterized by perifollicular and follicular papules and pustules as well as scarring in the shaved area. Very kinky hair tends to grow back into the skin surface or pierces the follicular epithelium, followed by inflammation when the hair is cut extremely short.

*Dissecting folliculitis* (DF) is also known as *perifolliculitis capitis abscedens et suffodiens* (of Hoffman), *dissecting cellulitis*, *dissecting perifolliculitis*, and *perifolliculitis capitis*. DF is a primary neutrophilic cicatricial alopecia. It manifests with perifollicular pustules, nodules, abscesses, and sinuses leading to scarring alopecia. The etiology of *dissecting cellulitis* is unclear. There are three implicated factors: infection, follicular occlusion, and immune cell-mediated chronic inflammation (61). DF occurs predominantly in black men aged 18 to 40 years (52). It can also occur in men of other ethnicities; women and children are rarely affected (62,63). Chronic and relapsing courses result in cicatricial alopecia which can present as hypertrophic or keloidal scars (64,65).

*Acne keloidalis*, also named *acne keloidalis nuchae*, *dermatitis papillaris capillitii*, or *folliculitis keloidalis*, is a chronic idiopathic, inflammatory process leading to hair loss and hypertrophic scarring in papules and plaques (65). The exact etiology of *acne keloidalis* is unknown. Probable participating factors include constant irritation from shirt collars, excoriation and seborrhea, localized infection, shaving of the neck, coarse hair, and autoimmunity (52,66). *Acne keloidalis* occurs predominantly in black men between 14 and 25 years of age (21). The initial lesions are dome-shaped, firm, skin-colored follicular papules and pustules, which are located mostly on the occipital scalp and the nape of the neck, although they may also be found on the vertex and parietal area (21,67). As the disease progresses, papules and pustules may enlarge and coalesce into keloid-like plaques, associated with variable hair loss (Figure 42.4).



**Figure 42.3** Young African American women with early female pattern hair loss, (Courtesy of Dr. Jerry Shapiro.)



**Figure 42.4** African American men with *acne keloidalis nuchae* displaying keloid-like plaques and pustules. (Courtesy of Dr. Jerry Shapiro.)



*Folliculitis decalvans* (FD) is a common form of primary cicatricial alopecia comprising 10.7%–11.2% of all cases with cicatricial alopecia (68,69). The etiology of FD remains unclear. It may be a complex combination of a bacterial infection, particularly *Staphylococcus aureus*, a hypersensitivity reaction to “superantigens,” and a defect in host cell-mediated immunity regulation (68,70,71). FD predominantly occurs in young and middle-aged adults with a slight preference of the male gender. FD seems to occur more frequently in African-Americans compared to Caucasians (68,69). The primary lesions are painful or pruritic follicular pustules or papules (70). With progression, more pustules and papules, as well as crusting and nodules, can be seen. Associated with the inflammatory activity, one or more round to irregular patches of scarring alopecia develop. The patches look like ivory pseudopelade skin centrally with surrounding follicular pustules and crusts at the margins of active lesions (51,72) (Figure 42.5).

CCCA is a lymphocytic primary cicatricial alopecia that primarily affects African-American Women. It can rarely be seen in Caucasians (sometimes called “central elliptical pseudopelade”) and African-American men. CCCA presents with a patch of scarring alopecia similar to Pseudopelade of Brocq on the central scalp, which slowly progresses centrifugally. It remains unclear if chemical processing, heat, traction, or other traumas contribute to the development of this condition (21,51,53) (Figure 42.6). The etiology of cicatricial alopecias is poorly understood. Inflammatory cicatricial alopecias seem to be less frequent in people of Asian ancestry, suggesting that the shape of the follicle and hair shaft plays a role in the pathogenesis.



**Figure 42.5** Caucasian female patient with extensive folliculitis decalvans. (Courtesy of Dr. Jerry Shapiro.)



**Figure 42.6** African American women with central centrifugal cicatricial alopecia (CCCA). (Courtesy of Dr. Jerry Shapiro)

## BODY HAIR

Very little data is available on ethnic differences in body hair. The density in chest hair in men has been reported to be highest in Caucasians, especially in those with a darker skin color. Blonde Italian men showed denser chest hair compared to Scandinavian men. In general, growth of terminal body hair seems to be more prominent in men of Mediterranean descent compared to Nordic descent (73). Men of African and Asian ancestry were found to have considerably low body hair compared to Caucasian men (73). Axillary hair growth in Caucasians was found to be much denser than in Japanese of both sexes (18). Dense terminal hair growth of the external ear or pinna (hypertrichosis pinnae auris) is very commonly seen in men of East Indian descent. Eighty-five percent of Caucasian men also show some degree of terminal hair growth in this area as opposed to 55% of men of African ancestry. The mode of inheritance of this form of hypertrichosis seems to be Y-linked (74).

Growth of terminal body hair is of concern for most women. However it is very difficult to decide whether the hair growth is excessive and should be classified as Hirsutism or within the range of normal variation. Hirsutism is highly unusual in Asian women, despite the fact that levels of androgens may even be higher (18).

## Eyelashes

Na et al. performed a study to determine differences in eyelash shape and growth rate in Asian and Caucasian volunteers. Lateral digital photographs of upper eyelashes were taken and curl-up and lift-up angles of the upper eyelashes were measured from lateral views. Additionally, the phototrichogram technique was used to determine total number, length, and thickness of upper eyelashes. The central portion of the eyelashes or whole eyelashes was clipped and images were taken immediately and 7 days later to obtain the growth rate and anagen ratio. The number and thickness of the cuticular layers were measured by electron microscopy. Asian eyelashes revealed lower lift-up and curl-up angles, fewer numbers, and a thicker transverse diameter compared to Caucasian lashes.

No statistical difference was found in length or growth rate. The duration of the anagen phase was estimated at about 2 months. The eyelash anagen rate obtained was approximately 17.8%. The number of cuticular layers was greater in Asians (8.0) than in Caucasians (6.5), but no statistical difference was found in the thickness of a single cuticle layer between the two groups (75). No data is available for African eyelashes. Eyelashes of people of African descent seem to be curlier compared to those of Asians or Caucasians.

### Villus Hair

Very little is published on the size and distribution of vellus hair. Hwang and Baik obtained skin samples from different body sites of Korean adults to determine the density and distribution of hair follicles and sweat glands. The average hair density was found to be 36/cm<sup>2</sup> on the back, 33/cm<sup>2</sup> on the thorax, 40/cm<sup>2</sup> on the upper arm, 18/cm<sup>2</sup> on the forearm, 28/cm<sup>2</sup> on the thigh, and 11/cm<sup>2</sup> on the calf (76). Cyanoacrylate surface biopsies have been utilized to study vellus hair parameters in seven different body sites in Caucasian, Asian, and African-American volunteers with comparable height and body mass index (77,78). Hair density, hair shaft diameter, size, and surface area of the follicular orifices and the volume of vellus hair follicles have been measured and calculated on microscopic images in combination with digital image analysis. The highest hair density was found on the forehead; significantly lower densities have been determined on the other body areas (upper arm > back > chest > thigh > forearm > calf). This overall trend was seen in all three ethnic skin types. Significant differences in vellus hair density were found on the forehead with Caucasians showing the highest average density (292/cm<sup>2</sup>) followed by African-American (189/cm<sup>2</sup>) and Asians (138/cm<sup>2</sup>). These differences correlate well with the ethnic differences found for scalp hair density. No significant differences have been found in vellus hair density were found in the other six skin areas. The diameter of the follicular orifice showed the greatest inter-site variation on the forehead of Caucasian volunteers, whereas Asian hair follicles on the forehead seemed to be more uniform in size. The percentage of the surface area taken by the follicular orifice was calculated by multiplying the average circle area of the orifice with the hair density per cm<sup>2</sup>. Caucasians showed the highest values in general with the forehead (1.28%) representing the highest percentage of follicular orifices. Hair shaft diameters were found to be the highest on the calf in all three skin types. However, significant differences among the groups were seen, with Caucasian showing the thickest hair shaft diameter on average (42 µm), followed by African-Americans (22 µm) and Asians (17 µm). Hair shaft diameters on the forehead, back, chest, upper arm, and forearm were below 20 µm with no significant differences among the ethnic groups. The thigh showed a trend similar to the area on the calf. The differences in follicle size and hair shaft diameter indicate higher androgen sensitivity in Caucasians. This hypothesis corresponds well with the higher incidence of androgenetic alopecia in the Caucasian population.

### CONCLUDING REMARKS

Hair of people of different ethnic ancestries shows great differences in size, shape, and color. The broad distinction between Caucasian, Asian, and African hair can hardly accomplish the great variety and different needs of human hair. A more elaborate distinction, as suggested by De la Mettrie

et al., seems to be more useful, especially for the development of hair care and styling products (16). Ethnic differences in hair are found not only in scalp hair but also in body hair and vellus hair. The shape and size of hair follicles seems to be a pathogenetic factor for the susceptibility to certain scalp and skin disorders. Further research is needed to provide more insight into the chemical and structural differences in human hair and the related clinical problems.

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# Ethnic Differences in Skin Properties

Rishu Gupta and Howard Maibach

## INTRODUCTION

Differences in ethnic skin properties have been minimally investigated and the available literature investigating these parameters is sparse. Variations in skin structure may contribute to differences observed in cutaneous diseases between ethnic groups. The current human skin model is largely based on physical and functional properties from Caucasian skin. However, physiological parameters in skin of different races and color can alter a disease process and if the fundamental disease process is not understood, appropriate treatments of diseases may not be considered. As patients are increasingly presenting with cutaneous concerns stemming from their unique skin physiology, clinicians and scientists are analyzing the skin proprieties that are fundamental to understanding these disease processes. However, the available data can be difficult to interpret, especially when comparing one study against another.

In this review article, we consolidate the data in an attempt to clarify the published studies and to help provide insight into the differences in skin structure and function between ethnic groups or skin types. The objective skin properties we present include transepidermal water loss (TEWL), water content (WC), blood vessel reactivity (BVR), corneocyte variability, elastic recovery/extensibility, pH gradient, lipid content, antioxidant status, pore size/architecture, and epidermal innervation.

## METHODS

A literature search was performed through EMBASE, PubMed, Science Citations Index Expanded, and the Melvyl Catalogue (CDL-Hosted Database of University of California, San Francisco, California) from 1967–2012. Keywords in searches included words pertaining to race (race, ethnicity, black, African, white, Caucasian, Asian, Hispanic, Chinese, Japanese) and dermatology (skin physiology, skin function, epidermal barrier function). A manual review of the reference list from the acquired articles was performed. Studies examining skin color and hair were excluded to maintain focus of the article on skin structure and function.

## TRANSEPIDERMAL WATER LOSS

The skin provides a critical barrier between the body's own internal environment and the external environment. One of the functions of the barrier is to regulate water loss, which is primarily performed by the stratum corneum, a layer within the epidermis. TEWL (transepidermal water loss) is an objective

measure that is defined as the total amount of water loss through the skin excluding loss due to sweat. In the presence of an insult such as trauma or irritants, the skin also triggers repair mechanisms involving lipid synthesis and secretion to minimize TEWL. TEWL is the most studied parameter when comparing differences in biophysical properties between ethnic skins.

In 1988, Wilson et al. observed higher in vitro TEWL values in black skin compared to white skin by taking samples from 22 cadavers matched for age and gender (1). Furthermore, Kompaore et al. and Sugino et al. found a significantly higher TEWL in Blacks and Asians compared to whites (2,3). In contrast, Berardesca and Maibach found no significant differences in baseline TEWL between Hispanics, blacks, and whites in two separate studies (4,5). Recently Fotoh et al. came to a similar conclusion as their study did not observe a significant difference in baseline TEWL between Caribbean blacks, Caribbean mixed races, and Caucasians (6). Luther et al. also found no significant differences in baseline TEWL between Caucasians, Africans, and Asians (7). However, in one of the largest number of subjects studied, Muizzuddin et al. reported the following significant differences in baseline TEWL values: Caucasians > African Americans > East Asians (8). The investigators also reported that African Americans exhibited superior skin barrier strength, since African Americans required the most number of tape strippings to raise the baseline TEWL past a threshold value (8). Similarly, Chu et al. also found that Caucasian females had significantly higher TEWL at baseline than African American females in a total of 151 patients matched by age (9). Comparing Japanese subjects to French Caucasians, Yamashita et al. observed statistically significant lower baseline TEWL values in the Japanese subjects (10).

In addition to baseline TEWL, several authors have investigated TEWL differences between races in response to skin irritation. Berardesca and Maibach found that blacks had 2.7 times higher TEWL than whites after topical application of an irritant, 0.5% and 2.0% sodium laurel sulfate (SLS) to untreated and preoccluded skin (5). This finding suggests that blacks are more susceptible to irritation, which contradicts previous reports that blacks are less reactive to irritable substances than whites based on observable erythema (5,11). Berardesca and Maibach investigated the TEWL values in Hispanics and whites and did not find a significant difference between the ethnic groups. They did however report higher values of TEWL, though not significant, in Hispanics compared to whites after exposure to SLS-induced irritation (12). In contrast, Berardesca et al. were unable to reproduce earlier

results, as the investigators did not find significant differences in baseline TEWL values between race (blacks, Hispanics, and whites) or anatomical site (4). Aramaki et al. also observed no significant differences in TEWL and barrier function pre and post exposure to SLS irritation between German and Japanese women (13). Additional studies by Astner et al., Tagami, Pershing et al., and Hicks et al. report differences in TEWL and skin irritancy between Caucasian and non-Caucasian skin (14–17).

Reid et al. compared TEWL between skin pigmentation types instead of ethnicity and found that skin types V/VI required a greater number of tape strippings than skin types II/III to achieve the same TEWL (18). The results signified that skin types V/VI exhibited increased skin barrier strength. Furthermore, in the same study, TEWL was found to recover more quickly for skin types V/VI. Gunathilake et al. agreed with this study by similarly observing that skin types IV/V required an increased numbers of tape strippings, compared to skin types I/II, to achieve a TEWL value three times of the baseline TEWL value. The investigators also found that skin types IV/V exhibited quicker barrier repair kinetics compared to skin types I/II as measured by the recovery of TEWL 24–48 hours after the tape strippings (19). In contrast, Berardesca et al. found that African Americans had higher TEWL values at baseline and after each tape stripping compared to Caucasian skin, although Caucasian skin similarly increased in TEWL values after the tape strippings (20).

The data regarding differences in TEWL in ethnic skin is conflicting (Table 43.1). Initially, the literature suggested that African Americans had an increased TEWL at baseline.

However, recent studies with larger sample sizes have reported conflicting results and have shown that Caucasians have a higher baseline TEWL compared to African Americans and even Asian subjects. There is also increasing literature available citing studies reproducing differences in TEWL and skin irritancy.

The evidence does not entirely validate the notion that water barrier function is dependent on the degree of pigmentation since there have not been any studies of acquired hyper- or hypopigmentation and its affect on barrier integrity. However, studies by Reed et al. and Gunathilake et al., that measure differences in skin type instead of by ethnic groups, have observed a superior water barrier function and its ability to recovery quickly in darkly pigmented subjects. If the integrity of the barrier function is dependent on the degree of pigmentation, then there are clinical implications on the ability of people with different skin types to recover quickly to environmental insults or to absorb topical therapeutic agents. Furthermore, TEWL may also be a factor of physiological or disease states and the health of participating subjects should be documented in future studies.

## WATER CONTENT

Water content is an indication of skin hydration and can be measured by multiple methods including skin conductance, resistance, capacitance, and impedance. Skin conductance is dependent on the changes in electrical properties of the stratum corneum based on skin hydration. For example, dry stratum corneum has a weaker electrical conduction, while hydrated stratum corneum is more sensitive to the electrical

**Table 43.1** TEWL

Study	Technique	No. of subjects	Site	Results
Wilson et al. 1988	In vitro	Blacks 10 (mean age 38.6y) Caucasians 12 (mean age 41.1y)	Inner thigh	TEWL Blacks $1.1 \times$ > Caucasians (mean corrected log TEWL 2.79 and 2.61 $\mu\text{g}/\text{cm}^2/\text{h}$ , respectively) [ $p < 0.01$ for both values]
Berardesca and Maibach 1988	In vivo – topical application of SLS (irritant)	Black men 10 (age $29.9 \pm 7.2\text{y}$ ) White men 9 (age $30.6 \pm 8.8\text{y}$ )	Back	No significant difference in TEWL between blacks and whites at baseline After SLS stress: TEWL blacks (untreated, pre-occluded, and pre-delipidized) > whites but only statistically significant ( $2.7 \times$ greater) for 0.5% SLS applied in the pre-occluded area ( $p < 0.04$ )
Berardesca and Maibach 1988	In vivo – topical application of SLS (irritant)	Hispanic men 7 (age $27.8 \pm 4.5\text{y}$ ) White men 9 (age $30.6 \pm 8.8\text{y}$ )	Upper back	No significant differences in TEWL between Hispanics and whites at baseline After SLS stress: TEWL Hispanics (untreated, pre-occluded, and pre-delipidized) > whites, but not statistically significant
Berardesca et al. 1991	In vivo	Blacks 15 (mean age $46.7 \pm 2.4\text{y}$ ) Whites 12 (mean age $49.8 \pm 2\text{y}$ ) Hispanics 12 (mean age $48.8 \pm 2\text{y}$ )	Volar and dorsal forearm	No significant difference in TEWL between site or race at baseline

(continued)

**Table 43.1** TEWL (continued)

Study	Technique	No. of subjects	Site	Results
Kompaore et al. 1993	In vivo – topical application of MN – vasodilator	Blacks 7 Caucasians 8 Asians 6 (ages 23–32y, all)	Volar forearm	MN given before tape stripping: TEWL blacks & Asians 1.3x > Caucasians ( $p < 0.01$ ); no difference between blacks & Asians After eight and 12 tape strips: TEWL Asians > blacks > Caucasians ( $p < 0.05$ ) [Asians 1.7x > Caucasians]
Sugino et al. 1993	In vivo	Blacks, Caucasians, Hispanics, Asians (no. of subjects, ages not specified)	Not documented	Baseline TEWL: Blacks > Caucasians $\geq$ Hispanics $\geq$ Asians
Reed et al. 1995	In vivo	Skin type V/VI: African American 4 Filipino 2 Hispanic 1 Skin type II/III: Asian 6 Caucasian 8 (ages 22–38y, all)	Volar forearm	Skin type V/VI required more tape strippings ( $66.7 \pm 6.9$ ) compared with skin type II/III ( $29.6 \pm 2.4$ ) to achieve the same TEWL, i.e. skin type V/VI had increased water barrier strength (integrity) Barrier function in skin type V/VI recovered more quickly
Warrier et al. 1996	In vivo	Black women 30 White women 30 (ages 18–45y, all)	Left and right medial cheeks, mid-volar forearms, lateral mid-lower legs	TEWL blacks < whites on cheeks (20% less) and legs (17% less) at baseline ( $p < 0.05$ ); also lower on forearm but not statistically significant
Berardesca et al. 1998	In vivo	Black women 8 Caucasian women 10 (mean age $42.3 \pm 5y$ , both)	Mid-volar forearm	After tape stripping: TEWL blacks 1.2x > Caucasians after 3 ( $p < 0.05$ ) and 6 tape strips ( $p < 0.03$ )
Aramaki et al. 2002	In vivo – topical application of SLS (irritant)	Japanese women 22 (mean age 25.84y) German women 22 (mean age 26.94y)	Forearm	No significant difference at baseline or after SLS stress
Tagami 2002	In vivo	Japanese women 120 French women 322 (ages 20–70y, all)	Cheeks and mid-flexor forearm	TEWL Japanese < Whites but not statistically significant
Hicks et al. 2003	In vivo	White 8 Blacks 6 (ages 18–40y, all)	Volar forearm	TEWL whites > blacks but not statistically significant
Grimes et al. 2004	In vivo – topical application of 5% SLS (irritant)	African American 18 White 19 (ages 35–65y, women, all)	Inner Forearm	Baseline: No significant difference After SLS stress: immediate increase in TEWL of white subjects, but increase no longer evident after 24 hr and found to be similar to African Americans (not statistically significant)
Pershing et al. 2006	In vivo – topical application of capsaicinoid analogs	Caucasians: Male 3 Female 3 Asians: Male 3 Female 3 (ages 19–63y, all)	Volar Forearm	Increasing concentrations of total capsaicinoid not associated with proportional change in TEWL, in all subjects Capsaicinoid concentration of 16mg/mL produced increased mean TEWL in Caucasians, decreased mean TEWL in Asians ( $p < 0.05$ )
Astner et al. 2006	In vivo – topical application of ivory soap (irritant)	Caucasians 15 (skin type II/III) African Americans 15 (skin type V/VI) (ages 18–49y, all)	Anterior forearm	Positive dose-dependent correlation between TEWL and irritant concentration: Mean TEWL Caucasians > African Americans ( $P < 0.005$ ) Relative increment of increase in TEWL after irritant: Caucasians > African Americans ( $P < 0.005$ )

(continued)



**Table 43.1** TEWL (*continued*)

Study	Technique	No. of subjects	Site	Results
Fotoh et al. 2008	In vivo	Sub-Saharan Africans or Caribbean black women 25 (mean age $24 \pm 4$ y) African or Caribbean mixed-race women 25 (mean age $24 \pm 7$ y) European Caucasian women 25 (mean age $12 \pm 4$ y)	Forehead and volar forearm	No significant differences in baseline TEWL between races at each site Baseline forehead TEWL significantly higher than volar forearm for each race ( $p < 0.001$ )
Gunathilake et al. 2009	In vivo	Skin type I–II: Germany 20 Skin type IV–V: Sri Lanka 20	Volar forearm	Barrier recovery kinetics, measured by TEWL at 24 and 48 hours after tape stripping, was significantly higher in skin type IV–V than skin type I–IIs
Muizzuddin et al. 2010	In vivo	African American women Skin type IV–VI 73 ( $35.1 \pm 7.5$ y) Caucasian women skin type II–III 119 ( $36.0 \pm 6.0$ y) East Asian women skin type III–IV 149 ( $30.2 \pm 5.8$ y)	Left facial cheek	Baseline TEWL: African Americans < East Asians < Caucasians ( $p < 0.001$ )
Chu et al. 2011	In vivo	Caucasian females 84 African American females 67 Age range 15–75y	Dorsal forearm Upper inner arm	Caucasian had a significantly higher TEWL matched by age ( $p < 0.05$ )
Luther et al. 2012	In vivo	Caucasian males 6 – skin type II–III ( $28.1 \pm 4.3$ y) African males 6 – skin type V–VI ( $27.3 \pm 3.2$ y) Asian males 6 – skin type IV ( $23.5 \pm 1.6$ y)	Volar forearm	No statistically significant differences in TEWL between the three ethnic groups
Yamashita et al. 2012	In vivo	Japanese Asian 43 ( $41.1 \pm 12.8$ y) French Caucasian 104 ( $40.4 \pm 14.4$ y)	Cheek Dorsal hand Upper arm	Japanese subjects had a lower baseline TEWL compared to French Caucasians

field. Resistance is the reciprocal of conductance. Capacitance values are indicative of water content as they are dependent on the high dielectric constant of water compared to other substances. Generally, capacitance and conductance values have similar trends with regard to observed values of WC for the skin, while resistance and impedance exhibit the opposite behavior. There are also several sources of potential error when measuring WC such as the electrolyte content of the stratum corneum, sweat production, filling of the sweat gland, number of hair follicles, and artifacts from applied topical agents.

Historically, in 1962, Johnson and Corah reported higher skin resistance levels in blacks compared to whites in both children and adults. Though the study did not provide a link between resistance levels and hydration, an inferred conclusion of the study can be that blacks had lower WC based on skin resistance values (21).

Berardesca and Maibach, in addition to measuring TEWL, investigated WC by capacitance pre and post exposure to topical SLS in blacks and whites. The authors did not find any significant differences in WC before or after SLS stress between the two ethnic groups (5). In a similar study involving Hispanics and whites, there were no overall statistically significant differences in WC values observed between the two groups (12).

Berardesca et al. studied WC by conductance in blacks, whites, and Hispanics on two different sites, the dorsal and the volar forearm. Regarding the dorsal forearm, blacks and Hispanics had a statistically significant increased WC than Caucasians. Sugino et al. measured WC with an impedance meter and found a higher WC in Asians compared to Caucasians, blacks, and Hispanics. Though the exact study parameters were not documented, the authors did correlate the high WC with higher ceramide and lower TEWL values that were also investigated in the same study (3). Warrier et al. reported a significantly higher WC on the cheeks of black women compared to whites. The authors did not observe differences between the ethnic groups on the forearms and the legs (22).

Additional studies by Manuskiatti et al., Sivamani, and Grimes et al. did not find differences in WC between ethnic groups that included Caucasian, African American, Hispanic, and Asian participants (23–25). Recently, Fotoh et al. and Luther et al. conducted studies that also did not observe statistically significant differences in skin hydration between ethnic groups (6,7).

Recent studies have reported differences in WC between ethnic groups with respect to sun-exposed sites. Diridollou et al. measured WC in 311 women with capacitance tests

and found that lighter skin tone groups such as Chinese and Caucasians had a higher dryness skin index on sun-exposed sites compared to African Americans and Mexicans (26). Chu et al. corroborated these results when comparing WC through conductance on the dorsal forearm and the upper inner arm between 84 Caucasian females and 67 African American females. In the study, African American females exhibited a statistically significant higher WC compared to Caucasian females at the sun-exposed dorsal forearm (9). Yamashita also found lowered water content in French Caucasians compared to Japanese Asians (10).

The results from the published studies regarding WC in different ethnic groups remain conflicting and inconclusive

(Table 43.2). Multiple studies were unable to find a significant difference in WC. There is evidence suggesting a possible racial variance in WC at sun-exposed sites, but this conclusion has yet to be validated. Based on the available literature, no conclusions with respect to ethnicity and WC can be made. Comparing WC measurements between studies is difficult because of possible confounding factors. Physical factors, such as sweat production and hair presence, can affect the measurements by modifying the quality of the skin electrode contact. Therefore, there may be variables other than race that determine the WC of a person's skin. Future studies with larger sample sizes and multiple methods for measuring WC can help assess the true relationship between race and WC.

**Table 43.2** Water Content

Study	Technique	No. of subjects	Site	Results
Johnson and Corah 1962	In vivo	St Louis: Black boys 22; black girls 32 White boys 65; white girls 55 (age 83–92mo, all) San Diego: Black men 16; black women 5 White men 16; white women 5 (age 23y, all)	First and third fingers of right hand	Skin resistance blacks > whites at baseline ( $p < 0.01$ ); i.e. blacks have lower water content
Berardesca and Maibach 1988	In vivo – topical application of SLS (irritant) – capacitance	Black men 10 (age $29.9 \pm 7.2y$ ) White men 9 (age $30.6 \pm 8.8y$ )	Back	No significant difference in WC between blacks and whites at baseline before or after SLS stress
Berardesca and Maibach 1988	In vivo – topical application of SLS (irritant)	Hispanic men 7 (age $27.8 \pm 4.5y$ ) White men 9 (age $30.6 \pm 8.8y$ )	Upper back	No significant differences between Hispanics and whites at baseline After SLS stress: Hispanics > whites when negative visual score was given for irritation ( $p < 0.01$ ) [large standard deviations]
Berardesca et al. 1991	In vivo	Blacks 15 (mean age $46.7 \pm 2.4y$ ) Whites 12 (mean age $49.8 \pm 2y$ ) Hispanics 12 (mean age $48.8 \pm 2y$ )	Volar and dorsal forearm	Blacks (13% less) volar < dorsal forearm ( $p < 0.02$ ) Whites (22% less) dorsal < volar forearm ( $P < 0.001$ ) Hispanics (11% less) dorsal < volar forearm ( $p < 0.05$ ) Black and Hispanics > whites on dorsal forearm at baseline Hispanics > blacks and whites on volar forearm at baseline
Sugino et al. 1993	In vivo – impedance	Blacks, Caucasians, Hispanics, Asians (no. of subjects, ages not specified)	Not documented	Asians > Caucasians, blacks, and Hispanics

(continued)

**Table 43.2** Water Content (*continued*)

Study	Technique	No. of subjects	Site	Results
Warrier et al. 1996	In vivo – capacitance	Black women 30 White women 30 (ages 18–45y, all)	Left and right medial cheeks Mid-volar forearms Lateral mid-lower legs	Blacks > whites on cheeks at baseline ( $P < 0.05$ ) No significant difference between races on forearms and legs
Manuskiatti et al. 1998	In vivo – capacitance	Black women 7 White women 5 (mean age $25.8 \pm 4.2$ y, both) Black women 5 White women 5 (mean age $64.7 \pm 3.8$ y, both)	Preauricle, posterior neck, dorsal upper arm, dorsal forearm, volar forearm, lower back, abdomen, thigh, lower leg	No significant differences between blacks and whites at baseline
Sivamani et al. 2003	In vivo – impedance, topical application of petrolatum and glycerin	White 22 African American 14 Hispanic 14 Asian 9 (ages 18–60y, all)	Volar forearm	Baseline: No significant differences in electrical impedance between age, gender, or ethnicity; impedance of proximal < distal forearm ( $P < 0.001$ )
Grimes et al. 2004	In vivo – capacitance	African American 18 White 19 (ages 35–65y, women, all)	Inner forearm	Baseline: African Americans < Whites, but not statistically significant
Diridollou et al. 2007	In vivo – capacitance	311 American women from four ethnic groups: African American, Chinese, Caucasian, Mexican Divided into two age groups Age 18–50y: African Americans 56; Chinese 44; Caucasian 41; Mexican 30 Age > 51y: African American 58; Chinese 45; Caucasian 22; Mexican 15	Ventral forearm Dorsal forearm	Dryness Skin Index is higher in lighter skin tone groups on sun-exposed sites (Chinese and Caucasian women) than the other ethnic groups
Fotoh et al. 2008	In vivo – capacitance	Sub-Saharan Africans or Caribbean black women 25 (mean age $24 \pm 4$ y) African or Caribbean mixed-race women 25 (mean age $24 \pm 7$ y) European Caucasian women 25 (mean age $12 \pm 4$ y)	Forehead and volar forearm	No statistically significant differences in hydration between the three groups Black women had a reduced cutaneous hydrophilicity compared to the mixed-race group and Caucasian group
Chu et al. 2011	In vivo – conductance	Caucasian females 84 African American females 67 age range 15–75y	Dorsal forearm Upper inner arm	African Americans > Caucasians with respect to the dorsal forearm ( $p < 0.05$ ) No significant differences in conductance between the ethnicities at the upper inner arm site

(continued)



**Table 43.2** Water Content (*continued*)

Study	Technique	No. of subjects	Site	Results
Luther et al. 2012	In vivo – not documented	Caucasian males 6 – skin type II–III (28.1 ± 4.3y) African males 6 – skin type V–VI (27.3 ± 3.2y) Asian males 6 – skin type IV (23.5 ± 1.6y)	Volar forearm	No statistically significant differences in skin hydration between the three ethnic groups
Yamashita et al. 2012	In vivo – conductance for Japanese; capacitance for French	Japanese Asian 43 (41.1 ± 12.8y) French Caucasian 104 (40.4 ± 14.4y)	Cheek Dorsal hand Upper arm	Japanese subjects had higher water content compared to French Caucasians

## CORNEOCYTE VARIABILITY

Keratinocytes produce corneocytes that differ in size and shape from the keratinocytes. Corneocytes are shaped in the form of a disk that allows them to have a larger surface area in the horizontal position. In Caucasians, it has been shown that the surface area of corneocytes differs by body site and that the surface area has an important role in determining the permeability of the skin to water as well as the percutaneous absorption of topical substances (27–29).

Corcuff et al. studied the corneocyte surface area and spontaneous desquamation between African Americans, Caucasians, and Asian Americans. The investigators did not observe a difference in the surface area between the groups but found that spontaneous desquamation, indicated by corneocyte count, was increased in African Americans by a factor of 2.5 compared to the other ethnic groups (30). The investigators concluded that the enhanced desquamation could partially explain the “ashing” phenomenon clinically seen in African Americans. Warrier et al. compared the desquamation index in 30 blacks and 30 white subjects, matched for age, on their cheeks, foreheads, and lower legs. Warrier et al. did report a significantly lower desquamation index in African Americans on the cheeks and forehead compared to Caucasians. This result contradicts the clinical finding of dry skin commonly seen in African Americans. No significant difference was found on the legs. The authors suggested that the differences found on the face may be due to the differences of the moisturizing properties of sebum between the ethnic groups (22). Manuskiatti et al. studied the desquamation index in a smaller population of African Americans and Caucasians. The authors found no significant differences in desquamation indices between African Americans and Caucasians at the posterior neck, dorsal upper arm, dorsal volar forearm, lower back, abdomen, thigh, and lower leg. Though they did find a significant difference at the preauricular area, the authors said that the difference might not be valid due to the small sample size.

Fotoh et al. measured the thickness of the corneocytes from spontaneous desquamation at the dorsal and volar forearm in three different ethnic groups comprised of only females: 25 Caribbean Africans, 25 Caribbean mixed-race, and 25 European Caucasians. No significant differences between the groups were observed (6).

Gunathilake et al. observed an increase in retention of corneodesomes in the upper layer of the stratum corneum

for darker skin types, potentially resulting in enhanced SC barrier function (19). Recently, Muizzuddin et al. compared the degree of cross-linking in corneocytes (maturation index) between African Americans, Caucasians, and East Asians on the left facial cheek. The authors found that the maturation index value, indicative of the degree of terminal differentiation, was the highest in African Americans compared to Asians. The investigators suggested that higher value could be positively associated with enhanced barrier integrity in African Americans (8). Furthermore, Yamashita et al. found a statistically significant increased size of corneocytes in Japanese Asians compared to French Caucasians. The authors concluded that the finding might validate the enhanced barrier function observed by the measurements of other values in the same study (10).

Instead of differentiating between ethnicities, Gunathilake et al. compared the cornified envelope thicknesses between pigment skin types of I/II and IV/V. The authors reported that the darker pigment skin types exhibited thicker cornified envelopes. The authors suggested that the thicker cornified envelopes in skin types IV/V could help the permeability barrier function of the epidermis (19).

The evidence regarding corneocyte variability between ethnic groups remains inconclusive (Table 43.3). Multiple earlier studies either found contradictory results with regard to degree of corneocyte desquamation or desquamation index between ethnic groups, or did not find statistically significant differences (6,22,23,30). However, recent studies suggest that parameters measuring different elements of corneocyte desquamation can play an important factor in enhanced barrier function for African Americans, Japanese Asians, and skin types IV/V compared to Caucasians and skin types I/II (10,19,23). Further controlled studies are required to adequately measure corneocyte desquamation and provide insight into its clinical implications in skin disorders including xerosis.

## BLOOD VESSEL REACTIVITY

Blood flow in the skin has been investigated to evaluate skin physiology, irritation, cutaneous pathology, dermatologic treatments, drug delivery, and wound healing. Early on, cutaneous blood flow was evaluated by visual assessment of the degree of erythema or pallor. However, newer techniques have been implemented to measure cutaneous microcirculation by

**Table 43.3** Corneocyte Variability

Study	Technique	No. of Subjects	Site	Results
Corcuff et al. 1991	In vivo	Black (mean age $33.5 \pm 7.5y$ ) Caucasian (mean age $31 \pm 7y$ ) Asian (mean age $26.5 \pm 7.5y$ ) (18–25 subjects per group)	Upper outer arm	No difference in corneocyte surface area spontaneous desquamation (corneocyte count) blacks $2.5\times >$ Caucasians & Asians ( $p < 0.001$ )
Warrier et al. 1996	In vivo	Black women 30 White women 30 (ages 18–45y, all)	Left and right medial cheeks Mid-volar forearms Lateral mid-lower legs	Desquamation index blacks $<$ whites on cheeks (18% less) and forearms (20% less) [ $p < 0.05$ ]; but no significant differences on the legs
Manuskiatti et al. 1998	In vivo	Black women 7 White women 5 (mean age $25.8 \pm 4.2y$ , both) Black women 5 White women 5 (mean age $64.7 \pm 3.8y$ , both)	Preauricle, posterior neck, dorsal upper arm, dorsal forearm, volar forearm, lower back, abdomen, thigh, lower leg	No difference in desquamation index between blacks and whites except at preauricular area ( $p = 0.02$ ) [which race greater not specified]
Fotouh et al. 2008	In vivo	Sub-Saharan Africans or Caribbean black women 25 (mean age $24 \pm 4y$ ) African or Caribbean mixed-race women 25 (mean age $24 \pm 7y$ ) European Caucasian women 25 (mean age $12 \pm 4y$ )	Forehead and volar forearm	No significant differences in the thickness of spontaneous desquamation of the corneocytes, desquamation index, between the ethnic groups
Gunathilake et al. 2009	In vivo	Skin Type I–II: Germany 110 San Francisco 14 Skin type IV–V: Sri Lanka 129 San Francisco 10	Volar forearm Dorsal hand	Skin type IV–V exhibited significantly thicker cornified envelopes and retention of corneodesmosomes in upper layer of SC compared to skin types I–II ( $p < 0.0006$ )
Muizzuddin et al. 2010	In vivo	African American women skin type IV–VI 73 ( $35.1 \pm 7.5y$ ) Caucasian women skin type II–III 119 ( $36.0 \pm 6.0y$ ) East Asian women skin type III–IV 149 ( $30.2 \pm 5.8y$ )	Left facial cheek	African Americans had the highest maturation index value, indicative of terminal differentiation, compared to the other groups ( $p < 0.001$ ) followed by Caucasians, then Asians
Yamashita et al. 2012	In vivo	Japanese Asian 43 ( $41.1 \pm 12.8y$ ) French Caucasian 104 ( $40.4 \pm 14.4y$ )	Cheek Dorsal hand Upper arm	Japanese subjects had a statistically significant increased size of corneocytes on the cheek only compared to French Caucasians ( $p < 0.01$ )

objective metrics. The techniques utilized by the presented studies include laser Doppler velocimetry (LDV) and photoplethysmography (PPG).

LDV is noninvasive and measures the flow of red blood cells based on the Doppler frequency shift in the laser light reflected from the blood cells in motion. The shift outputs a flow measurement that is related to the number of erythrocytes multiplied by their velocity in the skin microvasculature. PPG is another noninvasive method that measures blood flow in the cutaneous microcirculation. PPG utilizes a transducer to emit infrared light that is absorbed by the hemoglobin in the skin blood vessels. A photodetector detects and records the backscattered radiation that is dependent on the amount of hemoglobin in the skin. LDV and PPG have been utilized for studies on skin physiology, dermatological disorders, and systemic diseases.

Guy et al. investigated the response of cutaneous blood vessels to a topical vasodilator, methyl nicotinate, with LDV and PPG in six black and six white subjects. The investigators found no significant differences between the ethnic groups with respect to multiple measurements including area under the response-time curve and time to 75% delay of maximum response. Though the authors did note lowered PPG maximal responses in blacks, their overall conclusion was that the blood vessel reactivity between ethnic groups was similar (31).

Berardesca and Maibach compared the differences in blood vessel reactivity pre and post exposure to topical 0.5% and 2.0% SLS between ethnic groups in two different studies: one study between blacks and whites and the other between Hispanics and whites. Both studies did not reveal a significant difference in blood vessel response between either groups before and after application of SLS (5,12). However, Berardesca and Maibach utilized LDV to examine differences in blood vessel reactivity between blacks and whites to topical corticosteroid, a vasoconstrictor, instead of to topical SLS. The authors found that black subjects showed a 40% decreased area under the curve response, 50% decreased peak response, and a

decreased slope of decay after maximum blood flow compared to whites. The authors concluded that black subjects exhibited a decreased blood vessel reactivity compared to whites in response to topical corticosteroids (32). Gean et al. observed conflicting results in their study as they studied blood vessel reactivity by LDV in response to a vasodilator (methyl nicotinate) between blacks, Caucasians, and Asians. The investigators found a statistically significant increased area under the curve response in blacks and Asians compared to Caucasians (33).

Kompaore et al. evaluated lag time to vasodilation with LDV before and after tape strippings to remove the stratum corneum in black and Caucasian subjects. The investigators found an increase in lag time in vasodilation in blacks before tape stripping but in response to methyl nicotinate. After 8 and 12 tape strips, the lag time to vasodilation decreased in all three ethnic groups but a statistically significant decrease in lag time was specifically observed in Asians. The authors were unable to explain the reason behind the ethnic differences in lag time to vasodilation (2).

Aramaki et al. did not observe blood vessel reactivity measured by LDV in response to SLS-induced irritation between Japanese and German women (13). Hicks et al. also did not find statistically significant differences in LDV-measured blood vessel response to 1% and 4% SLS between black and white skin (17).

Overall, the evidence regarding blood vessel reactivity and its differences in ethnic groups is limited and contradictory (Table 43.4). Studies have reported both increased and decreased statistically significant differences in blood vessel response between ethnic groups. Many studies also did not observe blood vessel reactivity differences among ethnic groups. One limitation in the comparison of the studies is the differences in the topical substance used in each study (SLS vs. corticosteroid vs. methyl nicotinate). Further studies are needed to clearly elucidate the differences, as the results could explain disparities seen in irritation and dermatotoxicology/pharmacology among ethnic groups.

**Table 43.4 Blood Vessel Reactivity**

Study	Technique	No. of Subjects	Site	Results
Guy et al. 1985	Topically administered MN (vasodilator); LDV and PPG	Blacks 6 (age 20–30y) Whites 6 (age 20–30y) Whites 6 (age 63–80y)	Volar forearm	MN given: No significant difference in time to peak response, area under response-time curve, or time for response to decay to 75% of its max value PPG max response young black (40% less) < young White ( $p < 0.05$ )
Berardesca and Maibach 1988	In vivo – topical application of SLS (irritant); LDV	Black men 10 (age $29.9 \pm 7.2y$ ) White men 9 (age $30.6 \pm 8.8y$ )	Back	SLS stress: No significant difference between blacks and whites Blood vessel reactivity minimal in blacks from baseline to application of 0.5% SLS on untreated skin
Berardesca and Maibach 1988	In vivo – topical application of SLS (irritant); LDV	Hispanic men 7 (age $27.8 \pm 4.5y$ ) White men 9 (age $30.6 \pm 8.8y$ )	Upper back	SLS stress: Similar LDV response in Hispanics and whites

(continued)



**Table 43.4** Blood Vessel Reactivity (*continued*)

Study	Technique	No. of Subjects	Site	Results
Berardesca and Maibach 1989	Topically administered corticoid (vasoconstrictor); LDV	Black men 6 Caucasian men 8 (mean age 27 ± 3y, both)	Forearm	After vasoconstrictor given: 40% decreased area under the curve response blacks compared with whites ( $p < 0.04$ ) 50% decreased peak response in blacks compared with whites ( $p < 0.01$ ) Decreased decay slope after peak blood flow in blacks compared with Caucasians Less blood vessel reactivity noted in blacks
Gean et al. 1989	Topically administered MN (vasodilator); LDV	Blacks 5 Caucasians 5 Asians 5 (age 20–35y, all)	Upper 1/3 volar forearm	MN given: Area under the curve for LDV response versus time blacks > Caucasians for all MN concentrations ( $p < 0.05$ ) Area under the curve for LDV response versus time Asians > Caucasians for higher dose levels of MN ( $p < 0.05$ )
Kompaore et al. 1993	In vivo – topical application of MN – vasodilator; LDV	Blacks 7 Caucasians 8 Asians 6 (age 23–32y, all)	Volar forearm	MN given: Before tape stripping: no difference between the groups in basal perfusion flow, but lag time before vasodilatation was blacks > Caucasians > Asians ( $p < 0.05$ ) After 8 and 12 tape strips: lag time before vasodilatation decreased in all three groups, but significantly decreased in Asians > Caucasians > blacks ( $p < 0.05$ )
Aramaki et al. 2002	In vivo – topical application of SLS (irritant); LDV	Japanese women 22 (mean age 25.84y) German women 22 (mean age 26.94y)	Forearm	No significant difference at baseline or after SLS stress
Hicks et al. 2003	Topically administered SLS (irritant); LDV	White 8 Blacks 6 (all subjects in the age range 18–40y)	Volar forearm	SLS stress: No significant differences in LDV response between groups

## ELASTIC RECOVERY/EXTENSIBILITY

Berardesca et al. investigated elastic recovery and skin extensibility on the dorsal and volar forearm in blacks, whites, and Hispanics. The authors measured the biomechanical properties by applying torque parallel to the skin and subsequently measuring the ability of the skin to stretch (skin extensibility) and the time necessary for the skin to recover to its original state after releasing the torque (elastic recovery). They found that blacks had a 26% decrease in elastic recovery compared to whites on the volar forearm, but an increase in elastic recovery in blacks, though not significant, on the dorsal forearm. Furthermore, there were no differences observed in elastic recovery between whites and Hispanics on either site. The authors suggested that the superior recovery time on the dorsal forearm was related to the greater sun damage to the skin experienced by white skin and that black skin exhibited greater photoprotection with the presence of increased melanin (4).

The same study reported significant differences in skin extensibility between anatomical sites (dorsal < volar forearm) in Hispanics and whites, but not in blacks. With regard to race, blacks did exhibit greater extensibility than whites on the dorsal forearm but less extensibility than whites on the volar forearm. The authors concluded that the differences between anatomic sites for Hispanics and whites, but not blacks, correlated to the fact that blacks had superior photoprotection secondary to melanin and thus were less susceptible to changes in skin elasticity due to sun exposure. This reasoning also was applied to the finding of greater extensibility of blacks compared to whites on the dorsal forearm. However, this theory could not explain the increased extensibility of whites compared to blacks on the volar forearm (4).

Warrier et al. solely looked at elastic recovery in a larger sample of patients (30 in each group) between black and white women. They found that blacks had a statistically significant

greater increase in elastic recovery compared to whites on the cheeks. The investigators did not find a significant difference in elastic recovery between the groups on the legs (22).

The reported studies examining the biomechanical properties, such as elastic recovery and extensibility, between ethnic groups are contradictory (Table 43.5). These properties are dependent on the anatomic site and confound the data attempting to observe differences in ethnic group. Age may also play a factor in conflicting results, as Warrier et al. had a larger age range (18–45 years) while Berardesca enrolled subjects within a small age range (mean age was 46.7–49.8 years) (4,22). Therefore, based on the available studies, the data on effect of ethnic skin on elastic recovery or skin extensibility remains inconclusive.

### pH GRADIENT

Differences in pH between races and skin pigmentation are a continuing subject of investigation. Earlier, Berardesca et al. did not find differences in baseline cutaneous pH values between black and Caucasian women. However, the investigators did report that the pH significantly decreased in blacks after three tape strips (but not after 9, 12, and 15 tape strips indicative of the deeper layers) compared to Caucasians. The authors found the results difficult to interpret but suggested that the greater increase in TEWL observed in blacks after three tape strips could allow for an increase in hydrogen ion concentration.

Warriar et al. examined pH at baseline between black and white females but without the use of tape stripping. Blacks had a significantly lower pH than whites on the cheeks (22). The authors reported a difference on the legs as well between the groups, but the difference was not statistically significant. The authors believed the decreased pH observed on the cheeks for blacks to be related to sebum production and the potential notion that blacks have a higher amount of sweat glands (22). Luther et al. found no statistically significant differences of cutaneous pH on the volar forearm of Caucasians, Africans, and Asians. The study involved a smaller number of patients as the authors included six patients per ethnic group (7).

Recently, Gunathilake et al. investigated pH at the skin surface and melanocytes with respect to skin pigment type instead of race alone in a large number of patients. The subjects were divided into two skin type groups, skin types I/II ( $n = 124$ ) and IV/V ( $n = 139$ ), and by three geographical locations: Germany, Sri Lanka, and the United States (San Francisco). The investigators found that skin types IV/V had significantly lower pH values on the skin surface than skin types I/II. Furthermore, skin types IV/V exhibited lower pH values of the cell bodies and dendrites of melanocytes than skin types I/II. The authors concluded that pH played a significant role in pigment type differences in epidermal structure and function. Specifically, they attributed the enhanced epidermal barrier function and recovery in skin types IV/V, also reported in the same study, to the lower pH values. Furthermore, the investigators found that the topical application of two poly-hydroxyl acids, to lower the pH of skin type I/II to that of the darker pigment skin types, resulted in accelerated epidermal barrier recovery and kinetics in the lighter skin types (19).

Earlier studies found limited differences in pH levels between ethnic groups (Table 43.6). However, the studies were smaller compared to the larger trial conducted by Gunathilake et al. The differences in pH between ethnic groups or skin types should be further investigated because of its possible affect on the epidermal barrier function and recovery. These differences could explain a person's propensity to develop cutaneous infections or eczematous dermatoses.

### LIPID CONTENT

Racial differences in lipid content in the skin have been increasingly studied. Initially, Reinerston and Wheatley utilized skin from black and white cadavers and found an increase in epidermal lipid and sterol content in blacks compared to whites. In contrast, Sugino et al. investigated ceramide levels between blacks, whites, Hispanics, and Asians. They found that blacks had 50% lower ceramides, which was statistically significant, compared to whites and Hispanics (34). Harding et al. studied stratum corneum lipid content of the scalp in UK and Thai subjects who had dandruff. The investigators reported similar levels of scalp lipids in both groups (35).

**Table 43.5** Elastic Recovery/Extensibility

Study	Technique	No. of subjects	Site	Results
Berardesca et al. 1991	In vivo	Blacks 15 (mean age $46.7 \pm 2.4y$ ) Whites 12 (mean age $49.8 \pm 2y$ ) Hispanics 12 (mean age $48.8 \pm 2y$ )	Volar and dorsal forearm	No significant difference between races on dorsal forearm Significant dorsal < volar extensibility within whites and Hispanics ( $p < 0.0001$ and $p < 0.0002$ , respectively) black > white extensibility dorsal forearm ( $p < 0.01$ ) black < white extensibility volar forearm ( $p < 0.01$ )
Warriar et al. 1996	In vivo	Black women 30 White women 30 (ages 18–45y, all)	Left and right medial cheeks Mid-volar forearms Lateral mid-lower legs	No significant difference between races on the legs Elastic recovery blacks $1.5\times$ > whites on cheeks ( $p < 0.05$ )

**Table 43.6** pH Gradient

Study	Technique	No. of subjects	Site	Results
Warrier et al. 1996	In vivo	Black women 30 White women 30 (ages 18–45y, all)	Left and right medial cheeks Mid-volar forearms Lateral mid-lower legs	pH blacks (pH = 5.15) < whites (pH = 5.52) on cheeks at baseline ( $p < 0.05$ ) No significant difference in pH on the legs at baseline
Berardesca et al. 1998	In vivo	Black women 8 Caucasian women 10 (mean age $42.3 \pm 5y$ , both)	Mid-volar forearm	No significant difference in pH at baseline After tape stripping: pH significantly decreased in blacks after three tape strips, i.e. superficial SC layers No differences between races after 9, 12, and 15 tape strips, i.e. deeper SC layers
Grimes et al. 2004	In vivo	African American 18 White 19 (ages 35–65 yr, women, all)	Inner forearm	Baseline: African Americans < whites, but not statistically significant
Gunathilake et al. 2009	In vivo	Skin Type I–II: Germany 110 San Francisco 14 Skin type IV–V: Sri Lanka 129 San Francisco 10	Volar forearm Dorsal hand	Skin type IV–V had significantly lower/acidic surface pH values compared to skin type I–II ( $p < 0.0001$ ) Skin types IV–V had a lower pH value of the cell bodies and dendrites of melanocytes than skin type I–II ( $p < 0.05$ )
Luther et al. 2012	In vivo	Caucasian males 6 – skin type II–III ( $28.1 \pm 4.3y$ ) African males 6 – skin type V–VI ( $27.3 \pm 3.2y$ ) Asian males 6 – skin type IV ( $23.5 \pm 1.6y$ )	Volar forearm	No statistically significant differences in cutaneous pH between the three ethnic groups

Fotouh et al. studied sebum quantity and measured the lipidic index in Caribbean, Caribbean mixed-race, and European Caucasian women (6). The authors did not see a significant difference in these metrics between the three groups. Luther et al. also did not reveal any ethnic differences of sebum content between Caucasian, African, and Asian males, though their sample size was fairly small (total  $n = 18$ ) (7).

However, recently, studies have pointed to a difference in ceramide and lipid composition of the epidermis between racial groups. Gunathilake et al. found that darker skin pigment types IV/V exhibited increased density of lamellar bodies in the stratum corneum and increased epidermal lipid content compared to skin types I/II. The authors attributed these results to the explanation of the superior barrier function and recovery the authors also discovered in the same study (19). Muizzuddin et al. found that African Americans had significantly fewer lipid ceramides, specifically C18 phytosphingosine-based ceramides, than Caucasians and

Asians. The authors only quantified C19 phytosphingosine bases but suggested that these levels were representative of the total amount of ceramides in the epidermis. The investigators concluded that the lower ceramide levels could be characteristic of dry and scaly skin representing possible impaired water retention (8). Jungersted et al. specifically measured the ceramide/cholesterol ratio in African, Asian, and Danish subjects. They found that Africans had a statistically decreased ceramide:cholesterol ratio and the findings correlated with the global incidence of atopic dermatitis found in these groups (36).

The data regarding lipid content in the skin remains inconclusive as studies arrived at different conclusions (Table 43.7). However, larger recent studies have indicated possible differences in lipid content that can reveal information about the development of pathological disorders between ethnicities. Further studies are needed to validate these conclusions.



**Table 43.7** Lipid Content

Study	Technique	No. of subjects	Site	Results
Reinertson and Wheatley 1959	In vivo	Cadavers: Black man 1 White man 3 Living: Black man 1 White man 1 (age 49–68, all)	Cadavers: Abdomen Living: Back and thigh	Lipid and sterol content in total epidermis blacks > whites
Sugino et al. 1993	In vivo	Blacks, Caucasians, Hispanics, Asians (no. of subjects, ages not specified)	Not documented	Ceramide levels blacks (50% less) < whites and Hispanics ( $p < 0.05$ )
Harding et al. 2002	In vivo	UK 41 Thai (dry season) 31 Thai (humid season) 31 (age 20–40y, all)	Scalp	UK and Thai subjects demonstrated similar levels of total lipids
Fotoh et al. 2008	In vivo	Sub-Saharan Africans or Carribean black women 25 (mean age $24 \pm 4y$ ) African or Caribbean mixed-race women 25 (mean age $24 \pm 7y$ ) European Caucasian women 25 (mean age $12 \pm 4y$ )	Forehead and volar forearm	No significant differences in the lipidic index and sebum quantity between the ethnic groups
Gunathilake et al. 2009	In vivo	Skin Type I–II: San Francisco 14 Skin type IV–V: San Francisco 10	Volar forearm Dorsal hand	Skin type IV–V exhibited increased density of lamellar bodies in the stratum granulosum and increased epidermal lipid content compared to skin types I–II
Muizzuddin et al. 2010	In vivo	African American women skin type IV–VI 73 ( $35.1 \pm 7.5y$ ) Caucasian women skin type II–III 119 ( $36.0 \pm 6.0y$ ) East Asian women skin type III–IV 149 ( $30.2 \pm 5.8y$ )	Left facial cheek	African Americans had significantly fewer ceramides (C18 phytosphingosine based ceramides) than Caucasians and Asians ( $p < 0.001$ )
Jungersted et al. 2010	In vivo	African 18 (median age 27y, range 22–34y) Asians 25 (median age 24y, range 20–39y) Danish 28 (median age 25y, range 20–38y)	Volar forearm	African subjects had the statistically significant lowest ceramide/cholesterol ratio compared to both Asian and Danish subjects ( $p < 0.001$ ). Asian subjects had the highest ratio
Luther et al. 2012	In vivo	Caucasian males 6 – skin type II–III ( $28.1 \pm 4.3y$ ) African males 6 – skin type V–VI ( $27.3 \pm 3.2y$ ) Asian males 6 – skin type IV ( $23.5 \pm 1.6y$ )	Volar forearm	No statistically significant differences in sebum content between the three ethnic groups

## ANTIOXIDANT STATUS

The antioxidant system in the skin is important in neutralizing free radicals generated by oxidative stress that can be a result of UV radiation or other stressors. The free radicals can be cytotoxic and have many consequences, including impaired barrier function or development of cutaneous carcinoma (37). The presence of the photoprotective melanin has always been thought to be the main reason for the lowered incidence of skin cancers in ethnic skin compared to Caucasian skin. However, differences in antioxidant levels may also play a critical role.

Recent studies have investigated the potential ethnic differences in antioxidant activity within the skin. Yamashita et al. measured the levels of the antioxidant catalase in 43 Japanese Americans and 104 French Caucasians. Japanese subjects had statistically significant higher catalase activity than French subjects. The authors correlated the increased catalase activity with the superior hydration and barrier function in Japanese skin also reported in the same study (10). Luther et al. were unable to find a statistically significant difference in cutaneous levels of antioxidants such as  $\beta$ -carotene and lycopene between Caucasians, Africans, and Asians. The sample size in this study was limited, as 18 subjects were enrolled in the study.

Antioxidant activity between ethnic groups has only recently been investigated and reported, but the clinical implications can be great (Table 43.8). The detailed classification of differences in antioxidant capacity between ethnic skins can reveal information about ethnic variations in dermatological disorders as well as incidences of cutaneous carcinoma and should be further investigated.

## FACIAL PORE SIZE

Sugiyama et al. investigated the differences in pore sizes between Caucasian, African, and Hispanic females (Table 43.9). The investigators found that Asians had the smallest pore areas on the cheek compared to the other groups. African Americans had the most severe impairment of architecture around the facial pores. The authors suggested that impairment of

surrounding epidermal architecture affected pore size in each ethnic group and ultimately affected the visual appearance of facial pores (38).

## EPIDERMAL INNERVATION

Reilly et al. analyzed the racial differences of skin innervation and nociceptor activity in response to irritancy. The investigators did not find any significant differences in epidermal innervation between Caucasian, Japanese, and Chinese subjects in response to capsaicin (39).

## CONCLUSION

Ethnic variations in skin properties have currently not been adequately defined. Different studies analyzing properties in similar ethnic groups often report conflicting results from the previous literature. Also, only a limited number of studies have been conducted with an appropriate sample size and controls for confounding factors to adequately elicit valid conclusions. A significant proportion of the studies only look at black and white skin and do not incorporate other racial groups such as Asian, Hispanic, Indian, Mediterranean. One obstacle in analyzing the studies is the classification of race or ethnicity. Anthropologists segregate racial groups based on the notion that racial variations underwent a natural selection process resulting in changes in genetics to help each group to adapt to their particular environments. Race can also be defined subjectively based on one's own perception of oneself and their presumed ancestry. The ambiguity of these subjective labels can obscure the interpretation of these studies in defining true variations inherent in skin structure and physiology. Future studies into genetics would assist in providing insight on the published data and help draw conclusions. Among many factors, future research studies should involve larger numbers of subjects, investigate the differences by Fitzpatrick skin types, control for geography/climate, incorporate skin disease status, and document skin care use of the subject. Currently, there is enough data to suggest that there are inherent ethnic

**Table 43.8** Antioxidant Status

Study	No. of subjects	Site	Results
Luther et al. 2012	Caucasian males 6 – skin type II–III ( $28.1 \pm 4.3y$ ) African males 6 – skin type V–VI ( $27.3 \pm 3.2y$ ) Asian males 6 – skin type IV ( $23.5 \pm 1.6y$ )	Volar forearm	No statistically significant differences in skin levels of antioxidants, $\beta$ -carotene and lycopene, between the three ethnic groups
Yamashita et al. 2012	Japanese Asian 43 ( $41.1 \pm 12.8y$ ) French Caucasian 104 ( $40.4 \pm 14.4y$ )	Cheek Dorsal hand Upper arm	Japanese subjects had a statistically significant higher catalase activity than French subjects ( $p < 0.01$ ) Japanese subjects had significantly lower values of protein carbonylation of the stratum corneum than French subjects ( $p < 0.05$ )

**Table 43.9** Facial Pore Size

Study	In vivo	Subjects	Site	Results
Sugiyama-Nakagiri et al. 2009		Caucasian females 20 African females 20 Asian females 20 Hispanic females 20 All subjects in the age range (20–39y)	Cheek	Asians had smallest pore areas than other ethnic groups African Americans had the most severe impairment of architecture around facial pores

differences in critical skin properties that affect the function of the skin. Describing and detailing these differences will lead to a better understanding of dermatological diseases in ethnic groups and ultimately to more effective treatment options.

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# Changes in Female Hair with Aging: New Understanding and Measures

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## INTRODUCTION

Hair is among the most defining aspects of human appearance and often a major initial driver of perceptions of youth and beauty. Ubiquitous hair-related changes in women associated with aging are often distressing and met with feelings of anxiety or depression which can progress to a lost sense of identity (1–5). Women with alopecia not only face the loss of their hair, but often feel isolated, embarrassed to seek care, and may be frustrated by misinformation, misdiagnosis, or poor treatment options.

In both men and women there is an increasing incidence of hair loss with advancing age (6–12). Women may uniquely encounter various hormonal and physiologic changes that can lead to alterations in the appearance, quality, and quantity of the hair fiber and hair follicle or pilo-sebaceous unit. Androgens, menopausal-related hormonal changes, and age-related senescent signals are all biological changes that can lead to significant alterations in the female pilo-sebaceous unit. Although these changes are complex, our ability to measure changes in hair parameters has improved dramatically. With these improved measures and a better understanding of the complex molecular signals governing hair biology, there is a greater opportunity to develop pharmacologic and/or cosmetic treatments that may specifically benefit women.

## CLINICAL MANIFESTATIONS Female Pattern Hair Loss

Female pattern hair loss (FPHL) is considered the equivalent of male pattern baldness or androgenetic alopecia (Figure 44.1A) (13). There is a reported prevalence of approximately 50% in Caucasians (14), and a much lower prevalence in Asians (15), Native Americans, and African-Americans (16). In both men and women, thinning typically begins in the teens, twenties, and thirties as a result of androgen-mediated follicular miniaturization (14,17), although the linkage to androgens is less clear in females than males (18). However, there is increased recognition that there may be non-androgen causes of hair thinning in women that have no counterpart in men (19). Thus, the term female pattern hair loss has been favored to encompass the clinical phenotype of hair loss in the central and temporal scalp region that may occur in genetically predisposed women resulting from androgens as well as hormonal changes due to menopause and potentially other metabolic or extrinsic factors (20,21). Women with FPHL usually first notice a gradual thinning of their hair, mostly on the top of their heads, and their scalp becomes more visible (11). Over time, the hair on the sides may also become thinner. The patient may notice that her “ponytail” is smaller or that her longer hair looks “skimpy” at the ends. This

thinning of scalp hair can vary in extent but it is extremely rare for a woman to become bare on top. With more extensive thinning there may be a “Christmas tree” pattern of thinning on top of the scalp with hair loss most notable behind the frontal hair-line (Figure 44.1B) (22). Miniaturized hairs are characteristic (23).

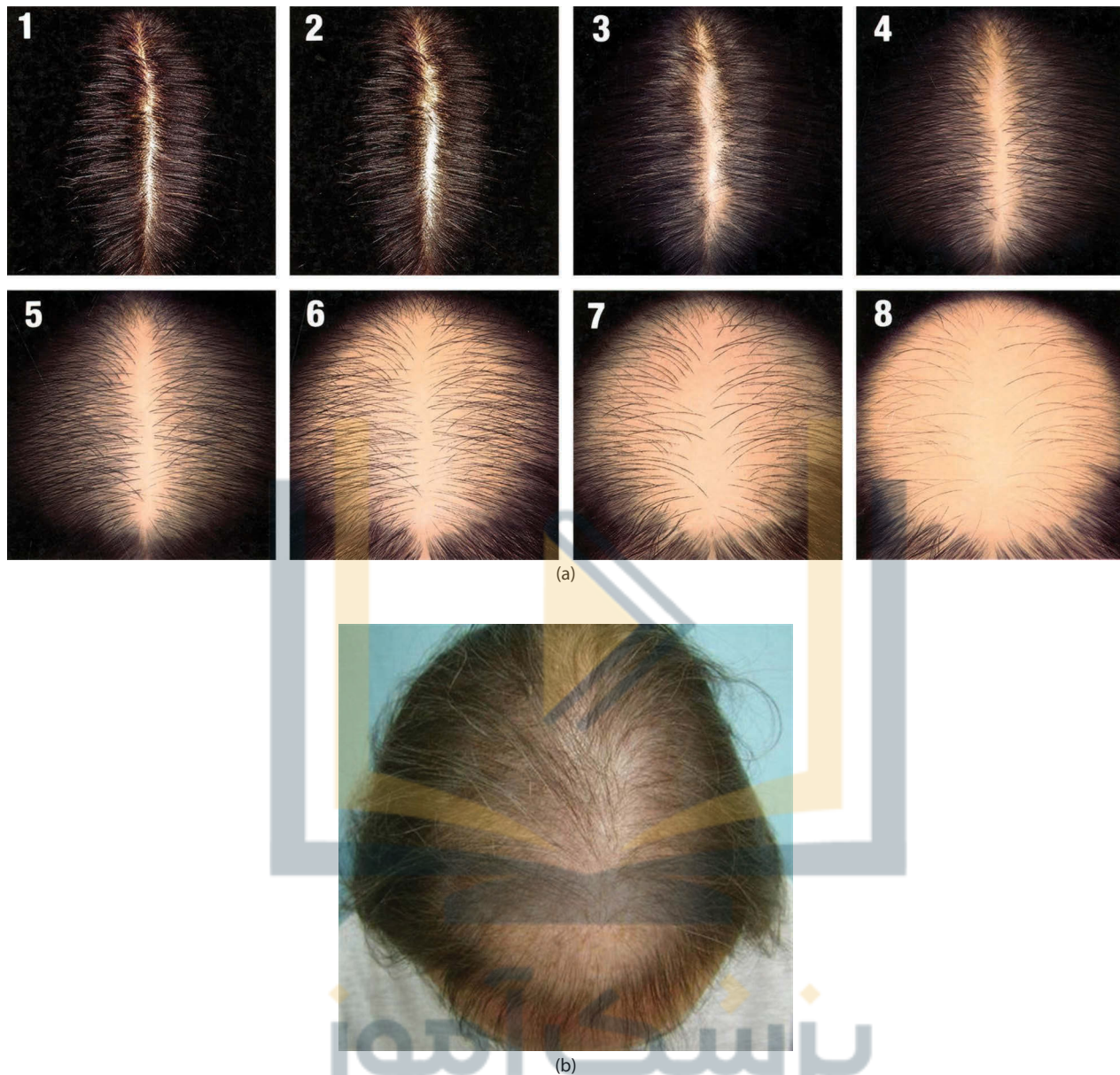
## Senescent or Chronogenetic Alopecia

Senescent alopecia (SA) is defined as hair thinning that does not become apparent until after approximately 50 years of age and in a person with no family history of androgenetic alopecia (8,24,25). Alternate terms include “chronogenetic,” “late onset,” or “age-related” hair thinning. Such hair thinning is often identified as a marker of systemic senescence. In support of this concept, it is observed that patients with progeria, who have genetically programmed premature senescence, show a phenotype of hair loss (26). In reality, senescent alopecia likely frequently coexists with androgenetic alopecia or FPHL. Clinically, the hair thinning in senescent alopecia is described as being diffuse when seen in its “pure” form but having both a diffuse and patterned thinning when seen in combination with androgenetic alopecia (25,27). Clinically and histologically, there is follicular downsizing or miniaturization and subsequently the hair shaft becomes narrower (28). Changes in hair density have also been associated with aging in women with and without perceived hair loss (29). Interestingly, imaging data supports that women who self-report having thin or thinning hair have significantly less hair and a faster rate of hair loss with age (29).

## Telogen Effluvium/Chronic Telogen Effluvium

Normally, the majority of scalp hair is in the anagen or growth phase, with a small percentage of hairs in the resting or telogen phase, normally being shed at a low daily rate (100–200 hairs shed daily) (30). Under certain circumstances a higher percentage of hairs cycle into the resting phase, and a sudden onset shedding, or telogen effluvium (TE), may be noted. Postpartum TE is a commonly observed clinical phenomenon that highlights the impact hormonal changes can have on the hair cycle (31). With age, both the number of hairs in anagen and the duration of anagen may be decreased, thus leading to an alteration and increase in the number of hairs shed daily (29).

Chronic TE is another condition that results in diffuse scalp hair loss and is typically seen in women in their fourth to sixth decades of life (32). It often presents with an abrupt hair shedding that can have a fluctuating course lasting at least 6 months and may continue for 6–7 years (32). Although chronic TE is usually self-limiting, the volume of hair typically does not return to premorbid volume and may be a comorbid manifestation of FPHL or SA.



**Figure 44.1** Female pattern hair loss. (a) Savin patterns of hair loss (13). (b) Clinical perspective showing diffuse hair loss with preserved frontal hairline. (From Olsen EA, *J Am Acad Dermatol*; 40(1):106–9, 1999.)

### Hair Shaft Changes

The hair shaft is made primarily of keratin bundles that are compacted together and surrounded with an outer cuticular layer to form a rope-like structure that is flexible yet strong. Sulfur crosslinks within and between the keratin fibrils provide for the longitudinal strength of the hair. The outer cuticle resembles overlapping shingles on a roof and forms the “armor” that protects the underlying hair shaft and provides “torsional” strength and resistance to bending (33). The quality and caliber of the hair shaft decreases with age and can significantly affect how the hair is perceived (34). Studies suggest that with age there is increased damage to the fragile substructure (endocuticle) of the hair shaft cuticle leading to increased fragility of the hair. As many hair care products interact negatively with the hair surface cuticle, various hair care practices that include chemicals and heat can also diminish the integrity of the hair shaft (35). Damage or loss

of the outer cuticle or “weathering” can lead to alterations in the optical properties of the hair (loss of luster) (36), decreased manageability (“flyaway hairs” and hair tangling), or hair breakage (37). If breakage occurs, it can happen anywhere along the length of the hair, causing a “shaggy” or “skimpy” appearance and visible and tactilely perceptible blunt (broken) hair tips.

Ultraviolet light can lead to oxidative changes of the hair shaft, resulting in damage to the hair color (photobleaching) as well as weathering of the hair structure through changes including lipid oxidation, disulfide bond cleavage, tryptophan degradation, protein degradation, and cysteine acid formation (38–40). The result is an increase in fiber porosity, increase in surface roughness, and decreased mechanical strength. With age and greying, the absence of the natural protective pigments in the hair leads to increased ultraviolet damage including loss of mechanical strength and an increase in surface roughness (34,38).



## Hair Greying

Hair greying is one of the most characteristic changes of aging. It is often quoted that, as a rule of thumb, 50% of people are 50% grey by the age of 50 (28). However, recent epidemiologic studies of men and women of various races suggests a far lower number—only 6%–23% of people have 50% grey hair at age 50 (34). Compared to people of Caucasian descent, those of Asian and African descent showed less grey hair (34). The age of onset of hair greying is thought to be influenced by genetics. Grey hairs have been noted to have different size (shaft diameter) and mechanical properties and are often perceived as dry and less manageable (41).

## MECHANISMS Androgens

Pathophysiologically, androgens mediate and drive the follicular transformation in androgenic alopecia. There is a substantial increase in the local, or follicular, transformation of testosterone to dihydrotestosterone by the enzyme 5- $\alpha$  reductase (12). Dihydrotestosterone, which has a five times higher affinity for the androgen receptor compared to testosterone, triggers specific genes that then lead to the gradual miniaturization of genetically programmed hair follicles (13).

The effects of androgens on the follicle have, for the most part, been studied in men under the age of 50. Hence, further work will be required to define the role of androgens in female hair loss. It has been suggested that the various clinical patterns of androgenetic alopecia in men and women may reflect quantitative differences in levels of androgen receptor and steroid-converting enzymes in specific scalp regions at different ages (42).

There is increased recognition that androgen excess can occur in patients with various conditions such as metabolic syndrome, polycystic ovary, and obesity and that these changes can lead to alterations in the hair follicle (43). Androgen excess may be a result of changes in sex binding hormones or due to an increase in peripheral conversion.

## Menopause/Estrogens

Menopause is defined as either the permanent cessation of menses or the lack of menses for 12 consecutive months (44), with the major hormonal changes during menopause being the near cessation of ovarian estrogen production. The definition and appreciation of perimenopause remains controversial, but recent reviews (45) suggest that hormonal changes begin much earlier than previously understood, resulting in symptoms such as night sweats, mid-sleep waking, and other physiological changes. Perimenopause, or the transition to menopause, spans a variable period of time when estrogen levels can be erratic before they decrease to the low, stable levels of menopause (45). This transitional phase occurs on average 5 years prior to the onset of actual menopause but can start as early as 10 years prior (46). While it has been recognized that estrogen is an important modulator of hair growth, the details of the molecular regulatory pathways have not been well characterized. Hair changes, including thinning of the hair shaft, temporally equate with the earlier onset “perimenopausal” period (29) which usually falls in the early 40s but may begin as early as 35 years of age. The mean age women undergo complete menopause is 51; thus women spend about one-third of their life in the postmenopausal period (44). However, if one includes perimenopause, where many of the reported negative symptoms begin to be reported, the period where a woman

lives with the symptoms of menopause may be as much as half of her life.

Estrogen is synthesized in the ovary as well as in a number of peripheral tissues and acts via estrogen receptors which belong to a superfamily of nuclear receptors. There are two estrogen receptors, alpha (ER alpha) and beta (ER beta). The relatively recent discovery of ER beta has broadened the range of potential estrogenic target tissues and has also redefined prior concepts of estrogen activity and signaling. In the human hair follicle, immunohistochemical studies have shown ER beta to be the predominant receptor (47,48). Similar to other estrogenic target tissues, the biologic activity of estrogen in the hair follicle likely depends on a complex interplay of signals that may differ depending on the relative distribution and location of the two ERs, as well as the activity of the peripheral converting enzyme, aromatase (49–51). Several studies have demonstrated the influence of estrogen on the murine and other mammalian hair cycle, however it is clear that the distribution, expression, and biologic activity of estrogen receptors in murine models may be quite different than in humans (51–57). In vitro studies have shown that organ culture of human scalp hair follicles exposed to estradiol results in decreased growth, whereas cells of the dermal papilla responded with proliferation (58,59). Estradiol has also been noted to induce aromatase activity in human scalp follicles, one possible mechanism by which it may exert biologic activity (60). Since hair growth is influenced by numerous hormones, growth factors, transcription factors, and cytokines, many of which are known to be modulated by estrogens, it is plausible that an intricate orchestration of these pathways occurs in response to estrogen. Further clarification and study of estrogen effects in different tissues, species and genders is ongoing (49,50,61).

## Senescent Signals

Although androgenetic alopecia and senescent alopecia share many clinical and histologic features, the mechanisms by which follicular downsizing and miniaturization occur have recently been shown to be distinct (62). Microarray comparison of age-matched subjects with androgenetic alopecia, senescent alopecia, and normal controls without hair loss has shown that androgenetic alopecia is associated with altered expression of genes known to be required for hair follicle cycling. In contrast, the transcriptional profile of senescent alopecia reveals changes in the complex phenomenon of alternative splicing, oxidative stress response, and apoptosis, which are characteristic of aging tissues (62). This difference in mechanism has significant implications in terms of treatment of hair loss at different ages. Further characterization of these senescent pathways may lead to attractive therapeutic targets for treatment of senescent alopecia, but may also prove to be useful markers of other systemic senescent processes.

## Pigmentation

Much like the skin, the pigment of the hair shaft is derived from melanocytes, which transfer melanin via melanosomes. However, unlike the skin, in which there is continual production of pigment, the activity of the melanocytes surrounding the hair follicle is intermittent and is tightly linked to follicular cycling. Hair pigmentation occurs only during the growth, or anagen, phase, which typically lasts 3–5 years. With each hair cycle, various factors may impact the fidelity of hair pigmentation, and these changes become most notable after the first 10 cycles or so (63). Hairs with absent pigment are seen as white, whereas those with a dilution, or a mixture of pigment



and white hair, are seen as grey. Interestingly, white hairs may have increased thickness and greater hair growth rate than pigmented hairs (64). Studies have shown that grey hair is associated with a decrease in follicular melanocyte population and a decrease in melanin content (65). A buildup of reactive oxygen species along with a decreased ability to handle oxidative damage has also been implicated in the process of greying (63,66,67).

## EFFICACY MEASURES OF HAIR REGROWTH IN HUMANS

### Density

#### Overview

A key weakness in hair research remains to reliably and predictively measure the effect of aging, hormone changes, or treatment on hair quantity and quality. Most methods require sampling from a small (1–5 cm<sup>2</sup>) site then extrapolating to the entire scalp. This can lead to significant error, as hairs are under differential control across the scalp (29,42). A further complication is the non-random, highly irregular pattern of scalp hairs, making repositioning crucially important and mandating a permanent relocation mark (tattoo) for consistent analyses (68). There are methods utilizing global photography, but they suffer from variability resulting from styling, color, and humidity, among others. Here we review common methods to measure changes in hair density and diameter, focusing on detecting subject perceptible changes in hair amount from any cause.

#### Direct Manual Hair Count

Early hair growth studies began in the 1980s and used the direct hair count method (69–78). Changes were determined by counting all vellus, intermediate, and terminal hairs in a 1-inch diameter (5.1 cm<sup>2</sup>) midvertex area. This was labor intensive and prone to systematic bias by missing small or minimally pigmented hairs. The primary benefit was that the hairs most noticeable by subjects were ones most reliably quantified.

#### Macrophotographic Manual Hair Count

In the 1990s, manual hair counting was updated by counting dot maps representing hairs present in enlarged color macro photographs (79–92,93). This involved clipping all hairs in a 1-inch diameter area (5.1 cm<sup>2</sup>) to 1 mm at the anterior edge of the thinning vertex, with a dot tattoo to maximize relocation. Photographs must be taken with a dedicated, preset camera system to maintain reproducibility. The photographs are enlarged to color transparencies and converted to dot maps of visible hairs. This revealed more small or nonpigmented hairs, and was a step forward in precision. However, it is reliant on labor-intensive human counting and subject to bias from the individual counter. Also, exact repositioning remained difficult, increasing the signal-to-noise ratio and adding variability.

## Automated or Semi-Automated Phototrichograms

#### Overview

Macro photographs improved hair count methodology but manual analysis remained cumbersome and labor intensive. Hence, digital image acquisition and computational analysis enabled more sensitive, precise, and time-saving methods using fully or semi-automated systems (68,94–96).

At baseline, imaging sites are tattooed and the hair clipped. A water drop is used to minimize light scattering due to scalp flakes or texture. This is essential to enable semi or fully automated counting of hairs via identification of straight edges. Water has a similar refractive index to hair and so minimizes the appearance of nonpigmented hairs. This has the disadvantage of underestimating miniaturized, vellus, or grey hairs, but the advantage of biasing to the pigmented, terminal hairs which are most likely to be subject noticeable. A baseline image is acquired as a reference, and at each subsequent visit the probe is repositioned to achieve alignment with the partially transparent baseline image (Figure 44.2). Anagen hair counts can be determined by counting hairs that lengthened in images taken 24 hours later. Image analysis algorithms can also be employed to measure hair diameter.

#### TrichoScan®

TrichoScan is an automated digital phototrichogram tool to analyze macro scalp images (97–99). It is reported to analyze multiple hair parameters including density (hairs/cm<sup>2</sup>), diameter (pixels converted to microns), growth rate (mm/day), and anagen:telogen ratio. TrichoScan combines epiluminescence microscopy (ELM) with automatic image analysis. Briefly, the site is identified, tattooed, and clipped as above. The clipped hair is dyed black to increase the hair–scalp contrast, complicating the analysis by counting grey hairs the same as pigmented hairs. For density and diameter determinations, the hairs are colored immediately after clipping. For growth rate and anagen:telogen ratio, the hairs are colored 3 days after clipping.

Automated algorithms select color components, reject artifacts (bubbles and reflections), determine the threshold, define the hairs, eliminate the tattoo, and analyze the hair from each region. This method is routinely used in clinical studies for assessing growth-promoting substances as well as to study diffuse hair loss in various hair-related disorders (97,99–101).

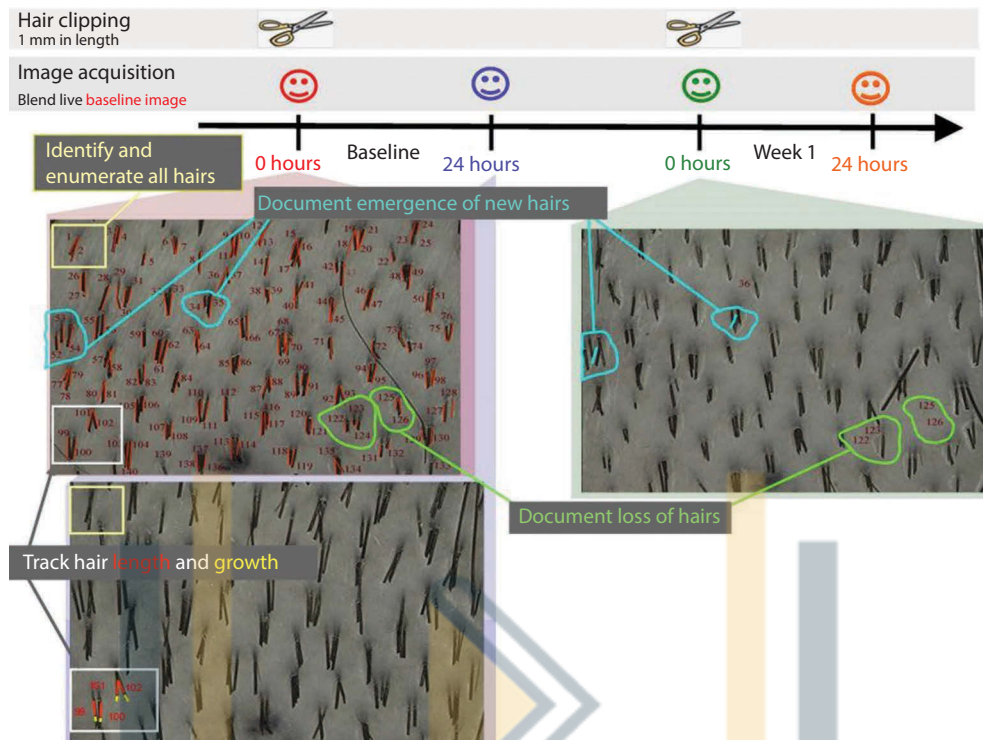
TrichoScan has been used extensively and has improved the speed of analysis, enabling use of more subjects and assisting in stronger, more powerful clinical designs. However, as with any new method, the precision and accuracy remain controversial. van Neste and Trueb compared TrichoScan to manual counting and found significant differences between the “gold standard” of manual counting and the computer-assisted analyses (102). Further work will be necessary to fully understand the positive and negative aspects of automated analysis. As technology improves, so will automated methods.

#### Canfield EpiLume®

Canfield EpiLume is a macro digital image capture system frequently paired with the Canfield Hair Metrix image analysis system or TrichoScan image analysis to measure changes in hair growth properties, including treatment effects (103). The photographic system leverages a fixed focal camera for imaging scalp sites prepared as above and then images are processed using a validated image analysis system (104)

## Hair Diameter

The perception of hair mass or volume (hair *amount*) is a function of both density and diameter (105), but careful consideration reveals that changes in hair diameter could be as or more influential than changes in hair density. For example, an 80-μm hair has almost twice the mass or volume of a 60-μm hair of the same length because it has approximately double the cross-sectional area. Therefore in two samples with the same number of hairs, the one with 80-μm shafts has almost twice the



**Figure 44.2** Macro-photographic image analysis. Optional imaging parameters available in manual or semi-automated systems.

hair *amount* as the one with 60- $\mu$ m shafts. To put this in context, a 10% drop in density will result in a 10% change in *amount*, while a 10% change in diameter will result in a 20% change in *amount*—twice the effect of the same change in density.

Hair diameter can be obtained from multiple methods, including linear density (106) phototrichogram (94,95), laser fiber analysis (Diastron®), and optical fiber diameter analysis (OFDA) (Figure 44.3) (107). As human hair is variable in diameter even across a single fiber, methods have been developed that assess either single points along the fiber or averages for the entire fiber. Further, the shape, or cross-sectional area, can be highly variable and account for multiple hair “feel” parameters. All of these factors must be considered when choosing an optimal method.

#### Linear Density

The linear density diameter is a calculation based on the number of fibers in a sample, the length of the sampled fibers, and an average density for human hair (106). This method provides an accurate average diameter but does not take into account variations in diameter along fibers or variations in fiber shape.

#### Phototrichogram

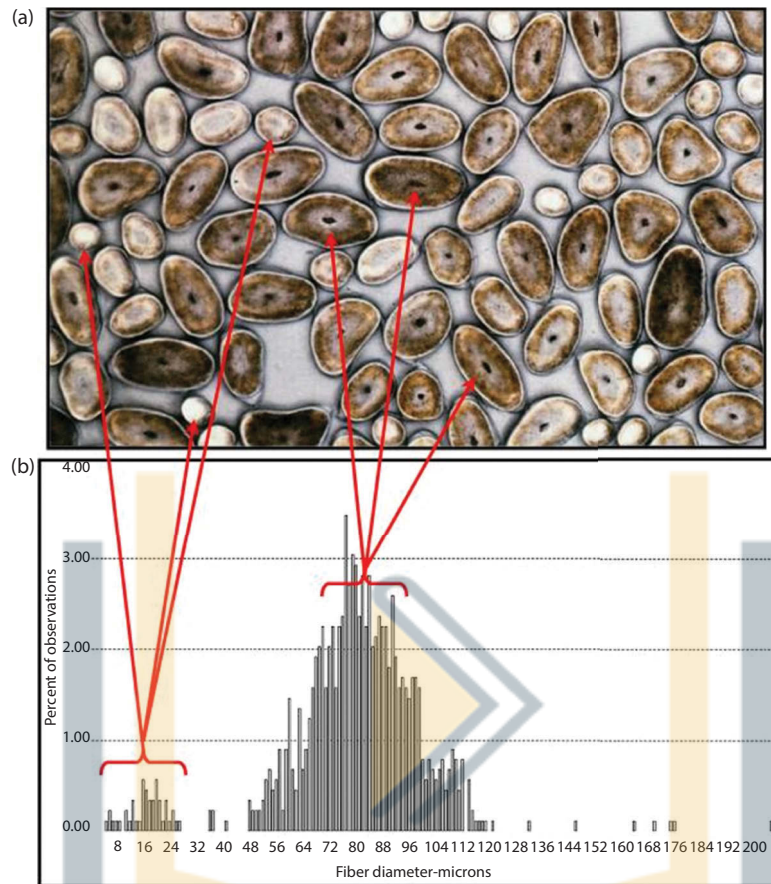
Current high resolution phototrichograms can be used to measure diameter (68). This method enumerates the fibers and counts the total hair area, then calculates the average diameter of each hair. This method is limited to average diameters as the resolution of current camera technology limits the size of a pixel in an image to approximately 1–4  $\mu$ m. This means many hairs must be averaged for accurate and meaningful results. This method also measures hair approximately 1 mm from the scalp and so is useful for measuring the effect of treatments, as newly grown hair is all that is contained in that short fragment.

#### Diastron

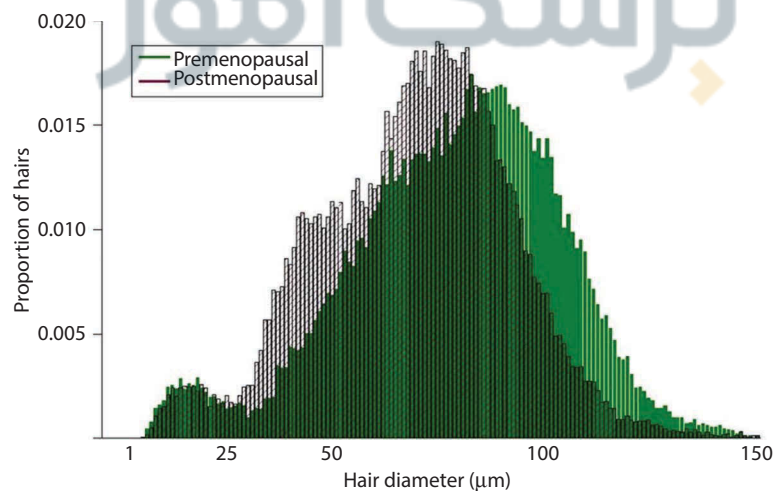
The most common method is the laser method using the Diastron device. Description of the full utility of this method is beyond the scope of this review, but ample information can be found on the Diastron company website. Briefly, single 4-inch hair fibers are mounted in a holder and rotated while in the beam of a laser. This is highly precise and provides detailed information on single fibers. In addition to average diameter, it provides fiber shape (ellipticity) and can be applied to multiple sites along the fiber. The limitation is the low throughput relative to the diversity of human scalp hair. It also requires 4 inches of hair, so about 8 months growth, necessitating extended clinical trials to measure hair changed during the study.

#### Optical Fiber Diameter Assessment

OFDA allows measurement of many hundreds to thousands of fibers per sample (107–109). Briefly, 2-mm snippets are cut from each sample, washed, equilibrated, and cast onto glass slides. The slides are read by an optical laser which finds the 2-mm axis and measures the perpendicular. As this technique allows for measurement of many fibers per sample, it is valuable for understanding effects of treatment across larger sites and the effect on the diameter distributions. As it measures short segments, it can be used to evaluate the effect of treatment of less than 1 month. Recently OFDA was used to measure differences in hair diameter in pre- and postmenopausal women, demonstrating its utility in human clinical studies (Figure 44.4) (29). The limitation is that variability is not determined along the fiber and no information is gleaned about fiber shape or ellipticity. The primary advantages are throughput and breadth of analysis (29).



**Figure 44.3** Optical fiber diameter analysis showing diversity of human hair shapes. (a) Bundle of hair fibers from Caucasian female embedded and cross-sectioned. (b) Same bundle of hair fibers examined by OFDA. Arrows represent different populations of hair diameters (108). (With kind permission from Springer Science and Business Media: Hair growth parameters in pre- and postmenopausal women. In M. R. Trüeb, MR and Tobin, JD, eds., *Aging Hair*, 2010, pp. 49–60, Mirmirani, P, Luo, F, Youngquist, SR, Fisher, BK, Li, J, Oblong, J, Dawson, TL.)



**Figure 44.4** Optical fiber diameter analysis of pre- and postmenopausal female scalp hair. Scalp hair collected from 1 cm<sup>2</sup> area of frontal or occipital scalp (108). (With kind permission from Springer Science and Business Media: Hair growth parameters in pre- and postmenopausal women. In M. R. Trüeb, MR and Tobin, JD, eds., *Aging Hair*, 2010, pp. 49–60, Mirmirani, P, Luo, F, Youngquist, SR, Fisher, BK, Li, J, Oblong, J, Dawson, TL.)



## Combination Measures

As hair quantity is determined by the hair's density (#/cm<sup>2</sup>) and diameter (μm), hair loss and growth result from changes in either or both. Therefore an ideal hair-measuring technology would combine these two measures into a single more descriptive and sensitive "hair amount" metric (29,105)

### Cross-Section Trichometer

A new device has recently been developed, the cross-sectional trichometer. This measure captures hairs from a predetermined area and compresses them into a precise caliper. Care must be taken for accurate sample selection, as small changes in the number of fibers have a strong influence. Control of the site is accomplished with a 2×2-cm dye marker. All hairs inside the demarked 4-cm<sup>2</sup> area are captured, providing highly reproducible results. Future work could provide a noninvasive, fast, and accurate measure of hair "mass" or "volume," which would be predicted to be highly relevant to the perceived efficacy in hair loss treatment (105).

### Hair Weight

Hair weight and hair number have been demonstrated to be valid parameters for assessing efficacy of both minoxidil (17,72) and finasteride. Briefly, a representative site is selected on the thinning frontal/parietal scalp and all hairs clipped to 1 mm. In subsequent visits, the procedure is repeated. All the hairs from each collection are manually counted, with pointed versus blunt tipped hairs separated, then each sample is weighed. In addition to hair weight and hair count, hair width and length may be assessed by projection microscopy. While extremely precise, this methodology remains highly labor intensive.

## Global Assessment

### Global Macro Photos

While precise technical measures of hair growth are important for evaluation of pharmacologic intervention, it is also important to understand whether technically measurable changes lead to changes perceptible by the subject and/or investigator (110–112). These evaluations are based on global, or whole-head, macro photography and rating using perceptual scales by the subjects and investigators. While it is crucial to measure perceptible changes, it is extremely difficult due to numerous and aforementioned complicating factors. The investigator must tightly control humidity, hair style, hair color, background and clothing colors, lighting, camera type and magnification, among others. It is also vital to observe and control these features in presentations of hair benefit claims and data, as manipulation of any of these facets can lead to inaccurate interpretation.

### Subject Self Perception

Patient assessment is measured by administration of a validated questionnaire based on seven parameters: four on efficacy and three on appearance. These parameters include visible scalp, hair appearance, hair growth, slowing of hair loss, and satisfaction with the hair appearance and the frontline (93). Patients may also evaluate changes by reviewing randomized pre- and post-treatment photographs (68). It is common for self-perception to lag far behind technical assessment, likely due to the amount of time required for new hairs to grow to a length appreciated by the subject and that the affected area may not be easily visible to the subject. For instance, technical effects of minoxidil can be measured in 8–12 weeks, but self-perception requires 9–12 months.

### Expert Assessment

Investigators assess subjects using a standardized seven-point rating scale (–3 to +3) after referring to a baseline photograph as a reference (93). Generally, investigator assessment time to noticeability falls between technical and self assessment.

Changes can also be evaluated by an expert panel. In this method, standardized color photographs were taken with the head in a stereotactic positioning device and then visually graded by trained, calibrated experts (111). Paired baseline-to-post-treatment slides are then independently and blindly reviewed by an expert panel using vertex and frontal views (68,93). Expert graders have much higher sensitivity than subject assessments and usually are able to identify effects sooner. However, the perception of the individual under treatment must remain the ideal, and be the focus of development of new therapies.

## DISCUSSION

Complex biologic mechanisms regulate hair growth and characteristics over the course of a woman's life. With age, there is a waning of many of these functions with an associated increase in self-perceived thinning (29). Our current understanding is that hormonal controls (including androgens and estrogens) as well as age-related physiological changes influence scalp hair including alterations in hair diameter and density. Prior studies have shown that hair fiber diameter increases to 40 years of age, reaches a plateau, then decreases with advancing age (29). Further, hair diameters for Caucasian women are significantly higher pre- versus postmenopausal for frontal but not occipital scalp. This suggests that menopausal status and estrogen alteration affect scalp hair diameter. In contrast, hair density in women decreases throughout teen and adult life, with no abrupt change near the perimenopausal period. This suggests senescent as opposed to hormonal signals lead to follicular dropout and decreased hair density.

Using the combined measure of hair density and diameter, hair amount, to more accurately quantify the perception of hair loss shows that hair amount in Caucasian women peaks at 35 years of age. The values are similar at ages 25 and 45, only 6% and 5% less than the maximum. However, at age 50 hair amount is 11% less. As the amount of hair changes little from age 25 to 45, normal loss is less likely to be noticed until after the diameter begins to decline in the mid 40s, consistent with the increased self-perception of hair loss in midlife women.

In addition to density and diameter, other factors affect the self-perceived appearance and amount of a woman's hair with advancing age. While several of these reported changes are subjective, a variety of quantifiable factors contribute. Nonbiologically regulated factors include length, style, color (from dying and bleaching), curvature (chemical waving), and damage (through breakage and volume) (11,113). Biologically regulated facets include density and diameter, color (due to pigmentation), and curvature. Hair density is a function of factors including anagen:telogen ratio and growth rate. The relative importance of each of these factors on the perception of hair loss is not fully understood.

While the methods described in this review are all appropriate for assessing changes in hair growth in humans, no single method addresses all questions relating to hair growth properties or the efficacy and mode of action of hair loss treatments. Therefore one must always consider the primary objective and utilize an appropriate array of techniques to address the question posed.

The continual rapid advancements in optics and computers will continue to improve the study of basic hair biology and treatments, increasing both sensitivity and speed. Hair count techniques have progressed from manual counts to macrophotography. Recent developments using automated methods have increased the capability and sensitivity to monitor hair loss and treatment responses, including measures of density, diameter, growth rate, and anagen:togen ratio (99). Software and algorithms have increased accuracy and speed, giving these methods the capacity necessary for analysis of data from large clinical trials.

While new quantitative techniques facilitate field execution and decrease the time, effort, and costs required in material screening trials, the conduct of large pivotal phase III trials to establish the efficacy and safety of hair regrowth active substances for FDA submission still requires a duration long enough for subjects to self-perceive a benefit. Furthermore, these techniques do not replace exploratory methods targeted at understanding the mechanism of new interventions. Methods such as biopsies combined with imaging or molecular DNA/RNA or biochemical marker techniques are still needed to derive an understanding of how and why these treatments work.

For clinical design, the use of both active and placebo controls and having adequate subject numbers and diversity are critical for properly evaluating any changes in hair biology, including therapies. For example, considerations in subject enrollment include ensuring that the population covers a range of hair loss progression (Norwood/Hamilton scale scores for men, Ludwig scale scores for women) and accounting for critical variables such as age, ethnicity, medical conditions, and medications that can affect hair biology. The selection of the scalp site is also critical for study outcome. For example, in women the effect of FPHL is more pronounced on the frontal than on the occipital scalp so studies with measurements from the frontal region will likely be more sensitive (109).

Since the above objective measurements do not necessarily translate immediately into patient-perceived benefits, subjective perception measures by both patients and investigators are necessary in treatment evaluation, especially in pivotal long-term trials, to insure that clinically meaningful benefits have been produced. A self-perceived benefit is the key to successful hair regrowth products.

This chapter has focused on describing the fundamental changes associated with hair loss in normal women with age and the methods used to objectively quantify those changes. We have addressed methods for tracking hair density and diameter, as they are the variables most likely to be noticed by subjects seeking treatment. Historically, treatment regimens have focused on scalp hair number density as this was perceived to be the parameter most influencing hair loss. However, recent research is indicating that characteristics beyond density play a crucial role in subjects' satisfaction with their hair. For example, hair diameter has been found to play a critical and highly influential role in both the perception of hair loss and in the efficacy of treatment (6,10,105,109). In another study, grey hair was found to be a more significant contributor to apparent age than hair thinning, and therefore addressing greying may be an important intervention for improving patient satisfaction (5). Given the importance of these hair characteristics, in the next few years new methods must be developed to measure these endpoints. A final consideration for addressing patient hair concerns is the strong linkage between scalp health and

hair quality, and it is likely that measures of scalp health will begin to be used to link skin and hair biology (114).

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# Menopause, Skin, and Cosmetology

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## INTRODUCTION

The hormonal disorders of menopause affect the whole organism, including skin and skin appendages. Women can avoid or limit some side effects of menopause with hormonal replacement therapy (HRT), and an appropriate cosmetology may complement the favorable effects of HRT on the skin (1,2).

Skin changes at menopause, in perimenopause, and in postmenopausal women include changes that can be related to the complex process of aging and others, among them signs of cutaneous hyperandrogenism. These changes affect not only skin, but also mucous membranes, hair, and other appendages. As far as skin and skin aging are concerned, HRT may be used with some success. However, HRT only very partially prevents or corrects what happens in terms of skin, mucous membranes, and appendages with menopause, that is, the so-called hormonal aging of the skin (1–4).

## MENOPAUSE AND HRT

Menopause is a natural biologic process, not an estrogen deficiency disease. Menopause represents the permanent cessation of menses resulting from loss of ovarian follicular function (5). Menopause can occur spontaneously or be induced through a medical intervention, i.e. surgery, chemotherapy, radiation therapy. Aging of the female reproductive system begins at birth and proceeds as a continuum. It consists of a steady loss of oocytes from atresia or ovulation, which does not occur at a constant rate, as evidenced by the relatively wide range (42–58 years) for spontaneous menopause. Menopause is defined as the anchor point after 12 months of amenorrhea following the final menstrual period, which reflects a near-complete but natural diminution of ovarian hormone secretion. In the Western world, menopause occurs at an average age of 51.4 years. Although there has been an increase in life expectancy over the last century, the age of menopause has not changed, unaffected by improving nutrition and reduction of disease.

The term *premenopause* only refers to the whole of the (reproductive?) period prior to the menopause (since birth?) and therefore should be abandoned. *Perimenopause* (or menopause transition) begins with variation in menstrual cycle length and ends with the final menstrual period. For most women, the transition lasts approximately 4 years. *Postmenopause* is the span of life dating from the final menstrual period and is defined as stage 1 (early: the 5 years following final menstrual period) and stage 2 (late, with a duration variable since ending with the woman's death). *Climateric* syndrome defines the symptomatology associated with the reproductive transition

of perimenopause/menopause. This (and some changes in postmenopausal women such as osteoporosis) may be prevented with HRT (5).

Estrogen-based therapies for postmenopausal women may be divided into two categories: estrogen replacement therapy (ERT) and HRT, a combination of estrogen (for instance, estradiol [E2]) and of progestin. Progestins reduce the risk of endometrial adenocarcinoma, which is significantly increased in women with a uterus who use unopposed estrogen. However, in some women, these progestins may favor the development of cutaneous signs of hyperandrogenism, such as seborrhea, acne, alopecia, and facial hirsutism (1,2,5).

Porphyrias and lupus erythematosus (LE) are the only dermatologic conditions in which HRT should not be used (1,2,6,7). Although a few studies suggested that estrogen replacement does not increase the risk for lupus flares in postmenopausal women with LE (8–10), HRT and ERT may be responsible for clinical and biologic accentuations in women with lupus, and for a first manifestation of the disease in women with no past history. Melasma was evidenced in women under HRT (11).

The initiation of hormone therapy may be proposed around the menopause to treat menopause-related symptoms or to reduce the risk of osteoporosis or fractures in select postmenopausal women, or both. The benefit-risk ratio for HRT is favorable close to menopause but decreases with aging and with time since menopause in previously untreated women (12).

## SKIN AGING AND MENOPAUSE

Skin aging is a progressive and complex process, which corresponds to at least two major components. Intrinsic or chronobiologic aging, on one hand, affects all tissues, while photoaging, that is, helioderma, only affects skin in sun-exposed areas. Helioderma is not influenced by hormonal status.

## SO-CALLED HORMONAL SKIN AGING

Around menopause, during premenopause and early postmenopause, the skin usually becomes thinner and rougher (Figures 45.1 and 45.2). So-called “dry skin” is only rough skin, which is due to chemophysical alterations of the stratum corneum at the skin surface level, not to changes in dermal or epidermal hydration. Menopausal skin in non-sun-exposed areas is characterized by a diminution in skin thickness that affects both the epidermis and the dermis (Figure 45.3), and

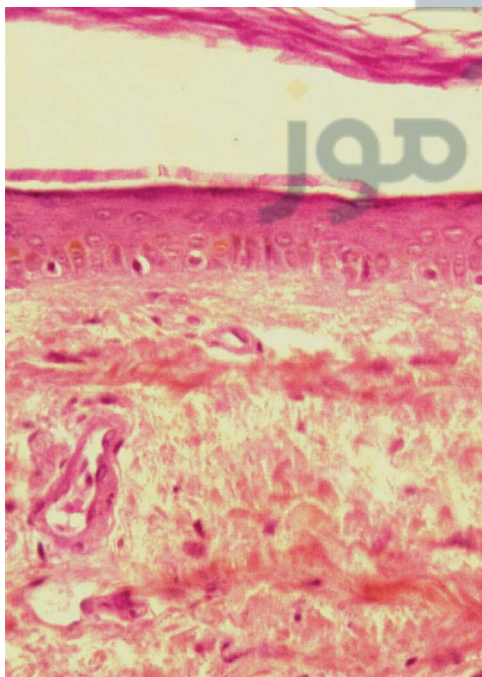




**Figure 45.1** Menopausal skin thinning.



**Figure 45.2** Menopausal “dry skin.”



**Figure 45.3** Epidermal thinning and compact stratum corneum.

by a decrease in some constituents of the dermal extracellular matrix such as collagens and glycoaminoglycans (13–15). Some 30% of skin collagen is lost during the first 5 years after menopause, that is, early postmenopause. This decrease in dermal collagen also parallels bone loss in postmenopausal women (16,17).

What is also generally observed is an increase in percutaneous water loss correlated to alterations in the hydration and the lipid constitution of the stratum corneum, which becomes more compact (1,3,4). Taken together, this leads to alterations in skin extensibility (increased) and elasticity (decreased) (18).

The correction of some skin changes in postmenopausal women with HRT supports the existence of what has been called hormonal skin aging.

### HORMONE REPLACEMENT THERAPY AND SKIN AGING

The observation of cutaneous change correction in postmenopausal women under ERT suggests that some skin alterations noted with aging are due to the decrease in estrogens. Studies in postmenopausal women indicated that the skinfold thickness is maintained with long-term hormone therapy (19). In some initial studies it was suggested that oral estrogen therapy can prevent epidermal thinning for at least 3 years after castration (20).

More recent studies (ultrasonography and skin biopsies) indicated that skin atrophy and dermal atrophy in sun-unexposed areas are corrected with HRT (21,22). No effect on the epidermis could be evidenced (21,22). HRT corrects or prevents the decrease in dermal thickness and in dermal collagen content (15,16,23). These effects of HRT are systemic and do not depend on the way, oral or percutaneous, estrogens are administered. However, the previous observations of the correction of the epidermal atrophy following castration with estrogen substitution (20) were not confirmed (21,22). Estrogens may also affect skin surface lipids, epidermal hydration, sebum excretion, wrinkling, and skin elasticity in postmenopausal women (24–27).

### SKIN AGING AND OTHER HORMONE REGIMENS DHEA

No effect of DHEA could be found in terms of correction or prevention of skin aging in pre- or postmenopausal women (28).

### Estrogens in Topical Treatments

When topically applied to the skin, estrogens have been shown to induce a partial correction of some skin aging changes without evidence of systemic effect (29). Estriol and Estradiol ointments improved elasticity and reduced the wrinkle depth, but no control is available in this open 6-month study (30). However, the effects of conjugated estrogen (Premarin cream) were studied in a randomized, double-blind, parallel group study: 54 women applied 1 g of either Premarin cream or placebo cream to the face daily for 24 weeks (31). Skin thickness was measured by B-scan ultrasonic echography and skin microrelief by profilometry. Skin thickness (dermal plus epidermal) increased in the treated group. Premarin was also significantly more effective than placebo in improving facial fine wrinkles. There was no effect on other parameters (skin roughness, laxity, and hyperpigmentation) (31). Topical estradiol was

also shown to increase the amount of dermal collagen (32,33) and stimulate collagen synthesis (33).

### Phytoestrogens, Isoflavones

Genistein has been shown to be an inhibitor of UVR-induced skin carcinogenesis (34). The effects of isoflavones and other phytoestrogens on skin atrophy, dermal collagen are under investigation to determine their possible use in the prevention of skin aging in postmenopausal women. Animal studies showed an increased collagen metabolism in animals treated with a preparation of genistein and daizein (35).

### OTHER SKIN CARE AND MENOPAUSE

Taken together, these data indicate that HRT, alone or in combination with preparations with estrogens or isoflavones, may be used to minimize skin changes due to estrogen deficiency. However, because of the possible adverse effects, such as the increased risk of breast cancer and cardiovascular disease, HRT cannot be recommended today to treat skin aging. On the other hand, cosmeceutical care has an important role to play for the menopausal woman (36). Tretinoin, glycolic acid, and ascorbic acid-containing products have been shown to change age and/or sun-related skin damage (37–41). They may therefore be considered as medicines, which may complement the action of HRT. Collagen injections, botulinic toxin, peeling, and resurfacing lasers are designed for photoinduced wrinkles and solar keratosis, conditions where HRT has no action. Surgery is necessary for cutaneous ptosis of the face and neck.

### OTHER SYMPTOMS Genital Discomfort

Vulvar atrophy (Figure 45.4), vaginal atrophy, and genital dryness may be observed in postmenopausal women. Vaginal



Figure 45.4 Postmenopausal vulval atrophy.

dryness may respond well to HRT. However, HRT is not effective on vulvar atrophy, and topical estrogens have to be used (1,40).

### Hirsutism or Hypertrichosis

True hirsutism, if absent before menopause, is uncommon after, but facial hirsutism, or hypertrichosis is not uncommon (41). Facial hirsutism (Figure 45.5), either in perimenopause or after menopause, may be related to an idiopathic skin hyperandrogenism, even in the absence of biologic evidence of hyperandrogeny (2,40). Together with alopecia, excess in seborrhea, and to a lesser degree acne, this hypertrichosis may also be related to progestin intake (either in women under HRT, or progestin therapy in premenopausal women), to DHEA, or to tibolone (42). When true hirsutism develops, whether alone or with other patterns of hyperandrogenism, it may correspond to an ovarian or adrenal tumor (43).

True hirsutism, but not facial hypertrichosis, responds well to antiandrogens (cyproterone acetate or spironolactone) (44). HRT has no or little effect. Facial hirsutism needs epilation (by laser or any other method). Eflornithine (45), a potent inhibitor of polyamine metabolism, may also be used (Vanical cream). The 5 $\alpha$ -reductase inhibitor finasteride was also shown to improve cases of facial hirsutism when topically applied, with decreased hair growth and thickness (46).

### Alopecia

Hair loss may only occur in postmenopause alone or in association with facial hirsutism, but is not infrequent in premenopausal women after 40. When mild and with a progressive installation, this so-called androgenogenetic alopecia (AAG), or female-pattern hair loss (Figure 45.6), has to be distinguished from other causes of progressive hair loss in women: hypothyroidism, iron deficiency (47), lichen planus (48), or more commonly, senile alopecia (2).

Neither ERT nor HRT can prevent hair loss, and antiandrogens, such as cyproterone acetate or spironolactone, should be recommended in association with ERT and topically applied minoxidil (44,49). Finasteride, which blocks the reduction of testosterone into active diOH testosterone, does not prevent progression of hair loss in postmenopausal women with female-pattern hair loss (50). However, the recent report of the possibility of an improvement of some AAG in postmenopausal women with hyperandrogenism supports the hypothesis that not all types of female hair loss have the same



Figure 45.5 Postmenopausal facial hirsutism.





**Figure 45.6** Postmenopausal alopecia.



**Figure 45.7** Climacteric hyperkeratosis of the palms.

pathophysiology (51). Psychotherapy is often necessary, and in some cases hair transplantation and scalp surgery.

## OTHER TREATMENTS

Topically applied androgens, not estrogens, can be considered in cases of genital hair loss. HRT has no effect on genital or axillary depilation. Neither HRT nor other hormonal regimens have any proved effect on postmenopausal nail changes.

Climacteric keratodermas (Figure 45.7) are very poorly understood and no data are available concerning the effect of hormone replacement (52).

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# Section VI

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Cosmetological Treatments



بزشک آموز



پزىشک آموز



## Mesotherapy

Maria Pia De Padova, Gabriella Fabbrocini, Sara Cacciapuoti, and Antonella Tosti

### INTRODUCTION

Mesotherapy was first developed in 1952 by Dr. Michel Pistor, a French physician, for the management of pain and vascular disorders (1), who coined the term *mesotherapy*. He defined it as treatment of the mesoderm (the primary germ layer that develops into connective tissue, muscle, and circulatory system). In 1976, he described mesotherapy as "... little volume, few times and in the right place" (2).

Pistor founded the French Society of Mesotherapy in 1964 and The French National Academy of Medicine officially acknowledged mesotherapy as a medical specialty in 1987. In the meantime, mesotherapy became popular in most parts of Europe and South America, and more recently in the United States and Asian countries.

### TERMINOLOGY

Mesotherapy, broadly defined, represents a variety of minimally invasive techniques that consists of the intra- or subcutaneous injection of variable mixtures of natural plant extracts, homeopathic agents, pharmaceuticals, vitamins, and other bioactive substances in small quantities through dermal multi punctures. The term *mesotherapy* is derived from the Greek words *mesos* (meaning "middle" or "mean") and *therapeia* (meaning "to treat medically," i.e. injecting into the middle layer of skin or *intradermotherapy*). Therapeutic effects of chemical products used for mesotherapy are prevalently used for skin rejuvenation and local fat deposits.

### INDICATIONS

Mesotherapy is reported to have a wide array of applications, especially in the field of cosmetic dermatology. Skin-rejuvenation/glow, lift, and fat reduction are the most common.

Mesotherapy can be performed for:

- Chronoaging: mild-moderate (Glogau I/II)
- Photoaging: mild-moderate (Glogau I/II)
- Prevention of aging
- Preparation for sun exposure
- Smokers

Many products are available on the market to perform mesotherapy for skin rejuvenation. Some contain only one active ingredient and others are mixture of different compounds (Table 46.1). Some authors believe that injection of a single ingredient can provide better results than a cocktail injection: the side effects resulting from the interactions among different ingredients can be reduced and the active ingredients, injected alone, can be more effective (3).

Mesotherapy is also proposed for reduction of local fat deposits or cellulite and for body contouring.

### MESOTHERAPY FOR FAT DEPOSITS

Cellulite is an abnormal accumulation of fat above the fascia resulting in dimpling appearance of skin resembling an orange peel (4). It is most commonly seen in the buttocks and thighs of females. It is seen in both normal and obese persons. It is believed to occur due to abnormality in the venous lymphatic system. According to literature, the treatment of local fat deposits and cellulite is based on the agents reported in Table 46.2 (5).

The two different types of mesotherapy used for cosmetic fat reduction are lipolytic stimulation and ablative mesotherapy. Lipolytic stimulation is based on activation of lipolysis in fat cells. There are at least three general mechanisms by which lipolysis can be increased: (1) inhibition of phosphodiesterase or the adenosine receptor (6–7); (2) activation of the beta-adrenergic receptor, and (3) inhibition of the alpha-2 receptor. Aminophylline, isoproterenol or forskolin, and yohimbine are thought to act on all the three different lipolytic signalling pathways (8). Isoproterenol, aminophylline, and yohimbine should have additive effects as they act at different points in the same physiological pathway. Ablative mesotherapy is based on the destruction of fat cells using a detergent. Injectable phosphatidylcholine and deoxycholate are the major components used in ablative mesotherapy. Phosphatidylcholine is able to:

**Table 46.1** Products Used for Mesotherapy

Hyaluronic acid alone (1.35%–3%)
Hyaluronic acid 0.2%, 1%, or 3% plus other active ingredients
Polynucleotide macromolecules
Organic silicium
Autologous cultured fibroblasts
Growth factors
Homeopathic products

**Table 46.2** Agents Contained in Solution for Treatment of Cellulite

Lipolytic	L-carnitine, caffeine, isoproterenol
Venostatic	Rutin, aminophylline, pentoxiphylline
Anesthetic	Procaine (also improves circulation)
Connective tissue breakdown	Collagenase, hyaluronidase
Drainage	Artichoke, ginkgo biloba

1. Penetrate the adipocytes and break down fat, which is then carried to the blood stream and excreted via the kidneys and bowel (9). Since phosphatidylcholine is viscous, an emulsifying agent, deoxycholate, is added to facilitate its injection. This chemical has been shown to independently cause lysis of the adipocytes (10).
2. Promote lipolysis by stimulating  $\beta$  receptors and inhibiting  $\alpha_2$  receptors present on the adipocyte membrane (11).
3. Cause inflammatory cytokine-mediated necrosis and reabsorption of adipocytes. Once the inflammation subsides, new collagen is formed, leading to retraction of the loosened tissue (12).

Phosphatidylcholine induces lipolysis via the activation of cyclic-monophosphate and the activation of beta adrenergic receptors. Gluteofemoral adipocytes have a significantly lower number of beta adrenergic receptors as compared to other localized adiposities. Deoxycholate alone is recommended for use in small localized fat deposits, with a phosphatidylcholine and deoxycholate combination reserved for larger treatment areas (13). The observation that post-injection resolution of inflammation is faster with the phosphatidylcholine and deoxycholate mixture compared to deoxycholate alone supports these recommendations (14). Depth of injection varies from 6 mm to 12 mm at a dose of 250 mg (15). Although the therapeutic effects of this procedure are linked to the properties of the molecules listed above, an essential part in the treatment of this condition is a low glycemic diet and regular, preferably aerobic, physical exercise.

## MESOTHERAPY FOR SKIN RENJUVENATION

### Areas of Application

- Face (cheeks, chin, forehead)
- Neck
- Low neckline
- Back of hands
- Abdomen
- Arms and legs (inner surface)

Although mesotherapy for skin rejuvenation is an easy-to-perform technique, contraindications and disadvantages are always to be considered (Table 46.3).

### Technique

#### Classical Mesotherapy

For the procedure, common disposable syringes are used with a 4 mm × 0.4 mm (27G) needle (Leble needle) that permits correct intradermal injection in any cutaneous surface. With this needle, it is easy to avoid telangiectasia and skin lesions. The 13 mm × 0.3 mm (30G) needle, however, is more appropriate for skin biostimulation.

The injections can be performed using three different techniques, always keeping the needle with an inclination of 45°:

- **Picotage.** One drop of the product is injected into the superficial dermis. The injections are spaced at 2 mm, and the needle penetrates 2 to 2.5 mm. The physician maintains a constant pressure on the plunger.
- **Cross-linking.** Consists of vertical injections, with complete penetration of needle, followed by horizontal injections (1-cm distance between lines). The product is injected during the extraction of the needle from the dermis. This technique is particularly useful for cheeks and low neckline in patients with advanced stage of chronoaging.
- **Linear threading.** Either vertical or horizontal injections are performed (Figure 46.1). Vertical injections are useful to prepare the nasolabial and glabellar wrinkles 10 to 15 days before injecting dermal fillers and botulinum toxin. Horizontal injections are useful in treating neck wrinkles. To reduce the burning sensation, the physician can apply EMLA cream or use mesotherapy products containing lidocaine chlorhydrate 1 hour before treatment.

#### Microtherapy and Electroporation

Microtherapy and electroporation are new methodologies that offer some advantages over classical mesotherapy. Microtherapy is a procedure that facilitates the intradermal injection of drugs. The use of extra-fine needles permits the

**Table 46.3** Mesotherapy for Skin Rejuvenation: Contraindications and Disadvantages

Contraindications	Disadvantages
Allergy to ingredients of the cocktail	Only for mild–moderate aging
History of hypertrophic scars	Mild erythema, slight itching/burning sensation 5 min after injections
Bleeding abnormalities and/or anticoagulant therapy	Small hematomas
Pregnancy/ breastfeeding	Possibility of allergic reactions
Autoimmune disorders	Lack of controlled clinical trials
Epilepsy	Lack of guidelines according to the evidence-based medicine
Diabetes	
Herpes simplex virus type 1 (HSV-1) infection in active phase	
Bacterial infections	
Inflammatory skin disorders (acute phase)	



**Figure 46.1** Linear threading, horizontal injections.

drugs to reach the papillary level of the dermis, thus avoiding damage to the skin's superficial nerves and vessels. This reduces risks of undesired side effects. Some of the advantages of this procedure are the following:

- Painless injections, because the needle does not reach the nerves
- Reduced hematomas, since only capillaries and not venules are touched
- Slow absorption of the drug because it does not reach the venules of the deep dermis and thus barely enters the circulation, remaining locally active for a long time
- No scars in the deep dermis that might cause undesired sequelae

Dermo-electroporation is a very interesting methodology with manifold applications in dermatology and cosmetology. This innovative procedure diffuses pharmacologically active substances through the horny layer, which represents the most

limiting factor in the optimal penetration of topical agents. The horny layer represents the main block to the penetration of hydrophilic molecules and/or of molecules having a high molecular weight (16,17). The method is based on the application of an electric impulse to the epithelial surface. The electric impulse is able to generate a transmembrane potential difference of 0.5–1.5 V, giving rise to the phenomenon of poration of cell membranes. At the same time, it causes an electrochemical perturbation of the horny layer, with a consequent increase in its permeability. It is well known that a specific molecular weight and lipophilic properties can be passively diffused in the horny layer and reach the dermal region (18). The procedure is absolutely painless and safe for the patient. The system is characterized by a 100-V condenser that generates a typical reversible exponential wave and by a metallic activation chamber in which is placed a conductive gel containing the active molecules. The electropores allow cell penetration of molecules that usually do not penetrate because of their high molecular weight. The aqueous channel is rather large (1–2  $\mu\text{m}$ ), permitting the passage of molecules such as medium-molecular-weight polysaccharides (hyaluronic acid), peptides (soluble collagen), and glucosides. The first step of the treatment is to remove the horny layer with crystal microdermoabrasion, followed by superficial peeling. The second step consists of the introduction of the revitalizing substances (hyaluronic acid, vitamins, peptides, etc.) in the conductive gel, which is then ionized in the ionization chamber and applied to the area to be treated by a light massage (19). The treatment lasts about 30 minutes, including the pre-peeling (cavitation) stage, and can be repeated every 1–2 weeks for a cycle of 10 sessions at most.

### Results

Mesotherapy is believed to improve the global appearance of the skin and it can be an excellent complement to all other rejuvenating procedures. Two or three treatments are necessary to see results: in general the skin appears firmer, brighter, and better moisturized. (Figures 46.2–46.5)

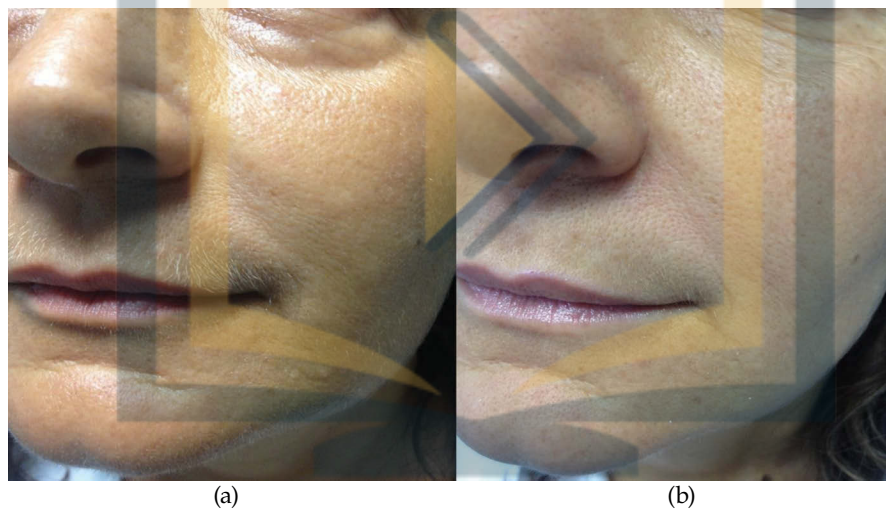


**Figure 46.2** A patient (a) before and (b) 2 weeks after mesotherapy.





**Figure 46.3** A patient (a) before and (b) after mesotherapy and filler injection.



**Figure 46.4** A patient (a) before and (b) 3 weeks after mesotherapy.



**Figure 46.5** A patient (a) before and (b) 3 weeks after mesotherapy

## CONCLUSION

In recent years, mesotherapy has gained popularity due to its noninvasive, painless nature, but to date, the mode of action of many products used in mesotherapy is either doubtful or unknown and there are no clear-cut guidelines on the dosage and efficacy of the products. Safety of molecules that are injected is not established for all compounds and phosphatidylcholine, for instance, is not approved for mesotherapy in most areas of the world. Thus, continued research and well-designed controlled scientific studies are required to enhance the claims of effectiveness of these new products and techniques. It can be hopeful to have guidelines and recommendations regarding their use for aesthetic applications.

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## Microneedles and Cosmetics

Raja K. Sivamani and Howard I. Maibach

The field of transdermal drug delivery has significantly advanced over the past decade (1). Traditionally, drugs are delivered either orally or through the use of a hypodermic needles, but both have significant drawbacks. Oral drug delivery is affected by the acidity of the stomach, poor intestinal absorption, and first pass hepatic metabolism, which contribute to lower bioavailability. Hypodermic needles are painful and lead to patient discomfort and anxiety. Transdermal drug delivery is an alternative delivery method that introduces drugs by bypassing the skin barrier to allow for either systemic or local drug delivery. Transdermal drug delivery approaches do not mechanically penetrate as deeply as hypodermic needles.

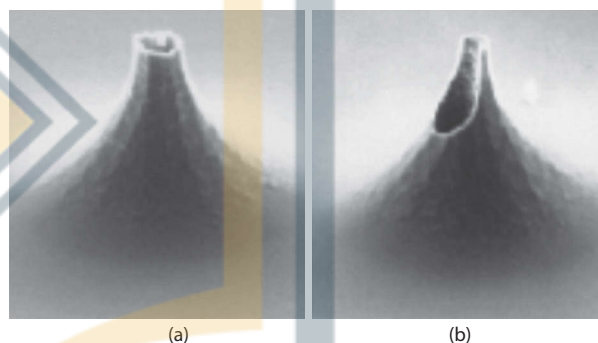
A major barrier in transdermal drug delivery is the stratum corneum. It is the outermost layer of skin that is composed of keratinized dead cells. Transdermal drug delivery systems aim to bypass the stratum corneum since it is usually the rate-limiting barrier in transdermal delivery. The stratum corneum is lipophilic in nature and is resistant to the passage of hydrophilic substances or the passage of molecules that are larger than a few hundred Daltons. In general, multiple different approaches are taken to bypass the stratum corneum (1). Microneedles enhance transdermal drug delivery by creation of physical conduits or channels through the stratum corneum.

### MICRONEEDLE FABRICATION

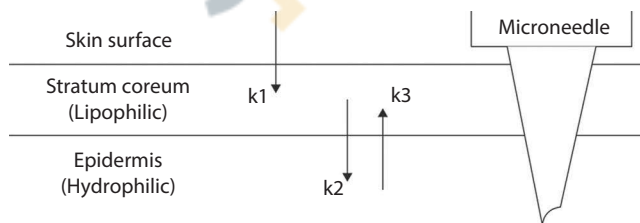
Microneedles are miniature needles that are created using lithographic techniques. Most are designed to penetrate the stratum corneum and enter into the epidermis without entering the underlying dermis. By doing so, they avoid nerve endings in the dermis, rendering insertion painless (2–6). However, in the case of cosmetic uses where dermal remodeling is desired, deeper microneedles are used such that they enter the dermis and are painful with application.

Microneedles have been developed in several different designs, including out-of-plane and in-plane (7). Out-of-plane needles are designed such that the microneedle is perpendicular to the surface (Figure 47.1). In-plane microneedles are parallel to the surface but are more difficult to place into arrays. Because cosmetic applications have focused on the out-of-plane microneedle, this chapter reviews their use in cosmetics. In-depth discussions of in-plane microneedles can be found elsewhere (8). One exception is where in-plane microneedles are used to develop out-of-plane microneedle sheets, which are then converted into a microneedle roller (9). Microneedle rollers have been used for cosmetic applications and this will be discussed later. Out-of-plane microneedles are further subdivided into solid and hollow microneedles. Solid microneedles do not have an internal conduit for infusion of drugs and are used to either create physical holes in the stratum corneum or to coat the microneedle such

that the drug is delivered from the surface of the microneedle upon insertion. Hollow microneedles have a conduit (Figure 47.1) that allows for either bolus or continuous infusion after the microneedle is inserted (Figure 47.2). Microneedles are made out a variety of materials including metal (10), titanium (11,12), glass (13–15), polymers (9,14,16–19), and even sugars (3,16,20).



**Figure 47.1** Out-of-plane silicon hollow microneedles. (a) Symmetric hollow microneedles are designed so that the opening is right at the point of insertion. (b) Asymmetric microneedles are designed with the opening offset from the point of insertion.



**Figure 47.2** Schematic of hollow out-of-plane microneedle injection. The ability of a substance to move from the skin surface into the stratum corneum is determined by  $k_1$ . The rate of movement from the lipophilic stratum corneum into the more hydrophilic epidermis is determined by the ratio  $k_3/k_2$ . Drug solutions injected by microneedle bypass the transitions indicated by  $k_1$  and  $k_2$ . In the case of lipophilic drugs, some of the drug may partition back into the stratum corneum due to the “effective partition coefficient”  $k_3/k_2$ . (From Sivamani RK et al., *J Dermatol Treat*; 20(3):156–9, 2009.)



## MICRONEEDLE STRATEGIES FOR DRUG DELIVERY

The strategy for drug delivery depends on the design of the microneedle. Because solid microneedles do not have an internal conduit, they cannot be used to infuse drug through the needle. Instead, solid microneedles are used in three different strategies. The first strategy is to create micropores in the stratum through insertion and removal of the microneedle array, and it has been shown that these pores remain open for up to 24 hours before they close (6). Drug solutions can then be applied topically such that they can traverse these transiently open channels to bypass the stratum corneum, and some studies have utilized iontophoresis to accelerate movement through these transiently open channels (21–23). A second strategy is to coat microneedles with a drug prior to insertion. The third strategy is to create dissolving microneedles that dissolve upon insertion to deliver a drug payload. Both the strategy of coating microneedles or encapsulating a drug payload into a dissolving microneedle is limited to drugs that are stable to undergo the coating or encapsulation process (24). Hollow microneedles have an internal conduit and can be used to infuse drugs as a bolus (25,26) or as a continuous infusion (27) after insertion.

Utilizing these various strategies, microneedles have been employed to deliver vaccines (28–30) or drugs in animals including insulin (10,14,16,27,31), erythropoietin (16), desmopressin (11), and methotrexate (32). Microneedles have also been testing in humans with *in vivo* studies that have shown the ability to inject nicotinic acid derivatives through hollow silicon out-of-plane microneedles (25,26) and insulin through a hollow glass micropipette tip (33).

## COSMETIC USES

### Solid Microneedles

The length of the microneedle greatly influences its potential applications. Short microneedles are defined as those that do not penetrate past the epidermis and their role is for enhancement of penetration past the stratum corneum. Because short microneedles will not penetrate into the dermis, there is typically no bleeding and minimal pain. On the other hand, long microneedles are able to penetrate past the stratum corneum into the superficial dermis. This subset of microneedles typically causes pain and bleeding.

Several studies have investigated the use of solid microneedles (Table 47.1) for use in cosmetics and several microneedle-based devices are available commercially, including both short and long microneedles. Their applications have included topical anesthesia, increased penetration of topical hair growth products, photodynamic therapy, and revision of scars. An example of a microneedle roller with short microneedles is shown in Figure 47.3.

Previous studies have shown that pretreatment with solid microneedles allows for topical anesthesia to be quicker in onset (34,35). Both of these studies indicated that anesthesia was accelerated within 30 minutes. One showed that in more pain-sensitive subjects, the anesthesia acceleration was noted by 10 minutes (35).

Several studies have investigated the use of microneedles for enhancement of hair growth. One study evaluated the use of a polymer-based microneedle roller to enhance the topical delivery of l-ascorbic acid for hair growth in mice. In this study, the strategy employed was to create microchannels and then topically apply l-ascorbic acid. Microneedle roller pretreatment



**Figure 47.3** Microneedle roller. Stainless steel microneedles are placed in a cylindrical arrangement. This photo depicts rollers that are too shallow to penetrate to the dermis but can create holes in the stratum corneum.

enhanced cutaneous permeation by approximately 10-fold and showed that this enhanced hair growth in mice (36). A human study in eleven women with female pattern hair loss was conducted as a split-scalp study where a growth factor mix that consisted of basic fibroblast growth factor (2.5 µg/mL), insulin-like growth factor-1 (1 µg/mL), vascular endothelial growth factor (2.5 µg/mL), stem cell factor (2.5 µg/mL), keratinocyte growth factor-2 (2.5 µg/mL), superoxide dismutase-1 (5 µg/mL) and Noggin (2.5 µg/mL) was compared against a saline placebo control treatment (37). The subjects received weekly treatments for 5 weeks with a motorized microneedle that was set to a penetration depth of 0.5 mm. The mean hair shaft size ( $p < 0.05$ ) and the hair counts ( $p < 0.001$ ) both increased by 15% after 5 weeks.

Photodynamic therapy (PDT) frequently requires the local application of a photosensitive drug in conjunction with incident light to selectively destroy cells, presumably through locally generated singlet oxygen (38). In an attempt to more efficiently deliver topical photosensitive drugs, silicon microneedle patches were used to create transient microchannels in nude mice and deliver either 5-ALA (39) or Meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate, a preformed photosensitizer (40). Both of these studies delivered the photosensitizing drug by placing a transdermal patch over the area that had been transiently perforated with a microneedle patch. Both studies found that the photosensitizing drug was delivered in greater quantities, to a greater depth, and in a shorter time in comparison to sites that were not perforated with the microneedle patch. The greater depth of penetration is likely the result of faster penetration past the stratum corneum, and future studies should assess the kinetics of the penetration. As such, one potential advantage of microneedle based therapy would be to reduce incubation times for PDT. One clinical study evaluated the role of short microneedles with PDT for photorejuvenation and noted clinical improvement of photodamage (41). However, there was no control group where the subjects did not receive microneedle pretreatment and therefore it is not clear how much extra benefit the microneedles may have provided.

A commercially available microneedle device is the Dermalroller® (Dermalroller S.A.R.L.) (42), which is a hand-held roller with rows of stainless steel solid out-of-plane microneedles. Two claims regarding the Dermalroller are that it can be used to enhance transdermal drug delivery and it can be used to reorganize underlying collagen in the dermis (42). The first claim for enhancing transdermal drug delivery works on the strategy of creating transient microchannels in the stratum corneum to allow for topical delivery of various drugs, sera, or vitamins necessary for the particular cosmetic

**Table 47.1** Clinical Studies of Microneedles Used for Cosmetic Treatments

Device	Material	Study design	Use	Results/Notes
Microneedle roller (35)	Stainless steel	Split-body controlled study	MN pretreatment to facilitate topical anesthesia (lidocaine 4%)	MN treatment has faster onset of anesthesia at 30 min In pain-sensitive subgroup, MN accelerated anesthesia by 10 min
Microneedle array (34)	Not indicated	Controlled study with sham microneedles	MN treatment after topical application of dyclonine 1%	MN treatment had faster onset of anesthesia
Microneedle array (52)	Glass	Controlled study comparing microneedle vs hypodermic needle	MN-based injection of 2% lidocaine	MN-based injection was similar to hypodermic needle-based injection
Microneedle roller (40)	Stainless steel	Uncontrolled clinical study	MN pretreatment and 5-ALA-based PDT led to even application of 5-ALA	Note improvement in facial scars and hyperpigmentation No control group
Motorized microneedles (37)	Steel	Split-body controlled study	Topical application of growth factor mix (bFGF, IGF-1, VEGF, SCF, KGF-2, SOD, Noggin)	15% improvement in hair shaft size and hair counts after 5 weeks on MN-treated side
Microneedle roller (45)	Titanium	Uncontrolled clinical study	MN treatment of hypertrophic burn scars	Scars pretreated with topical vitamin A and vitamin C for 4 weeks Histology showed increased collagen and elastin production Scars improved in clinical appearance No control group
Microneedle roller (46)	Stainless steel	Uncontrolled clinical study	MN treatment for acne scarring	Improvement in rolling scars No hyperpigmentation. No control group
Microneedle roller (44)	Stainless steel	Randomized, controlled, unblinded study	MN vs 100% TCA CROSS peel treatment of acne scarring on the face	MN superior to 100% TCA CROSS for rolling scars; no difference for boxcar scars; worse for icepick scars Transient postinflammatory hyperpigmentation noted in six patients
Microneedle roller (47)	Stainless steel	Uncontrolled clinical study	MN treatment for the atrophic scars in acne	Noted improvement in rolling and boxcar scars. Authors note 81% had "excellent" improvement No MN-induced hyperpigmentation No control group
Microneedle roller (48)	Stainless steel	Uncontrolled clinical study	MN treatment of acne scars ( $n=4$ ), posttraumatic scars on lower extremity and trunk ( $n=6$ ), and striae on upper leg ( $n=1$ )	Scars improved in clinical appearance Histology showed elastin increasing in most, but three subjects had decreased elastin No control group
Microneedle roller (49)	Stainless steel	Split-face controlled study	MN treatment of atrophic acne scars	MN-treated side had greater improvement in acne scarring at 6 months

**Abbreviations:** MN, microneedle; TEWL, transepidermal water loss; TMP, meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate; ALA, aminolevulinic acid; PDT, photodynamic therapy; TCA CROSS, trichloroacetic CROSS peel

treatment. This is supported by a study that evaluated the use of the Dermalroller in human skin *ex vivo* to show that the use of the Dermalroller significantly enhanced transdermal drug penetration (43). Increased penetration was noted with an increase in the microneedle size. In support of the second claim, one study that compared a microneedle roller composed of 1.5-millimeter needles to trichloroacetic acid cross

showed that the microneedles increased collagen production (44). Interestingly, the Dermalroller is claimed to be painless. However, a claim that the microneedles could cause microinjuries to the dermis to stimulate reorganization of the collagen would require penetration into the dermis and would stimulate the pain fibers in the dermis. Clinically, the Dermalroller leads to bleeding at the insertion and treatment

sites. This indicates that the microneedles penetrate into the dermis since the epidermis does not contain blood vessels. It is likely that the Dermaroller penetrates into the dermis and causes pain, necessitating the use of anesthetics prior to use. Further clinical studies will better clarify the pain that results from use.

Another commercially available device is the MTS-Roller™ (Clinical Resolution Laboratories, Inc.), which is another roller composed of an array of microneedles similar to the Dermaroller. The MTS-Roller is used to create transient microchannels in the stratum corneum to deliver two proprietary formulas for wrinkles and for hair loss. However, no clinical studies are publicly reported regarding the use of the MTS-Roller for these indications. The company lists two studies of MTS-Roller for the stimulation of collagen reformation in the dermis and for comparative investigation against intense pulsed light (IPL) as a stimulus for collagen synthesis. Similar to the Dermaroller, the MTS-Roller is claimed to induce collagen reformation through injury to the dermis. Autologous platelet rich plasma is obtained from the patient and then this is delivered to the treatment side to induce more collagen synthesis, but there are no published clinical studies to support or refute this claim. The MTS-Roller is claimed to be painless; however, the MTS-Roller likely reaches the dermis. Because the dermis contains nerve endings, a formal clinical study would better elucidate the mechanisms involved in the use of the MTS-Roller.

Multiple studies have evaluated the use of long microneedles in the treatment of scarring (44–49). Microneedle-based scar revision in acne scars appears to be more effective for rolling and boxcar scars rather than icepick scars (44,46,47). In a comparative study in the treatment of acne scarring, microneedle-based scar revision was found to have similar results to 100% trichloroacetic acid chemical reconstruction of skin scars (CROSS) peeling (44). Microneedle-treated areas were assessed by histology, with increased collagen and elastin production (45,48) with normalization of the papillary dermis in hypertrophic burn scars (45).

One study has evaluated the role of coated microneedles that dissolved after insertion to deliver ascorbic acid and the retinoid retinyl retinoate (50). The study was designed as a split-body design where the wrinkles at the lateral canthus (crow's feet) were treated with a loaded microneedle while the other side was left untreated as a control. The authors showed that both ascorbic acid- and retinyl retinoate-coated microneedle-based delivery lead to an improvement of the wrinkles of the lateral canthus in comparison to the control treatment.

### Hollow Microneedles

Only a few animal (27,51) and clinical (25,26,33) studies of hollow microneedles have been performed. Currently, there are no commercial cosmetic devices that utilize hollow microneedles. One study showed that injection of anesthesia through hollow microneedles was comparable to a hypodermic needle (52). However, microinjection through microneedles offers many exciting opportunities including the injection of botulinum toxin, photodynamic therapy, localized stimulation of hair growth or hair loss, and localized melanin injection. Future research will determine the hollow microneedle's impact in cosmetic therapy. In particular, superficial and painless injections of botulinum toxin may be possible in light of the development of topical botulinum toxin delivery (53).

### SAFETY OF MICRONEEDLES

One of the initial concerns with microneedles was the biocompatibility of silicon or glass, since there are reports of silicon- and glass-related granulomas (54,55). Most microneedles are developed to only penetrate into the epidermis and not into the dermis. Therefore, any broken pieces of a microneedles will likely be discarded within 2 weeks, as this is the normal turnover time for the epidermis. In addition, microneedle fabrication has moved toward polymer- and sugar-based synthesis since silicon is expensive when considering mass production for commercialization. The polymers and sugars are biocompatible and many are designed to biodegrade. As microneedle technology continues to move toward biocompatibility, microneedle material-related toxicity will likely become less of a concern.

The stratum corneum is also a formidable barrier against infection. Microneedles physically breach this barrier, raising the possibility for cutaneous infections. Channels created by solid microneedles are open transiently for 24 hours or less (6), and microneedles create a lower bacterial burden than injection with traditional hypodermic needles (56). Transient insertions of solid microneedles likely present decreased infectious risk as compared to the traditional hypodermic needle. However, the use of hollow microneedles for extended infusions may theoretically elevate the risk for infection. There are no studies of the infectious risk of long-term infusions with hollow microneedles, and the risk of cutaneous infections will need to be addressed in future studies.

In one case, a "tram-track" like scar appeared after the application of microneedle treatment (57) and this may be result of aggressive microneedling over convex surfaces where there may be bony prominences (48). In another case report, a subject had a severe reaction to the cosmetic agent that was applied with the microneedle device. Therefore, topical substances should be tested prior to use with a microneedle device. Additionally, some advise the use of light pressure around the eyes to lessen the possibility of a hematoma or bruising (48). Postinflammatory hyperpigmentation is rare (46) and appears to be transient and respond to topical hydroquinone when it is present (44).

### CONCLUSION

The future of microneedle use in cosmetics is bright. With microneedles, it is now possible to transdermally deliver large peptides and molecules that would not have been possible previously. Because the short microneedles are typically painless upon insertion, this can greatly increase patient comfort and acceptance. Currently cosmetics research has focused on long solid microneedles. This may broaden to the use of hollow microneedles with continued research in this area. Continued collaboration between bioengineers and dermatologists will be critical to the continued evolution and growth of the microneedle's applications within cosmetics.

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# Photodynamic Therapy in Dermatology

Jacques Savary

Photodynamic therapy (PDT) is a therapeutic that has been used since the beginning of the 20th century to destroy tumorous skin lesions.

- 1903: von Tappeiner describes the first PDT with eosin and sun exposure to treat a carcinoma of inferior lip.
- 1913: Friedrich Metz-Beyer administers porphyrin to himself and becomes photosensitive.
- 1950: The absorption of porphyrin by cancerous cells that become photosensitive is highlighted.
- 1980: The interest for PDT increases but patient's photosensitivity is an obstacle.
- 1993: Publication of the first clinical essay on a-aminolevulinic acid (ALA) in PDT.
- 1995: Registration of the first ALA-modified patent.

This therapeutic was developed in Northern Europe essentially for the treatment of nonmelanocytic skin cancers. PDT then expanded to other European countries and North America for other indications than those dealing with skin carcinologic.

## PRINCIPLE

The basic principle in cancer research lies in the selective destruction of abnormal cells of target tissue by a chemical reaction activated by specific light, while preserving normal skin structures. This reaction results from the fixation of a photosensitizing agent on tumorous cells followed by its activation by a floodlight of a light visible at appropriate wavelength. This activation is followed by a phototoxic effect created by an irreversible oxidation mechanism that destroys tumorous cells.

The mechanism of this photochemical reaction is based on light effects on the photosensitizing molecule (1). To each kind of molecule matches a type of wavelength likely to be absorbed. In the basal state, this photosensitizing molecule presents an energy ground state (S8); under the effects of photons produced by the light, this molecule reaches a superior and unstable level of excitation. At this unstable level, the molecule holds an excess of energy that it quickly loses in three ways: heat liberation, fluorescence emission, and transition to a triplet intermediate stage. Turning back from this triplet stage to the basal stage is slow. It is during the triplet stage that the photosensitizing molecule interacts with cells' organelles by two mechanisms requiring the presence of oxygen in the targeted tissues:

- Direct mechanism: Production of toxic-free radical. In that case, the photosensitizing is damaged.

- Indirect mechanism: Transfer of energy to the oxygen. This fosters its transition to a singlet state able to oxidize amino acids, nucleic acids, and membranes' cell lipids. This mechanism prevails; in that case, the photosensitizing returns to its basal state likely to be excited by the light anew.

## Effects on Tissues

Cellular damage caused by this oxidative stress essentially affects cellular membranes and organelles of cell (mitochondria, endoplasmic reticulum, plasma membrane). These deteriorations lead to cellular necrosis (2). Histological signs of necrosis (eosinophilic cytoplasm, hyperchromatic nuclei) appear within an hour after the lighting.

This cellular necrosis is associated with apoptosis (3) phenomenon (cellular suicide). Apoptosis signs occur more lately; they can be seen 24 hours after the PDT.

Three "actors" are necessary for PDT:

- A photosensitizing molecule
- A light source
- Oxygen

## Photosensitizing

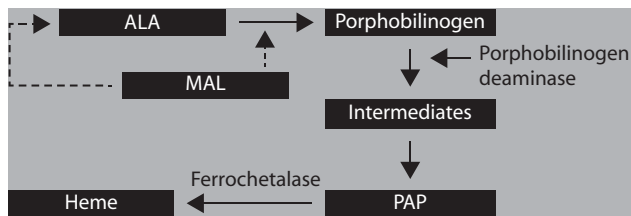
Several photosensitizing agents have been used at the beginning of the PDT, part of them were systemic sensitizing. These had no specificity and would lead to residual phototoxicity phenomena of the whole tegument imposing on patients extended resting from visible light. Thus, to be easily used in dermatology, photosensitizing requires specificities:

- To focus on tumorous cell
- Can be activated by a light penetrating into the skin
- To produce a significant amount of cytotoxic substances (free radicals and singlet oxygen)
- Can quickly be eliminated from the skin

The use of topical photosensitizing agents has been favored in dermatology. Most of them are five ALA drifts. In reality, ALA is not the photosensitizing. It is the precursor of an endogenous photosensitizing that physiologically exists in the skin and notably in keratinocytes: protoporphyrin 9 (Pp9). This porphyrin is an intermediate stage in the heme synthesis chain (Figure 48.1).

Physiologically, some mechanisms can autoregulate this heme synthesis by enzymatic systems, especially ferrochelatase, which transforms Pp9 in heme. During an ALA or methyl aminolevulinate (MAL) excess supply these autoregulation mechanisms are overwhelmed and a cellular accumulation occurs.





**Figure 48.1** Mechanism of action of ALA/MALA.

The accumulation increases in epidermic dysplasia (4,5). The real mechanism of this specific accumulation is not clearly solved. Several mechanisms are suspected:

- Intense penetration of the ALA in the epidermic dysplastic cells due to membranous modified transportation phenomena
- Increase of porphobilinogen desaminase activity
- Decrease of ferrochelatase activity

Nowadays, two kinds of Pp9 precursors are used in skin PDT: amino levulinic acid ALA (Levulan1, Kerastick1) and its precursor methyl aminolevulinic acid (Metvix1, Metvixial).

These two drifts have different properties; the MAL is more lipophilic than the ALA, which gives it better penetration in the skin. This indication will have an impact on the occlusion time necessary for the product to penetrate: 12 to 18 hours recommended for the ALA (Levulan) and 3 hours for the MAL (Metvix, Metvixia). Moreover, the specificity to the P9 accumulation in keratinocytes in differentiation is more important for the MAL than for the ALA, whereas for healthy skin, the Pp9 production is much more important after the ALA application than after the MAL one (6). The systemic diffusion of MAL is less important than the one of ALA for the mouse (7). Photoactive porphyrin synthesis detected by fluorescence for the mouse decreases faster after the MAL application than after the ALA one (8). After 24 hours, the fluorescence induced by the MAL has nearly disappeared.

As for the human healthy skin, the fluorescence decreases to 93.4% +/- 6.1% 30 hours after the cream removal to 20% MAL (9). The depth of MAL penetration measured by the fluorescence intensity is estimated to 2 mm after 3 hours of occlusion (10).

## Light Source

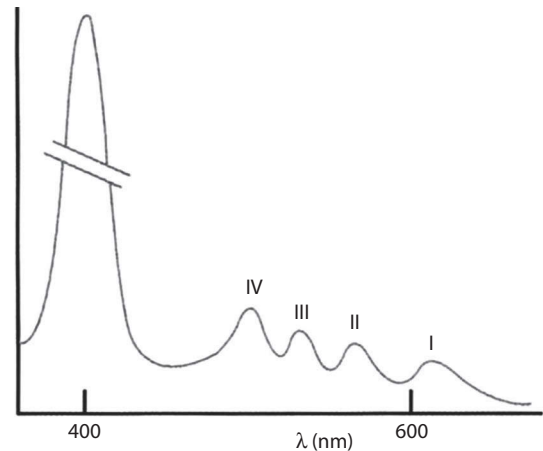
It requires two characteristics: activate the Pp9 and penetrate sufficiently into the skin to allow the destruction of the targeted lesions.

The Pp9 activation spectrum consists of several stripes of color (Figure 48.2). The maximal absorption stripe for which the Pp9 becomes more energetic lies in the blue at 410 nm, it is the Soret band. Other absorption peaks are of 510, 530, 580, and 630 nm, but they are less energizing (5).

The light penetration in the skin depends on the wavelength of the light. The longer the wavelength is, the deeper the penetration in the skin is. Thus, the blue penetrates the stratum corneum, whereas the red penetrates until 2 mm in the skin reaching the dermis hypodermis limits.

Red is preferred for the treatment of cancerous and precancerous lesions because of its better penetration into the skin. The merest absorption by Pp9 being countered by the use of a more important light energy.

Several sources can be used.



**Figure 48.2** Pp 9 activation spectrum.

- Sources of high energy
  - Lasers are attractive as they are monochromatic and very energetic. Although the lightening scope size is narrow, the exposure time is very short. As the pulse dye laser emits at 695 nm, it can be used to activate the Pp9.
  - Intense pulsed lights (IPLs) are very energetic polychromatic light sources. The light emission spectrum depends on the filters used, which allows to choose the inferior limit of the spectrum 550, 560, 580, and 590 nm.
  - For lasers, the exposure time to light is very short (on the order of millisecond kind), but the lightening scope size is broader.
- Sources of low energy
  - Slide projectors that emit between 570 and 1100 nm have been used by PDT pioneers.
  - Xenon halogens (630 nm) and fluorescent lights emit narrow spectrum and have a good energetic capacity.
  - Light-emitting diodes (LEDs) are the most frequently used. Their specificity is to emit on a very precise wavelength (variation between 5 to 10 nm). Those small diameter light bulbs are braced to one another to create lamps of which the surface is significant and the form adaptable to the targeted skin area. Their life span is long (10,000 hours) without any energy loss. They are even more attractive in that they do not emit infrared and so have no thermal impact.

Should a high- or low-energy source be chosen? A short lightening with a very strong energy will sharply activate the porphyrins, which will lead to an intense release of free radicals and of singlet oxygen responsible for the selective destruction of the targeted tissues. Nevertheless, Pp9 synthesis by keratinocytes recurs itself. Some authors advocate for the use of low-energy light sources that allow a long lightening and a continuous activation of porphyrin, which ensure the emission of less brutal and better distributed cytotoxic substances during the lightening (9). Some teams use low-energy sources made of LED (11).

One study (12) compared MAL PT with LED (530 nm, 37 J/cm<sup>2</sup>) versus MAL PDT with IPL (610–950 nm 80 J/cm<sup>2</sup>) for the treatment of actinic keratosis, the patient being his

own witness. Neither the complete remission rate at 3 months nor the cosmetic outcomes show a significant difference. On the other hand, the pain is less important with IPL as the lightening time is very short. There is no comparative study dealing with basal cell carcinoma (BCC) and Bowen disease.

#### *What Kind of Energy Can Activate Pp9 and Lead to Cell Destruction?*

The amount of necessary energy depends on the Pp9 ALA or MAL precursor, its concentration, and the lightening spectrum. The necessary energy had been evaluated for different topical (ALA, MAL) depending on the intensity of the fluorescence visible in a Wood lamp. Thus, the maximal fluence had been determined to obtain the maximal fluorescence. The latter will depend on the emission spectrum: the larger the spectrum (so outside Pp9 absorption peaks), the more important the fluence will have to be. For instance, for the MAL, the necessary energy is:

- 37 J/cm<sup>2</sup> for a narrow spectrum of 630 nm
  - 75 J/cm<sup>2</sup> for a spectrum from 570 to 670 nm
  - 85 J/cm<sup>2</sup> for a spectrum of 600 to 750 nm
- For LED, halogens, and fluorescent lamps, several indications will have an influence on this fluence (J/cm<sup>2</sup>): the power of the lamp (mW/cm<sup>2</sup>), the distance between the lamp and the skin, and the lightening time.

#### *Using Daylight to Activate Pp9: Daylight PDT (DL-PDT)*

Common adverse events are stinging and burning associated with pain. Pain is a real problem for the treatment of actinic keratoses (AK) on the face and scalp. The pain intensity is proportional to the area of the lesions, especially on face and scalp. The sudden activation of Pp9 accumulated during the 3 hours is responsible for the pain. Conventional PDT requires dedicated equipment and long time incubation (3 hours) between the photosensitizer application and the illumination. These elements can limit the availability for patients and increase costs.

Daylight PDT uses the visible light from the sun as a light source for photo activation of Pp9. The spectrum of daylight extends from 380 nm to 780 nm.

The Protoporphyrin IX absorption peaks are: in the blue light at 410 nm (Soret band), with others absorption peaks at 505 nm (green), 540 nm (yellow), 580 nm (orange) and 635 nm (red). All PpIX absorption peaks are within the visual spectrum of daylight.

The principle of this method is a continuous production and progressive activation of Pp9 avoids a sudden release of active oxygen in the skin. This photoactivation with low light intensity requires prolonged sun exposure. The patient has to be exposed less than 30 mn after the photosensitizer application and stay 2 hours exposed to the daylight. Studies on daylight PDT focus only on the treatment of actinic keratosis of face and scalp (13–16).

The protocol for daylight PDT with MAL in patients with actinic keratoses (AK) of the face or scalp (Table 48.1) is different than conventional PDT (cPDT).

### **Oxygen**

Oxygen is circulated by the blood. Therefore, the elements fostering lack of oxygen in the soft tissue will decrease the efficiency of the technique - cold, vasoconstrictors, sclerosis.

### **PDT INDICATIONS IN DERMATOLOGY** **Validated Indications**

They are at the center of studies allowing the registration of products. Today, only the MAL is registered in Europe since 2002 with the following indications:

- Actinic keratosis
- Nodular and superficial BCC when surgery is impossible
- Bowen disease

#### *Actinic Keratosis*

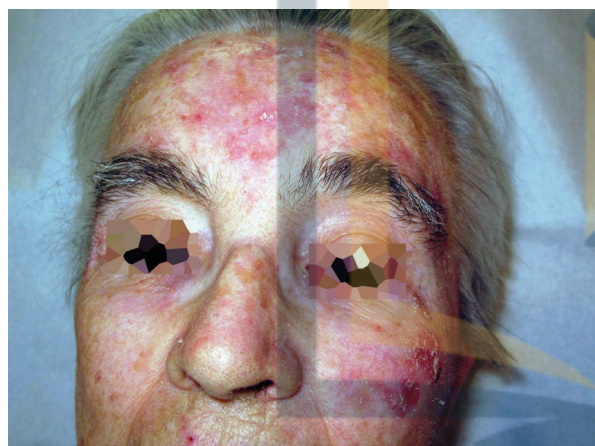
Those precancerous lesions are common on photo-exposed field of scalp, face, hands, and forearms (Figure 48.3). As they are often many on these areas, they create real fields of possible cancer development (Figure 48.4). The annual rate of transformation of AK in squamous cell carcinoma can change from one extreme to another. According to authors, it ranks from 0.25% to 16% (17). All the studies had been done with the MAL, which is the only available product on the market.

**Table 48.1** Protocol for daylight PDT

Pathology	Grades I and II actinic keratosis on the face and/or scalp. Fields of actinic damage including AK and subclinical lesions.
Period of the year when the treatment is possible	This period is a different function of the latitude. In northern Europe between March and October, in southern Europe and Australia all year long.
Weather condition	All weather conditions except rain, very cloudy weather, and if the temperature is suitable for the patient to stay comfortably outdoors for 2 h.
Treatment modality	<ul style="list-style-type: none"> <li>• Preparation of skin: thickness lesion reduction by keratolytic preparation (urea or salicylic acid) application the week before treatment.</li> <li>• Apply chemical (no mineral containing titanium dioxide, zinc oxide, which blocks visible light) sunscreen to the treatment area and to all sun-exposed areas of the skin.</li> <li>• Smooth curettage to remove scales and scabs.</li> <li>• Apply a thin layer of MAL to treatment areas, without occlusion.</li> <li>• Begin daylight exposure within a maximum of 30 mn after MAL application.</li> <li>• The patient stays 2 continuous hours outside.</li> <li>• After 2 hours the patient removes the residual cream, washes the skin, and protects the treated area with hat, cap, etc. for the rest of the day.</li> </ul>
After the treatment	<ul style="list-style-type: none"> <li>• Use moisturizer cream for 1 week after the treatment.</li> <li>• Evaluation of the treated lesions after 3 months.</li> </ul>



**Figure 48.3** Actinic keratosis.



**Figure 48.4** Fields of possible cancer development.

The studies done versus placebo (18) showed a slightly superior efficiency on complete cure rate (the lesion having completely disappeared).

Comparative studies were made versus cryotherapy (19,20), which is the reference treatment. Cryotherapy was performed using nitrogen spray and a double-freeze-thaw cycle; the MAL PDT was done regarding two protocols, either one session or two sessions with a week of interval. There was no significant difference in the complete response rate at 3 months and 5 years between cryotherapy and MAL PDT one session.

The difference between these two techniques lies in the healing result. In studies (19,20), cosmetic outcomes are seen as excellent and good according to more than 90% of the investigators. In comparison to cryotherapy, cosmetic outcomes are seen as superior for the MAL PDT.

- MAL PDT efficiency is significantly superior to the placebo: the complete answer rate per patient and per lesion increases under MAL PDT.
- MAL PDT is as efficient as cryotherapy, reference treatment of KA.
- A single session of MAL PDT is efficient on AK lesions and can be renewed if necessary.

- The MAL PDT cosmetic outcomes are considered superior as the cryotherapy ones.
- In majority, the MAL PDT treatment is considered very satisfactory by patients who had already been treated, and is preferred to former treatments.
- MAL PDT using red light is an appropriate alternative treatment for multiple actinic keratosis on a large skin surface (Figure 48.4).

In its conclusion (17), the International Society for Photodynamic Therapy (IPDT) considers that PDT can be considered as a first intention treatment in the treatment of AK.

Treatment of AK by daylight DL-PDT is in development; DL-PDT with MAL cream has labeling for this indication in some countries.

The first phase II studies were performed in Scandinavia and showed that DL-PDT with MAL is as effective as conventional PDT (C-PDT) in treating grade I and II AK on the face and scalp associated with a lower frequency of skin reactions and almost painless. Two phase II studies performed in Australia and Europa demonstrated a non-inferiority of DL-PDT versus C-PDT. The multicenter trial in Australia DL-PDT reported complete lesion response rate of 89% of mild AK on the face/scalp, and 93% for C-PDT. The similar protocol study in Europe demonstrated efficacy on mild and moderate AK (70% of lesions cleared after one session of DLPDT, versus a comparable rate 74% for C-PDT). In these two studies DL-PDT is significantly almost painless.

#### *Superficial BCC*

BCCs are the most frequent tumors occurring in human. The superficial forms are the less aggressive and are considered low-risk lesions outside the face H zone. Most of the time, these lesions are of large area or multiple with a risk of having significant of numerous scars after the surgical cure. PDT is a noninvasive treatment and the quality of its healing result has to be taken into account in the therapeutic decision-making process.

A European multicenter study carried out in seven countries compared the MAL PDT one session to cryotherapy (freezing of at least 20 seconds) in two groups of patients carrying BCC (21,22). The rate of complete answers at 3 months and at 30 months and 5 years per patient and per lesion did not show significant differences between the two groups. The healing quality assessed by investigators and patients is superior in the MAL PDT group. More patients had an excellent cosmetic outcome with PDT than with cryotherapy. Those recurrence rates are higher compared to surgery. That is why it is better avoiding risky face areas, but PDT offers a better cosmetic outcome than simple excision surgery (23).

PDT is a therapeutic alternative for the treatment of BCCs as surgery would be hard to perform because of the localization, the size, the number of lesions (Figure 48.5), the pathologic state of the patient, or because the healing risk would be disproportionate for a low-risk lesion. The other advantage compared to other nonsurgical techniques such as cryotherapy and electro curettage is that it does not leave any scar, which is satisfying to the patient (Figure 48.6). Moreover, in the case of recurrence, PDT can be repeated and surgery remains possible, as there is no skin modification.

In superficial CBC treatment:

- The clinical efficiency of the MAL PDT is equivalent to the one of cryotherapy.





**Figure 48.5** Multiple superficial basal cell carcinoma on a patient treated by X-ray therapy for Hodgkin disease.



**Figure 48.6** Cryotherapy scars for basal cell carcinoma.

- This efficiency is maintained in an equal way after five years of follow-up for both treatments.
- The PDT cosmetic outcome is better than the one of cryotherapy.

In its conclusions, the IPDT (17) considers the PDT as an effective and reliable treatment option for BCCs that offers excellent or good cosmetic outcomes. PDT offers an advantage in the treatment of large, extensive, and multiple lesions.

Photodynamic diagnosis (PDD) fluorescence diagnosis serves as a valuable noninvasive diagnostic and treatment tool. Following topical application of the photosensitizer, a large amount of Pp9 selectively accumulates in the neoplastic cells. When the skin is illuminated with a UVA light source (370–405 nm), the tumoral area shows a distinctive pink to red fluorescence on a background of blue light. This PDD is useful to detect tumors but is not sufficient to evaluate their margins. Margin evaluation with PDD is better than clinical evaluation but does not correspond with the real margins found with Mohs surgery (24).



**Figure 48.7** Bowen disease before treatment.



**Figure 48.8** Bowen disease after two sessions.

#### *Nodular Basal Cell Carcinoma*

The difficulty in the treatment of these lesions lies in the penetration of both the light and the photosensitizer in the superior lesions at 2 mm of thickness. For this kind of tumor, debulking is performed preliminary to the photosensitizing application. Several studies (25) show 3 months complete answers rate between 73% and 94% with the MAL PDT. At 5 years, recurrence rates reach 14%.

A comparative study between MAL PDT and surgery showed that the 3 months complete answers rate was not inferior for the PDT (91% versus 98% for surgery). At 60 months, the recurrence rate was 14% versus 4% for surgery (26).

According to patients, the cosmetic outcome is superior to surgery.

In its conclusions, the IPDT believes PDT is an effective and reliable treatment for thin lesions, with the advantage of good cosmetic outcome.

#### *Bowen Disease*

This squamous cell carcinoma in situ is frequent in elderly patients. It appears anywhere on the skin but mainly on the lower legs. Approximately 3% transforms into invasive carcinoma. There are often healing problems on leg localization for elderly patients regarding circulatory insufficiency. The treatment of reference is surgery when possible or 5-FU topically (Figures 48.7 and 48.8).

A European multicenter comparative random study (27) assessed the MAL PDT in Bowen disease treatment. The assessed therapeutics were MAL PDT or PDT placebo, 5-FU, and cryotherapy.

In Bowen disease treatment:

- MAL PDT efficiency is significantly superior to placebo.
- The answer rate per patient or per lesion is similar when the patient is treated by MAL PDT, cryotherapy, or 5-FU.
- Those results are maintained 24 months after the treatment; the lesion recurrence rate under MAL PDT is the same as under cryotherapy or 5-FU.
- The great majority of investigators believe that the cosmetic outcomes are excellent or good after the treatment by MAL PDT. These results are superior to those obtained after cryotherapy.

In its conclusions, the IPDT considers PDT to be an effective treatment for Bowen disease. This therapeutic can be considered as a first-line treatment for Bowen disease. After repeated treatments, nonresponders should be considered for surgery.

### Out-of-Label Indications

PDT had been used in multiple indications in studies started with varying protocols. These several studies had been taken up again in a consensual conference by North American authors (28).

#### Acne

The mechanism of action is not clearly elucidated. There is a clear diminution of *Propionibacterium acnes*, but the target of free radicals and singlet oxygen is essentially the sebaceous gland (29).

Atrophy or even destruction is demonstrated in biopsy after one PDT treatment. Comparison can be made with isotretinoin that induces a cellular apoptosis of sebocytes and reduces the sebum excretion rate. This glandular atrophy is not permanent and seems to be restored with time, but there is a lack of long-term histological data on sebaceous gland atrophy after PDT.

PDT is especially useful for inflammatory acne but side effects are sometimes serious. Observations report varying degrees of pain, erythema, edema, blistering, and acute flareup. This last side effect can be compared to isotretinoin. Optimization of efficacy-tolerance ratio is necessary.

Concentration of photosensitizer, time of application, occlusion or not, and fluence parameters are certainly different from those used to treat nonmelanoma skin cancer. Mavilia et al. report a significant improvement of inflammatory acne. It appears that the nature of photosensitizer has an impact on efficacy.

The acne indication had benefit from many studies, especially with the ALA PDT and several light sources. The consensual conference conclusions are as follows:

1. The best indications are inflammatory and cystic acne.
2. The improvement is limited in comedonal acne, except in a study with pulsed dye laser (PDL).
3. Acneiform flares appear after each treatment. They seem to depend on the photosensitizing concentration, the application time, and the light source. Nevertheless, these therapeutic protocols are not clearly defined yet.

#### Photoaging

This indication has also been submitted to several works in Canada and in the United States. Pp9 is prone to store in keratinocytes in multiplication. Therefore, PDT is particularly recommended in severe photodamage: severe elastosis associated with AK, early skin cancer, pigmentary changes, vascular changes (erythema, telangiectasia), and rhytides (30).

Photorejuvenation action of PDT was found after AK treatment and the skin on the treatment area appears younger, without rhytides, smooth with a best trophicity. This improvement of the appearance of skin corresponds to significant molecular changes in the photodamaged skin. Type I and type III procollagen mRNA significantly increased after treatment with ALA, PDT, and PDL versus PDL alone. Historically, increased proliferation and changes in epidermis thickness were observed. High-resolution echography shows an increase in skin thickness and a reduction of subepidermal low echogenic band thickness.

Clinically, there is an improvement of signs of photo-damage: mottled pigmentation, fine lines, roughness, and sallowness of the skin. There is no change in deep wrinkles, telangiectasia, and sebaceous gland hypertrophy.

The majority of North American studies deal with PDT with ALA and IPL with various protocols regarding photosensitizing concentration, time of application, and light energy. There is no doubt that the decrease of the concentration and of the application time diminishes side effects but it also implies a very weak synthesis of Pp9, which can damage the technique's efficiency. The use of IPL PDT versus IPL only would reduce the number of IPL sessions to obtain the same result on photoaging signs. European studies with MAL PDT also confirm the efficiency on photoaging (31).

#### PDT and Onychomycosis

Some case reports suggest that PDT may represent an alternative treatment in therapeutic strategy for onychomycosis. The nail plate and the hyperkeratosis nail bed are first removed, and the treatment is repeated between three and six times according to the studies. The MAL PDT protocol is the same as that in oncologic indications: MAL 20% 3 hours with occlusion, uncoherent red light (630 nm), 37 J/cm<sup>2</sup>. Patients do not report pain during or after irradiation or after.

Other out-of-label indications include:

- Warts
- Dyskeratosis: Hailey-Hailey; Darier
- Healing
- Extramammary Paget
- Psoriasis
- Hair removal
- Cutaneous leishmaniasis
- Cutaneous T cell lymphoma
- *Molluscum contagiosum*

### PDT IN PRACTICE

PDT a simple technique; non-operator-dependent, that can easily be performed at a medical office. The PDT session takes place in three stages:

- Lesion preparation: soft cleaning with the curette to remove loose scales and crusts, application of the MAL as a cream covering the lesion with a 1-cm margin at the edge. Cover with an occlusive dressing and then with a totally opaque dressing, especially on exposed areas.

- Keep the cream and the dressing on for 3 hours.
- Remove the dressings, wash the remaining cream with a compress and saline solution, and start the lightening. Parameters of LED lamps are generally already set. Depending on the lamps, it usually lasts between 8 and 10 minutes (Figure 48.9)

Post-treatment care is simple: the treated zone needs to be protected from light for at least 24 hours, then a dry dressing and application of emollient can be done daily.

Right after the procedure, the reactions are pain, erythema, and edema. Later, the following reactions may occur:

- Squamiae
- Crusts
- Suppuration
- Blisters
- Skin ulceration
- Skin pigmentation after sun exposure

### PDT and Pain

- All patients report an unpleasant burning, stinging, or prickling feeling.
- Around 20% of patients report pain ranking between 7 and 10 (on a pain analogical scale) requiring specific care (32).
- Pain appears at the beginning of the lightening and increases after the first few minutes, reaches a plateau, and decreases slowly once the lightening is stopped (33).
- The pain origin seems to be neurological as less GABA receptors intervene (34).
- This pain depends on (35):
  - Localization: more intense at the scalp and face than on the rest of the body (36)
  - Kind of tumor: more important for AK than for BCC and Bowen disease
  - Lesion size: the pain increases with the size
  - Fluence rate: pain is reduced with lower fluence rate but time exposure is increased.
- The physician has to take the pain into account during the lightening
  - Vocal anesthesia: do not leave the patient alone, talk to reduce his anxiety, modify the pain, and suggest a solution to reduce it.

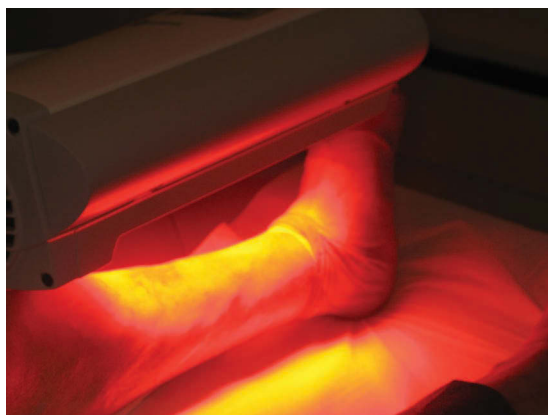


Figure 48.9 Lesion illumination.

- Physical means aimed at cooling down the pain are the most efficient: fan, water spray, liquid nitrogen spray. If the pain is too severe, the procedure can be interrupted for a few minutes to cool down the skin before restarting the lightening (24).
- Nerve block and local anesthesia without vasoconstrictive agent may be useful.

PDT should be considered as a new therapeutic resource that especially fits numerous AK on large area treatment. Future studies will have to confirm the PDT preventive aim in field cancerization. Regarding Bowen disease and CBCs, PDT represents a solution for broad and multiple lesions when surgery is hard to perform or risky. We can only wish that in a few years indications out of nonmelanoma skin cancer will benefit from studies able to define precise protocols.

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## Cosmetic Cryotherapy

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### INTRODUCTION

Most dermatologists and dermatologic surgeons limit themselves to warts and keratoses. Some also treat skin cancers. Many are unaware of the diverse range of conditions, pathologic and cosmetic, amenable to treatment with liquid nitrogen. Cryosurgical treatments, when applied correctly to appropriately selected patients, produce excellent cosmetic results, which justifies a description of these techniques in a cosmetic dermatology text. As liquid nitrogen is freely available and cheap, and can be used without local anesthetic, it is often thought of as “low-tech” and unable to compete with the newest lasers. On the contrary, cryosurgery is the equal of high-tech alternatives for therapeutic efficacy and aesthetic outcome for a wide range of conditions. Despite these advantages, cryosurgery tends to be underutilized. Cryosurgery developed as a dermatological treatment modality about a century ago, with the first cryogens being liquid air and compressed carbon dioxide snow (1). The following is a description of the specific cryosurgical techniques for treatment of an array of lesions seen by the cosmetic and dermatologic surgeon and describes the steps to be taken to achieve good cosmetic results.

### TECHNIQUE AND TERMINOLOGY

Various methods have been devised in the use of cryotherapy of lesions.

1. Open spray freeze technique
2. Applicator technique
3. Cryoprobe method
4. Thermocoupler method

### Timed Spot Freeze Technique

The spot freeze technique involves the use of a liquid nitrogen spray gun (Brymill Cryac Gun, Owen Galderma, Fort Worth, Texas, U.S.) that, through an appropriate nozzle, emits an open spray of liquid nitrogen (Figure 49.1). In general, the nozzle size is chosen according to the size of the lesion; however a “D” nozzle will be suitable for most benign lesions. The flask should be two-thirds full to ensure an even flow of cryogen. Overfilling the flask can lead to the valve icing over. The nozzle is held 1 cm from the skin surface at a 90° angle and the center of the lesion is sprayed until an ice ball forms that encompasses the lesion and the desired margin. The “designated ice field” may need to be marked out with a suitable pen, as freezing blurs lesion margins. Once the ice field reaches the desired size, freezing is stopped temporarily to allow palpation of the ice ball to ensure that the lesion is entirely frozen. Once satisfied of adequate ice

formation, the spray of liquid nitrogen may recommence and continue for the appropriate treatment time. During this time, the ice field size should remain constant, with the spray of liquid nitrogen adjusted with the trigger on the spray gun appropriately. If more than one freeze-thaw cycle (FTC) is needed, then complete thawing should occur before the next cycle. This usually takes longer than 60 seconds. This can be assessed by palpation between the finger and thumb again for the presence of an ice ball as well as waiting for the disappearance of the frozen-white surface appearance. Lesions greater than 2 cm in diameter are generally best divided into overlapping treatment fields to ensure that the timed spot freeze technique adequately treats all parts of a large lesion (Figure 49.2). Using only one treatment field may not adequately freeze the deep margins at the periphery of a large lesion. The open spray technique is extremely versatile and can be used for most easily accessible lesions. Variations do exist: the paintbrush method involves spraying starting from one side of the lesion and



**Figure 49.1** Liquid nitrogen cryosurgery equipment. Two standard machines together with pedestal containing a variety of sprays, spray needles, and probes.



**Figure 49.2** Bowen disease: showing method of freezing successive 2-cm overlapping circles to ensure uniformity of treatment of the whole lesion.

moving up and down across the lesion, and the spiral method, where treatment starts in the center of the lesion and moves outward in ever-increasing circles (2). These two techniques are particularly useful for larger lesions and when a light, superficial freeze is desired. Hyperpigmentation may complicate the treatment of some lesions. Hyperpigmentation is particularly common in people with Fitzpatrick type III and IV skin. Although usually temporary, it may take 3 to 4 months to resolve. In general before embarking on this treatment in people with darker complexions, a small test should be performed first.

Hypopigmentation is generally only seen after large doses of liquid nitrogen are applied to the skin, for example, in the treatment of skin cancer. In very fair people, this may go unnoticed. For patients with an olive complexion, to prevent a sharp demarcation between the hypopigmented treated area and surrounding normal pigmentation, “feathering” may be performed. This involves spraying the border of the ice field to ice formation to produce mild hypopigmentation that reduces the contrast with untreated skin. A better cosmetic outcome may be achieved when treating lesions within a single cosmetic unit by light spraying the entire cosmetic unit. Because any depigmentation so produced will be permanent, it is better to proceed cautiously when deliberately lightening the skin.

### Cotton-Tipped Dipstick

The dipstick technique involves dipping a cotton wool bud into a cup containing liquid nitrogen and firmly applying the bud to the lesion until a narrow halo of ice forms around the bud. This method is most effective with a cotton bud slightly smaller than the lesion to be treated and the cotton bud is home-made using loosely wrapped cotton wool around the wooden orange (or satay) stick. This holds more liquid nitrogen than prefabricated cotton buds. Pressure applied to the skin facilitates lower temperatures as a wider area comes into contact. The pressure can also be used to empty vascular lesions leading to a greater fall in temperature. As adenovirus is capable of survival within liquid nitrogen and other viruses may potentially survive and cross-contaminate one's store of nitrogen (3), redipping is therefore not recommended. Decanting a small amount of liquid nitrogen into a separate new disposable container for each patient treatment is preferred. Dipping a cotton bud into the flask of a spray unit leaves behind cotton fibers

that will eventually block the nozzle and lead to uneven flow of cryogen.

### Cryoprobe

Various types of cryoprobes are available; the choice of probe depends on the type and site of the lesion (4). Cryoprobes may be attached to the liquid nitrogen spray guns and are cooled by the stream of nitrogen. The probe is applied directly to the lesion. A thin layer of Vaseline or similar gel may be used on the tip of the probe to facilitate contact with the lesion and release of the probe on thawing. As cooling occurs through conduction of heat from the skin, comparatively longer periods of freezing are required with this technique. Once the ice forms, the probe and lesion is gently retracted to prevent further injury to the surrounding tissues. Direct pressure on a vascular lesion can also be used to empty the lesion and produce a greater fall in temperature, but the amount of pressure will influence the depth of freeze and lateral spread.

### Thermocouple Device

To treat malignant lesions, a temperature probe coupled to a digital thermometer that can read 10 to 75°C can be used. Local anaesthetic is injected in to the lesion, and a temperature probe is inserted into the estimated depth of the lesion. Usually a metal or styrene cone is used to concentrate the freeze. The liquid nitrogen is sprayed into the cone until the desired temperature is reached, usually  $-50$  to  $-60^{\circ}\text{C}$ . The process can be repeated until the desired destruction is achieved.

### MECHANISM OF ACTION

The mechanism of lesion destruction is similar for all cryogens and can be divided into four phases:

1. Heat transfer
2. Cell injury
3. Vascular stasis and occlusion
4. Inflammation

### HEAT TRANSFER

The rapid freezing of skin lesions depends on a quick transfer of heat from the skin to a heat sink, for example, liquid nitrogen. The rate of heat transfer is dependent on the temperature difference between the two, in this case 36 to  $-196^{\circ}\text{C}$ .

### TISSUE INJURY

On application of the cryogen, the initial event is extracellular ice formation. This commences at  $-10$  to  $15^{\circ}\text{C}$ . The transformation of water into ice leads to loss of water from the extracellular compartment. This concentrates the extracellular solutes and sets up an osmotic gradient across the cellular membranes. The movement of water across membranes is exacerbated by mechanical compression from extracellular ice crystals that damages the cell membrane. The movement of water out of the cell leads to an intracellular concentration of solutes. This damage is mostly reversible. Irreversible damage is due to intracellular ice formation and is dependent on the rate of cooling and the minimum temperature achieved. The faster the cooling and the lower the temperature, the greater the intracellular ice formation. Ice crystals do not form until temperature  $-5$  to  $-10^{\circ}\text{C}$ . The ice damages organelles (mitochondria and endoplasmic reticulum) and further concentrates electrolytes intracellularly.



The rate of thawing also influences the degree of damage, with long thaw times being associated with greater damage due to the accumulation of intracellular electrolytes. Rapid freezing and slow thaw maximize tissue damage to epithelial cells and is most suitable for the treatment for malignancies. In addition, repeat FTCs produce more tissue injury than a single freeze and thaw. The minimum temperature needed for destruction is cell-specific. Cryosurgery causes selective destruction of different cell or tissue types, depending on the temperature reached. The collagen-containing connective tissue types are more resistant to cryodamage than the epidermal cell types, especially melanocytes and deeper epidermal cell layers. Mild freezing causes dermoepidermal separation. Keratinocyte destruction requires a minimum temperature of at least  $-30$  to  $-40^{\circ}\text{C}$ , while melanocytes are much more sensitive, dying at  $-4$  to  $-7^{\circ}\text{C}$ . This fact is the reason for the resulting hypopigmentation following cryotherapy on darker skin individuals. Repigmentation often occurs with migration of melanocytes from the edge of the frozen zone or from undamaged melanocytes within hair follicles. Fibroblasts produce less collagen after a rapid thaw. Therefore, a rapid thaw may be more suitable for the treatment of keloids or benign lesions in areas prone to scarring.

### VASCULAR STASIS AND OCCLUSION

Cold temperatures lead to vasoconstriction and endothelial damage. At  $-15^{\circ}\text{C}$ , endothelium is damaged and platelet aggregation, along with microthrombus formation, leads to ischemic necrosis of the treated tissue in succeeding hours. A reflex hyperemia lasting minutes to hours also occurs, appearing as a purplish discoloration at the edge of the defrosting lesion.

### INFLAMMATION

Over the next 24 hours, inflammation develops in response to cell death and further contributes to the destruction of the lesion. Significant edema may occur, especially where the skin is loosely attached to underlying tissues, for example, the dorsum of hand and around the orbital region. Histological changes first become apparent 30 minutes after cryotherapy. Eosinophilia and vacuolation of the cytoplasm appear initially, and the entire cytoplasm becomes homogenized over the next few hours. This is accompanied by nuclear pyknosis. There is separation at the dermoepidermal junction, resulting in blister formation. At 2 hours, edema, focal capillary damage, and isolated microthrombi are seen; at 5 to 8 hours, there is segmental necrosis of blood vessels. There is initially a wet exudative wound, nature's own biologic dressing, followed by a dry eschar and, usually within a month, a well-healed hypopigmented scar.

### LESION SELECTION

Accurate diagnosis is essential to determine duration of the freeze required. An appropriate biopsy of the lesion should be performed if there is any diagnostic uncertainty. If a punch biopsy specimen is taken from the center of the lesion, information regarding the thickness of the tumor will also be gained. Vast experience in the treatment of numerous and varied skin lesions by cryosurgery has been accumulated over the past 30 years since this treatment was popularized by Zacarian for skin neoplasms (5,6). Much of this experience is summarized in Tables 49.1 to 49.4, while that pertaining to skin cancer can be found elsewhere (7).

**Table 49.1** Suggested treatment regime for disturbances of pigmentation and melanocytic lesions

Lesion	Technique	Time, number of FTCs	Margin	Sessions and interval	Response
Melasma	OS	Uniform ice formation $\times 1$	Feathering	4–6 weekly according to response	Moderate
Idiopathic guttate hypomelanosis	OS	5 sec, $\times 1$	1 mm	4–6 weekly according to response	Moderate to good
Tattoos	OS	30 sec, $\times 2$	1 mm	4–6 weekly according to response	54% improved
Freckles	P	Uniform ice formation, $\times 1$	Feathering	Usually only single treatment required	Variable
Lentigo simplex	OS or P	Light, $\times 1$	Feathering	Usually only single treatment required	Good
Solar lentigo	OS or P	5–10 sec, $\times 1$	Feathering	Usually only single treatment required	Good

Abbreviations: OS, open spray technique; P, cryoprobe technique; FTS, freeze-thaw cycle.

**Table 49.2** Suggested treatment regime for vascular lesions and nevi

Lesion	Technique	Time, number of FTCs	Margin	Sessions and intervals	Response
AIDS-related Kaposi sarcoma	OS	10–30 sec, $\times 2$	3 mm	3 at 3 weekly intervals	80% improved
Venous lake	P	10 sec, $\times 1$	1 mm	Usually only single treatment	Excellent
Cherry angiomas	P	10 sec, $\times 1$	1 mm	Usually only single treatment	Good
Angiokeratoma of Mibell	OS or P	10 sec, $\times 1$	1 mm	3 at 2 monthly intervals	Good
Angiokeratoma of the scrotum	OS or P	5–10 sec, $\times 1$	1 mm	3 at 2 monthly intervals	Good
Spider nevus	P	10 sec, $\times 1$	1 mm	3 at 6 weekly intervals	Good
Capillary hemangioma	P	5–30 sec, $\times 2$	1 mm	2–4 at 8 weekly intervals	Excellent
Cavernous hemangioma	P	5–30 sec, $\times 2$	1 mm	2–4 at 8 weekly intervals	Excellent
Pyogenic granuloma	P	15 sec, $\times 1$	1 mm	1–2 at 4 weekly intervals	Excellent

**Table 49.3** Suggested treatment regime for cysts, tumors, and nevi

Lesion	Technique	Time, number of FTCs	Margin	Sessions and intervals	Response
Acne cyst	OS or D PB to peeling	5–15 sec, × 1	–	2–3 at monthly intervals	Good to excellent
Milia	P	Ice formation × 1	1 mm	Usually only single treatment	Good
Myxoid cyst	P or OS	30 sec × 2	1 mm	1–3 at 8 weekly intervals	86% improved
Syringoma	P	Ice formation × 1	1 mm	2–3 at 1–2 monthly intervals	Good
Trichoepithelioma	P	Ice formation × 1	1 mm	2–3 at 1–2 monthly intervals	Good
Trichilemmal cyst	OS	Ice formation × 1	1 mm	2–3 at 1–2 monthly intervals	A minority respond
Steatocystoma multiplex	OS	Ice formation × 1	1 mm	2–3 at 1–2 monthly intervals	A minority respond
Skin tag	OS or forceps	5–10 sec, × 1	1 mm	Usually only single treatment	Excellent
Hidrocystoma	OS or P	Ice formation × 1	1 mm	2–3 at 1–2 monthly intervals	Small: good Large: poor
Dermatofibroma	OS or P	30 sec × 1	2 mm	1–3 at 1–2 monthly intervals	90% improved
Seborrheic keratosis	OS or D or P	Ice formation × 1	1 mm	Usually only single treatment	Excellent
Sebaceous hyperplasia	OS or P	5–15 sec × 1	1 mm	Usually only single treatment	Good
Chondrodermatitis nodularis helices	OS or P	15 sec × 1	2 mm	2–3 at 1–2 monthly intervals	15–20% improved
Verrucous nevus	OS	5 sec × 1	1 mm	Up to 5 at 1–2 monthly intervals	Excellent
Hyperkeratosis nevoid of the nipple	OS	20 sec × 1	1 mm	Up to 5 at 1–2 monthly intervals	Excellent
Acrokeratosis verruciformis	OS	5 sec × 1	1 mm	Several at 6–8 weekly intervals	Excellent
Dermatosis papulosa nigra	OS or P	Ice formation × 1	Nil	Several at 6–8 weekly intervals	Excellent, but may depigment
Benign lichenoid keratosis	OS	5 sec × 1	1 mm	2–3 at 6–8 weekly intervals	Good
Adenoma sebaceum	OS	5–20 sec × 1	1 mm	3–6 at 3 weekly intervals	Satisfactory

Abbreviations: OS, Open spray technique; P, Cryoprobe technique; FTS, freeze-thaw cycle; D, freeze-thaw cycle

**Table 49.4** Suggested treatment regime for various other conditions

Lesion	Technique	Time, number of FTCs	Margin	Sessions and intervals	Response
Keloid	OS or P	15–30 sec, × 1	1 mm	5–10 at 4–8 weekly intervals	Variable
Acne scar	OS	Face 5 sec, × 1 Back 5–15 sec, × 1	1 mm	1–3 at 4–8 weekly intervals	Good to excellent
Rhinophyma	OS	30 sec, × 2	Entire nose	4–6 at 8 weekly intervals	Satisfactory
Xanthelasma	OS	5 sec, × 1	1 mm	2–3 at 4–8 weekly intervals	Satisfactory
Alopecia areata	D	2–5 sec, × 1	Nil	4 at weekly intervals	Satisfactory
Porokeratosis plantaris discreta	OS	Ice formation × 1	2 mm	2 at 2 weekly intervals	90.5% improved
Elastosis perforans serpiginosa	OS	10 sec, X 1	1–2 mm	2 at weekly intervals	Excellent

Abbreviations: OS, open spray technique; P, cryoprobe technique; FTS, freeze-thaw cycle; D, freeze-thaw cycle.

## VASCULAR LESIONS

Spider nevi require only a light freeze. A 5-second single FTC is usually ample. Cryosurgery is a good alternative to fine wire diathermy for people with pale complexions, especially for diffuse lesions with more than one feeding vessel.

Diffuse telangiectasia, such as that associated with rosacea, can also be treated with good results and is a substantially cheaper alternative to the use of a pulsed dye laser. Pyogenic granulomas also respond well (Figure 49.3). Cryosurgery is also useful for palliation of HIV-associated Kaposi sarcoma.



(a)



(b)

**Figure 49.3** Pyogenic granuloma (a) before and (b) after cryospray.

Small individual lesions (and a 3-mm margin) can be treated with a 15- to 30-second single FTC, with an 80% complete response. Cryoprobes are useful when treating venous lakes, since they allow the operator to empty the lesion during treatment, which leads to lower tissue temperatures and higher cure rates. A single 10-second FTC is usually sufficient.

There is a great variation in the size and depth of cavernous and capillary hemangioma, and this is paralleled by the variation in freeze times required. For small thin lesions, a single 5-second freeze may suffice, while for larger lesions a 30-second double FTC will be required. Experience in the treatment of these lesions allows the operator to better judge the length of treatment required.

## ACNE

The first treatment to be advocated for acne was a solid carbon dioxide slush that acted as a peeling agent to reduce the oiliness of the skin and hastened the resolution of comedones and papules as well as improving depressed pitted scars. This was superseded by cotton-tipped applicators dipped in liquid nitrogen, which has also been used for larger acne cysts. Better results and better control can be achieved with the open spray techniques (8). Small inflammatory papules require a 2- to 5-second single FTC, while the large cystic lesions of acne conglobata may require a 15- to 20-second single FTC, depending on their size. Open spray liquid nitrogen has also been used as an alternative to dermabrasion and laser resurfacing for diffuse scarring (9). The skin surface is divided into squares, each about 4 cm on a side. Using the paintbrush technique, each segment is frozen for between 5 and 15 seconds, depending on the depth of the desired peel. Areas of hypertrophic scarring may require longer freezes because of the relative cold insensitivity of collagen, while areas around the eyes, where the skin is thin, only requires 5- to 10-second single FTCs. If more than one treatment is planned, 1 month is the suggested interval between sessions. Of the patients so treated, 95% were reported to have had good or excellent results, which were similar to those achieved by superficial dermabrasion, with the advantage of their use in the presence of active acne. Pigmentary changes are often seen in ethnic patients and care should be taken when using this technique on darker skin (10).

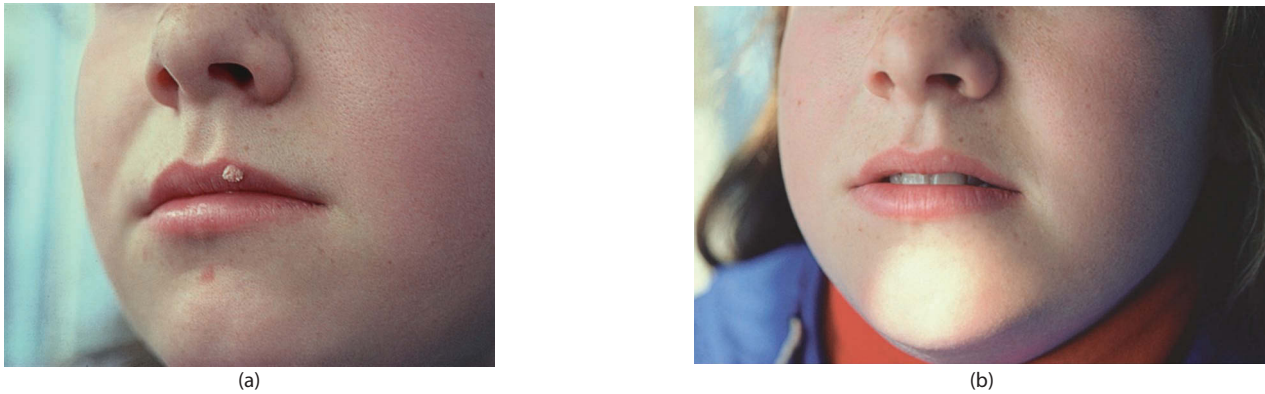
## SUN DAMAGED SKIN AND FACIAL PEELING

Solar keratoses, solar lentigines, solar elastosis, sebaceous hyperplasia, colloid milium, and the fine wrinkles of solar aging are all within the realms of unsightly sun-induced lesions amenable to cryosurgery. These lesions occur on highly visible sites, such as the face and hands, and may cause patients distress. Cryosurgery is an excellent option for limited disease. Many clinicians ignore sebaceous hyperplasia; however, a single 5-second FTC will often make these lesions disappear. The same applies to solar elastosis, solar lentigo, and many solar keratoses. For widespread changes, full-face cryopeels can be used for effective depth-controlled removal of actinic keratoses, pigmented lesions, and seborrheic keratoses. Healing begins immediately and is usually complete within 10 days. The skin is left smoother, pinker, and tighter, and the results are equivalent to those of a chemical peel; however, there is greater control of the depth of the ice field, so it can be adjusted to accommodate localized lesions (9).

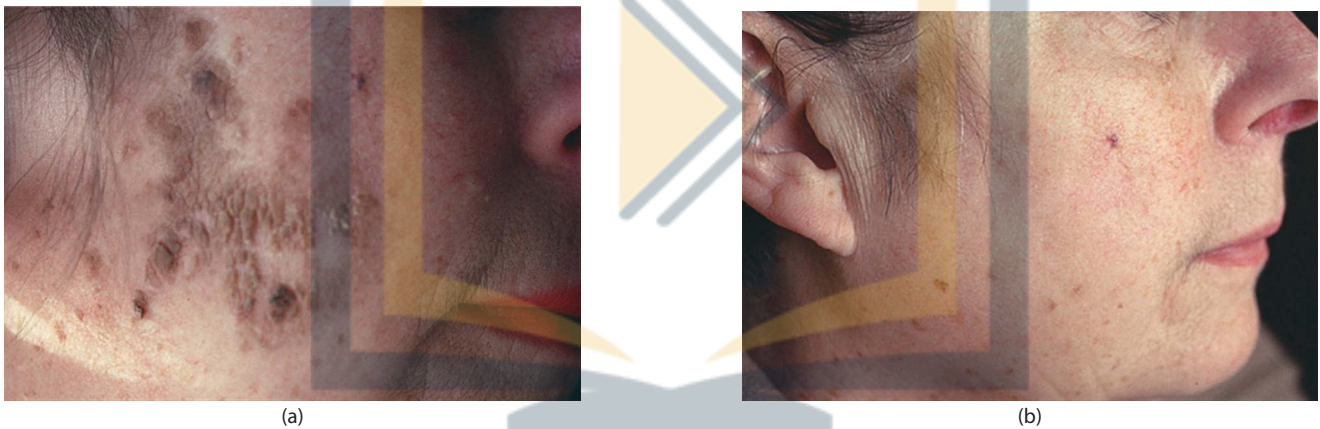
## VIRAL WARTS

These are due to the human papillomavirus. On different body sites, warts often show different morphologic types. The cryosurgical treatment scheduled will vary with the site and type: Common warts are mainly seen on hands, fingers, and knees. For flat lesions, a single FTC using the open spray for 10 seconds is generally sufficient for an initial treatment. For hyperkeratotic wart, it is often best to pare the keratosis prior to freezing. Periungual warts often require multiple freezes with gradually increasing dose. The spray should be directed laterally to avoid overfreezing the nail matrix and to prevent pterygium. Plane warts are smooth, small, flat-topped papules most commonly seen on the back of the hands and around the mouth. They are often multiple and can Koebnerize. Each wart should be frozen individually using the open spray with an E tip for 3- to 5-second single freeze. Plantar warts (verrucae) typically occur on weight-bearing areas of the sole. Prior to cryosurgery, they should be pared to remove excess hyperkeratosis, then frozen with the open spray and a B or C nozzle for 10 to 20 seconds depending on size and thickness. Repeat treatments at three to four weekly intervals are generally required. For recalcitrant lesions a double freeze thaw should be used.





**Figure 49.4** Filiform wart of upper lip (a) before and (b) after fine “needle” cryospray.



**Figure 49.5** Large seborrheic keratosis of right cheek (a) before and (b) after cryospray.

Filiform or digital warts are finger- or frond-like warts and are most common on the face, neck, and scalp (Figure 49.4). Each lesion should be sprayed with a fine “needle” to ice formation plus 10 seconds of continued spray. Treatment may need to be repeated after 2 to 4 weeks. The likelihood of recurrence is dependent on the number of lesions, the user, and the technique used. With a gentle technique cure rates are estimated around 64.4% and this is increased with more aggressive treatment (11). Aggressive treatments are more likely to lead to increased side effects. A less aggressive approach should be used where skin and subcutaneous tissue are thin (12).

### SEBORRHEIC KERATOSIS

Flat lesions can be effectively treated with a 5-second single FTC (Figure 49.5), but as keratin insulates the underlying epidermis from the cold, large hyperkeratotic lesions may still survive 30-second double FTCs. A recent pilot study examining 25 patients with SK treated with cryotherapy versus curettage found that 60% of patients preferred cryotherapy and rated their cosmetic outcome as better with cryotherapy (13). The main pitfalls of treatment are the induction of permanent

alopecia, if hair-bearing areas are treated, and the induction of transient hyperpigmentation. It is for that reason that dermatosis papulosa nigra occurring on pigmented skin is best treated cautiously, and preferably with a test patch of a single lesion.

### RHINOPHYMA

Cryosurgery has been used to treat rhinophyma (14). However, in our hands, it has proved to be less effective than dermabrasion or serial shaving. However, there have been a number of case reports detailing successful methods for treating rhinophyma. In one case report a patient was treated with two or three FTC over a number of sessions using the paintbrush method. Sessions were spaced fortnightly and combined with the use of spironolactone to reduce sebum excretion and pore size (15). Thirty-second double FTCs are recommended and can often be performed without anesthesia, or solely with EMLA cream. Multiple treatments are required, but are well tolerated. For mild cases, cryosurgery can still be considered as an inexpensive option with low risk and low morbidity that has shown some success.



**Figure 49.6** Tattoos (a) before and (b) 4 months after liquid nitrogen spray.

## TATTOOS

Good results have been seen after cryosurgery of tattoos in up to 50% of cases (Figure 49.6) (16). However, newer technologies including q-switched lasers are the preferred option for tattoo removal since cryotherapy can leave the patient with skin discoloration and ink retention (17). Cryotherapy provides complete, rapid, and cheap tattoo removal and for some patients desperate for the treatment there is no affordable alternative (18).

## KELOIDS

Many cryosurgeons disappointed by the apparent poor response of keloids to liquid nitrogen abandoned this form of treatment. Various techniques had been tried, including prophylactic and intralesional cryotherapy, with some success. Results varied between 20 and 75% scar reduction (19–23). The incidence of hypopigmentation in intralesional cryotherapy is lower than in contact cryotherapy (24). A prospective clinical trial published in 2010 examined surgical excision with post-operative radiotherapy versus cryotherapy with intralesional steroids. The study revealed that while surgery with cryotherapy and intralesional steroids had more side effects and a higher recurrence rate, it was still a good choice for new, small keloids (25). Currently randomized trials examining intralesional cryotherapy versus excision with corticosteroids or excision with brachytherapy are underway [NTR4151] (26).

## COMPLICATIONS

Inflammatory morbidity, inevitable side effects, and complications are difficult to separate with this modality of treatment (27). Table 49.5 shows some of the well-known complications of cryosurgery. Some degree of pain is universal, but its intensity is extremely variable. Syncope can occur if the pain is severe, and many prefer to treat patients (particularly young men) lying down. During the freeze time, pain is felt as burning, and during the thaw phase, when pain is commonly worse than during the freeze, it is felt as throbbing. The periungual region and the temples are the most persistent. Headache is an occasional sequela after treatment of sites close to bone, such as the forehead, temple, or scalp. Immediate hemorrhage, if occurs, is often prolonged, but can usually ultimately be stopped with pressure alone. This can follow by performing biopsies immediately prior to treatment, but can also occur if a pedunculated

lesion is manipulated while frozen, and cracks. Edema is the product of acute inflammation. Pronounced idiosyncratic edema may occasionally occur after short freezes. Edema is often more severe around the eyelids and lips (Figure 49.7). The edema can be partly inhibited by a single application of a potent topical steroid immediately following treatment (28), and if severe edema is anticipated then systemic corticosteroids can be used. Temporary hyperpigmentation is common in people with an olive complexion and may last 2 to 3 months. Careful sun protection following treatment may reduce this risk. For patients with Fitzpatrick types III and IV skin, even temporary hyperpigmentation may be unacceptable and treatment of a small test area is recommended. Hypopigmentation is virtually universal following tumor doses of cryosurgery, owing to the exquisite sensitivity of melanocytes to cold, and can occur unpredictably following lower doses. In pigmented skin, this will lead to an unacceptable cosmetic result; however, in fair-skinned people this is usually not a problem and can be dealt with by feathering. Any loss of pigmentation is permanent, but because the texture of the underlying skin is normal, it can be effectively disguised by cosmetics. Feathering is a technique described to minimize the contrast between normal and hypopigment skin. It involves a light spray around the outer margin of the ice field after the treatment. Alopecia will follow large doses of liquid nitrogen, and occasionally occurs at lower doses in an unpredictable fashion. Like pigment loss, any hair loss is usually permanent, and so cryosurgical treatment of lesions in the scalp and beard areas is generally only considered for small lesions. Scarring and wound contraction do not occur if the duration of the freeze after ice formation does not exceed 30 seconds, but can occur with higher doses (29). This is due to the relative resistance of fibroblasts and collagen fibers to cold, which leads to the preservation of the fibrous tissue network, which then acts as a scaffold on which wound healing occurs. Cartilage is similarly cryoresistant, allowing lesions on the ears and nose to receive full 30-second double freezes of liquid nitrogen without distortion of normal tissue contour (30). Sensory impairment following cryosurgery has been described in both the patient and the operator (31). Touch, pain, and cold sensations are all reduced and may take as long as 18 months to recover. The extent of sensory impairment is more pronounced with longer freeze times. Although this may be of advantage for repeat treatment of lesions or if analgesia is desired, patients must be warned of this complication if sensitive areas such as the fingertips are being treated. Neuropathy has been



**Table 49.5** Side effects of cryosurgery**Immediate**

Pain  
Headache  
Hemorrhage  
Edema and blister formation  
Syncope

**Delayed**

Infection  
Hemorrhage  
Excessive formation of granulation tissue

**Prolonged but usually temporary**

Hyperpigmentation  
Milia

Hypertrophic scars  
Alteration of sensation

**Prolonged and usually permanent**

Hypopigmentation  
Alopecia  
Atrophy  
Ectropion  
Notching of the eyelids, ear, or vermillion border.

**Figure 49.7** Left upper facial and periorbital edema 24 hours after two 30-second freeze-thaw cycles to left temple basal cell carcinoma of 2.4 cm in diameter.

described when treating tumors on the side of the neck, near elbow or knee, and on the side of the finger. This is temporary, persisting usually for 3 to 6 months. Contraindications to cryosurgery generally relate to intercurrent illnesses such as those listed in Table 49.6. Relative contraindications arise with certain lesions where the cosmetic result would be more favorable with different treatments, such as the beard areas or in patients with pigmented skin, or in sites where wound healing may be slow, such as the pretibial region. Cryosurgery can still be used on all these lesions as long as the operator acknowledges that the cosmetic outcomes will be diminished.

**CONCLUSION**

As can be seen from the broad range of conditions listed in the tables, distinguishing between the use of cryosurgery or cosmetic usage for treatment of disease is sometimes difficult. Many pathologies cause little functional impairment but cause psychological morbidity through their perceived unpleasant appearance. To dismiss these disorders as trivial or unworthy

**Table 49.6** Contraindications to cryosurgery

Agammaglobulinemia  
Blood dyscrasias of unknown origin  
Cold intolerance  
Raynaud disease  
Cold urticaria  
Cryoglobulinemia  
Pyoderma gangrenosum  
Collagen and autoimmune disease

and merely of cosmetic significance is to deprive the patient of a balanced informed opinion. For many of these conditions there are few published data that specifically address how to perform the actual treatment. This is because experienced cryosurgeons will be able to judge the treatment required on the basis of the pathology of the lesion, its thickness, and its site. For instance, a lesion on the lower leg of a person with venous insufficiency is likely to respond to shorter freeze times, and may have prolonged healing times. Even if all these treatments had been prospectively audited, more exact treatment protocols would be difficult to produce, since they would not allow for the many factors clinicians take into account when performing cryosurgery. One reason clinicians may obtain unsatisfactory outcomes is the use of inadequate treatment schedules. It is hoped that this description of the correct technique will ensure that others achieve results similar to those presented here. Cryosurgery is easy and can be learned quickly—but that is not the same as saying no training is required. As Zacarian, the father of modern-day cryosurgery, said,

A level of knowledge permitting an adequate understanding of the diagnosis and the pathophysiology of the condition to be treated must be a prerequisite. This is to be combined with a degree in skill in dermatocryosurgical procedures to allow the selection of those methods necessary to carry out the treatment plan. These skills must be acquired. There are no shortcuts (6).

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# Botulinum Toxins

Doris Hexsel

## INTRODUCTION

The cosmetic use of botulinum neurotoxin type A (BoNT-A) on the upper face was first reported by Carruthers and Carruthers in the late 1980s (1). The first studies of the cosmetic use of BoNT-A were published in the early 1990s and approximately 10 years later it was approved by the Food and Drug Administration (FDA) in the United States, and similar authorities in other countries. This represents one of the most important contributions to the approach of the aging face in recent years.

Easily applied by experienced physicians, BoNT-A injections are now perhaps the most frequently used cosmetic procedure. In the United States, BoNT-A injections were the most frequently performed cosmetic procedure from 1995 to 2010, and the one that increased at the greatest rate over time (2). Their popularity and success among physicians and patients can be related to their consistent positive results and safety (3,4), besides being a fast, minimally invasive (5), and low-risk procedure. Botulinum neurotoxin type A is now a recognized treatment for a wide spectrum of conditions characterized by relative over activity of one or a few muscles (m.). BoNT-A injections are effective in the treatment of localized hyperhidrosis, and recent publications report their effectiveness in other dermatological conditions. The relative simplicity of the procedure and the low rates of side effects or significant complications make this procedure increasingly attractive for cosmetic use.

Although botulinum neurotoxin type B may be cosmetically used and although there are varied botulinum toxin brands, this chapter discusses only the BoNT-A main preparations, such as Botox®, Xeomin®, and Dysport®.

## PHARMACOLOGY AND MECHANISM OF ACTION

*Clostridium botulinum* produces an exotoxin that is considered the most poisonous of all poisons (6). It is an anaerobic, gram-positive bacillus that forms spores and its strains are grouped into one of the eight serotypes: A, B, C, D, E, F, G, and H (7).

The currently available serotypes A and B result from modification of the protein structure and have been used for a variety of medical and cosmetic indications. Botulinum neurotoxin causes a temporary chemical denervation of the motor neuron in the treated muscle, resulting in therapeutic and cosmetic actions. It selectively inactivates these nerve terminals by blocking the release of acetylcholine, producing a temporary and ultimately reversible blockade of cholinergic transmission (8). In the neuromuscular junction, the blockade of the release of acetylcholine promotes a different spectrum of action (9) varying from muscle relaxation to muscular palsy, depending on the subtypes and doses used.

Different formulations of BoNT-A are available worldwide, which are neither identical nor interchangeable. Botox (Allergan, Inc., Irvine, California) is the trade name for onabotulinumtoxinA (ONA) (10). ONA is also known as Vistabel® in Europe and Vistabex®. Dysport (Ipsen Pharma, Boulogne-Billancourt, France) is the trade name for abobotulinumtoxinA (ABO), which is also known as Azzalure®. Xeomin (Merz Pharmaceuticals, Frankfurt, Germany) is the trade name for incobotulinumtoxinA (INCO), also known as Bocoture®. Table 50.1 shows the principal characteristics of the main commercial preparations of BoNT-As.

## RECONSTITUTION, HANDLING, DILUTION AND DOSE EQUIVALENCE

Available BoNT-A preparations are usually stored in a refrigerator and reconstituted with isotonic sodium chloride (0.9% saline solution) with or without preservative (5) prior to their use, except incobotulinum toxin.

The manufacturers of the commercial preparations of BoNT-A recommend their use within the first 4 or 8 hours after reconstitution, depending on the product, to ensure that the potency of the drug is maintained and to prevent the possibility of contamination of the vials. Manufacturers recommend storing BoNT-A in the refrigerator no longer than 4 hours after reconstitution. However, studies support the safety and efficacy of BoNT-A when the product is reconstituted up to 15 days (11) and 6 weeks before the injections (12,13). No signs of microbiological contamination were verified (11,13). Reconstitution of BoNT-A in smaller rather than larger volumes of saline solution is preferable. For cosmetic purposes, a higher concentration allows a low injection volume, which permits more precise placement of the neurotoxin. Tables 50.2 and 50.3 show the most common regular dilutions of Botox/Vistabel, Xeomin/Bocoture, and the recommended equivalent doses for Dysport/Azzalure.

A dose-equivalence of up to 1:2.5U between Botox or Xeomin and Dysport is adopted by the most experienced physicians in the use of both products, and is supported by recent studies (14,15). This dose equivalence is also inferred by comparing controlled studies that aimed to establish the optimal dose for glabellar area (16–18). Such studies demonstrated comparable results and optimal effects in treating glabellar wrinkles using doses of 20U of Botox (16) and 50U of Dysport (17,18). Hexsel and cols showed that injections of Dysport and Botox at the dosing ratios 2.0:1.0 U demonstrated similar field effects in both muscles and sweat glands. However, injections at an equivalence ratio of 2.5:1 U (Dysport:Botox) showed minimal differences but greater efficacy measured by diameter and area of the field of anhidrotic effect for Dysport when

**Table 50.1** Comparison of Different Commercial Preparations of BT Available in the Majority of Countries

	Botox® 100U	Vistabel® 50U	Dysport® 500 s.U	Dysport 300 s.U	Azzalure® 125 s.U	Prosigne® 100U	Xeomin® 100U
<b>Active substance</b>	BT-A complex (925 kD)	BT-A complex (925 kD)	BT-A complex	BT-A complex	BT-A complex	BT-A complex	BT-A neurotoxin (150 kD)
<b>Suggested equivalence for cosmetic uses (relative to Botox)</b>	NA	NA	Between 1:2 and 1:2.5U	Between 1:2 and 1:2.5U	Between 1:2 and 1:2.5U	1:1	1:1
<b>Mode of action: target protein</b>	SNAP 25	SNAP 25	SNAP 25	SNAP 25	SNAP 25	SNAP 25	SNAP 25
<b>Pharmaceutical form</b>	Lyophilized powder	Lyophilized powder	Lyophilized powder	Lyophilized powder	Lyophilized powder	Lyophilized powder	Lyophilized powder
<b>Reconstitution</b>	0.9 % NaCl solution	0.9 % NaCl solution	0.9 % NaCl solution	0.9 % NaCl solution	0.9 % NaCl solution	0.9 % NaCl solution	0.9 % NaCl solution
<b>Storage before dilution</b>	2–8°C or	2–8°C	2–8°C	2–8°C	2–8°C	2–8°C	25 °C
<b>Storage after dilution</b>	24h/2–8°C	24h/2–8°C	4h/2–8°C	4h/2–8°C	4h/2–8°C	4h/2–8°C	24h/2–8°C
<b>Shelf life (unopened)</b>	36 months	36 months	24 months	24 months	24 months	36 months	36 months
<b>Shelf life reconstituted</b>	Up to 24h depending on country approval	Up to 24h depending on country approval	4–8h	4–8h	4h	4h	Up to 24h depending on country approval
<b>Auxiliary substances</b>	albumin 0.5 mg /vial NaCl 0.9 mg	albumin 0.5 mg /vial NaCl 0.9 mg	albumin 0.125 mg lactose 2.5 mg	albumin 0.125 mg lactose 2.5 mg	albumin 0.125 mg lactose 2.5 mg	gelatin 5 mg, dextran 25 mg, and sucrose 25 mg	albumin 1 mg sucrose 4.7 mg
<b>pH-Wert</b>	5–7	5–7	5–7	5–7	5–7	5–7	5–7
<b>Toxin protein load in dose-equivalence range</b>	5 ng/100 U	2.5 ng/50 U	5 ng/500 U	5 ng/300 U	5 ng/125 U	4–5 ng/100 U	0.6 ng/100 U

**Table 50.2** Regular Dilutions of 1, 2, and 2.5 mL for Botox®, Vistabel®/Bocoture®, and Xeomin® and equivalent dilutions to achieve a 1:2.5U equivalence for Dysport®/Azzalure®

To achieve equivalence between Botox/Vistabel or Xeomin/Bocoture and Dysport/Azzalure of	If the vial of 100U (Botox or Xeomin) is usually diluted in	If the vial of 50U (Vistabel or Bocoture) is usually diluted in	The vial of 500U of Dysport should be diluted in	The vial of 300U of Dysport should be diluted in	The vial of 125U of Azzalure should be diluted in
1:2.5 U	1 mL	0.5 mL	2 mL	1.2 mL	0.5 mL
1:2.5 U	2 mL	1 mL	4 mL	2.4 mL	1 mL
1:2.5 U	2.5 mL	1.25 mL	5 mL	3 mL	1.25 mL

**Table 50.3** Regular dilutions of 1, 2, and 2.5 mL for Botox®, Vistabel®/Bocoture®, and Xeomin® and equivalent dilutions to achieve a 1:2U equivalence for Dysport®/Azzalure®

To achieve the equivalence between Botox/Vistabel or Xeomin/Bocoture and Dysport/Azzalure of	If the vial of 100U (Botox or Xeomin) is usually diluted in	If the vial of 50U (Vistabel or Bocoture) is usually diluted in	The vial of 500U of Dysport should be diluted in	The vial of 300U of Dysport should be diluted in	The vial of 125U of Azzalure should be diluted in
1:2 U	1 mL	0.5 mL	2.5 mL	1.5 mL	0.62 mL
1:2 U	2 mL	1 mL	5 mL	3 mL	1.25 mL
1:2 U	2.5 mL	1.25 mL	6.25 mL	3.75 mL	1.56 mL



compared to Botox (15). Another study from Hexsel and cols tested also the 1:1 dose-equivalence and showed greater diffusion for Botox compared to Dysport. These studies confirmed that, regarding the toxins, diffusion is a dose-dependent effect rather than related to intrinsic characteristics of each product (19).

Recently, an expert panel of French aesthetic physicians and biologists established a consensus on the clinical equivalence in efficacy and safety of Botox and Xeomin. They concluded both neurotoxins are clinically equivalent in terms of efficacy and safety, and that 1:1 conversion ratio can be used between them (20).

A recent study (21) presented similar results for the fields of muscular effect (WSS and ECMAP) between Dysport and Xeomin over time and larger FAE for Dysport compared with Xeomin at the 2.5:1 equivalence. The authors suggested that a lower dose equivalence between Dysport and Xeomin could be established, such as 2:1.

## CONTRAINDICATIONS, PRECAUTIONS, AND RECOMMENDATIONS

Contraindications and/or limitations for BoNT-A are listed in Table 50.4. Pressure at the site before and after treatment, application of cold, and the use of small syringes and fine-gauge needles can reduce pain and bruising at the injected sites (22). Hexsel and cols (23) presented a safe, economical, and effective

tool to cool the skin for analgesic purposes. It is a single-use disposable balloon filled with a frozen solution of 10%–20% isopropyl alcohol in water that is effective in reducing pain, ecchymoses, and hematomas in common dermatologic procedures. It is safer and more cost-effective than other tools (23).

Injections should be symmetrical regarding doses, muscles, and areas. This is important for the natural balance of the facial structures and to avoid asymmetries. Exception includes evident asymmetries, such as those caused by facial palsy.

Since the results are expected for 4 to 6 months, treatments are usually repeated twice a year, for maintenance of the results. A period of 15 to 30 days for touch-ups should be respected. It is important to avoid more than one touch-up and respect the minimal interval of 3 to 4 months between treatment sessions, due the risk of inducing the formation of antibodies. However, there is evidence of little resistance to BoNT-A in the cosmetic use (24). When BoNT-A is injected adjunctively to some surgical procedures, such as a facelift, blepharoplasty, and laser resurfacing, some physicians prefer to inject in the postoperative period (25).

The dose to be injected depends on the target muscle and can vary from patient to patient, and from one application to another, according to patient's needs, muscle mass and activity of the target muscles, gender, and number of previous treatments. The suggested doses by the consensus groups for Dysport (26,27) and Botox (28) are informed in Table 50.5 according to the area to be treated.

**Table 50.4** Contraindications for BoNT-A Injections

Pregnancy and breastfeeding
Active infection in the proposed area
Neuromuscular transmission disorders (myasthenia gravis, Eaton-Lambert syndrome, Rooke syndrome)
Hypersensitivity to components of the BT-A injection solution (BT-A, human albumin)
Medication that influences neuromuscular transmission such as quinine, calcium channel blockers, penicillamine, aminoglycoside antibiotics, pancuronium, galamine, tubocurarin succinylcholine
Medication that interferes with coagulation (e.g. acetylsalicylic acid, anticoagulants, vitamin E) and coagulopathies
Candidates with unrealistic expectations, unrealistic fears of the toxin, psychiatric disorder such as psychosis, mania, body dysmorphic disorder, and eating disorders.

Source: Wollina U, Konrad H, *Am J Clin Dermatol*, 2005; 6(3):141–50.

**Table 50.5** Suggested total doses of Botox or Xeomin® and Dysport® in Different Cosmetic Treatments

	Botox or Xeomin	Dysport
	Average Total Dose (U)	Average Total Dose (s.U)
Facial Indications		
Glabellar lines	12–40	30–70
Forehead lines	8–25	20–60
Crow's feet	12–30	30–60
Infraorbital rhytides	1–4	2–5
Bunny lines	4–8	10–20
Drooping nasal tip	2–6	5–10
Repeated nasal flare	1–4	10–20*
Perioral area	1–5	4–12
Mentalis/dimpled chin	4–10	10–20
Depressor angulis oris	4–8	10–20
Gingival smile	1–4	5–10(48)
Masseteric hypertrophy	15–40	60 for Caucasians and 120 for Asians
Platysmal bands	60	Maximum dose 50 per side
Décolleté wrinkles	50–100*	75–120

\*Doses suggested by the present author. Not defined by the consensus.

Patients should read and sign an informed consent form before application, and photographs should be taken before all cosmetic procedures, in order to evaluate the results. Photographs must be taken at rest and also with contracted muscles. Makeup should be removed and the skin cleansed before application.

## UPPER FACE

Cosmetic indications for the upper face include the treatment of glabellar lines, forehead lines, brow lifting, and crow's feet lines (26,28,29). The main cause of these wrinkles is the muscular action (28); they are thus considered "expression lines."

The direction of facial wrinkles and lines is usually perpendicular to the direction of the muscle fibers. The muscles of the upper face are intricately intertwined. In this area, location, muscle anatomy and muscle mass, as well as the way patients use their muscles vary greatly between individuals (30). A recent study (21) showed thicker and thinner muscles presented similar wrinkle severity scores at baseline. However, thicker muscles presented more severe wrinkles than thinner muscles 28 days after the injections. Authors suggest thicker muscles may need higher BoNT-A doses to achieve similar results of thinner muscles (21).

The frontalis m. have quite a variable anatomy and functioning, being responsible for raising the eyebrows, thus causing the horizontal forehead lines. Besides being important in the facial expression, aged people also use these muscles to amplify the visual field. Muscles controlling the frown include the corrugators m. and the orbicularis oculi m., which move the brow medially, while the procerus m. and the depressor supercilli m. pull the brow inferiorly. The orbicularis oculi m. is divided into three parts, the orbital, the preseptal, and the pretarsal portions.

## Glabellar Lines

Glabellar lines (Figure 50.1) are interpreted as presenting negative feelings, such as sadness, anger, and frustration (16,31).

A variety of different injection techniques and doses have been reported over the years. One or two injection sites may be used on the belly of the procerus m. This muscle can be treated with a single injection in the midpoint of an

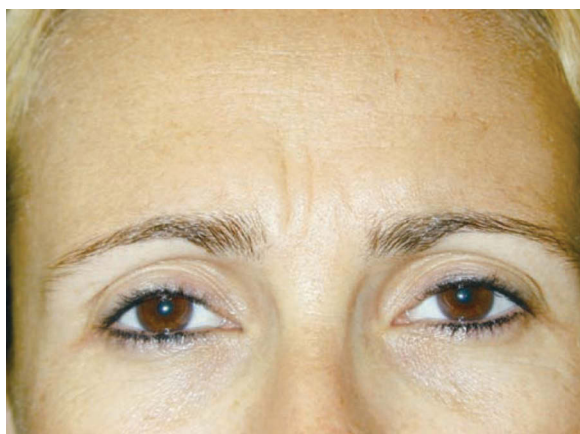
imaginary "X" formed by lines joining the inner brows and the contralateral inner canthus (26). If the frontalis m. is not treated, the middle to lateral portion of the eyebrows will be slightly raised by the opposing levator action of the frontalis m. (26) The conventional injection technique involves the observation of the medial aspect of the eyebrow, while the patient frowns. BoNT-A is slowly injected into the belly of each corrugator m., taking care to maintain the needle 0.5 cm from the upper orbital rim and internal to the midpupillary lines (26) (see Figure 50.2). The needle should be positioned perpendicularly and advanced slightly within the muscle fibers in a vertical direction toward the hairline (32).

The total doses of Botox (28) for glabellar lines range from 12 to 40 U, and from 30 to 70 s.U of Dysport (26). The doses should be adjusted based on wrinkle severity and on patient preference (26). Repeated injections of BoNT-A for glabellar lines were shown to be safe and efficacious, and no loss of effectiveness or cumulative adverse effects is reported (33).

The eyebrow is a mobile structure elevated by frontalis m. and depressed by brow depressors (orbicularis oculi m., corrugator supercilli m. and procerus m.). Botulinum neurotoxin type A treatment for glabellar lines causes an elevation of the medial and lateral brows (34) (Figures 50.3a and b), leading to a desirable arched shape and elevation of the brows (29) It was observed that the ideal brow shape in women is the lateral and medial elevation, instead of medial elevation only (29), whereas the rectilinear brow pattern is preferable for men. However, excessive elevation of the tail of the brows is undesirable by the majority of the patients.

## Forehead Lines

The frontal region should always be treated in association with the glabellar area to avoid increased compensatory use of glabellar m., which are mainly depressors (34,35). It is also important to preserve at least some frontal muscles movement, responsible for facial expression and lift of the eyelids and brows. Since the frontalis m. are also responsible for facial expressiveness, multiple injections of small doses of BoNT-A are used in this area to create only a weakening of the muscle instead of a total paralysis, preventing brow ptosis and allowing the patient to maintain some movement (32,36,37), especially in the upper part of these muscles. Besides, injection points should be high on the forehead and small doses are recommended for the first treatment (32). The total dose



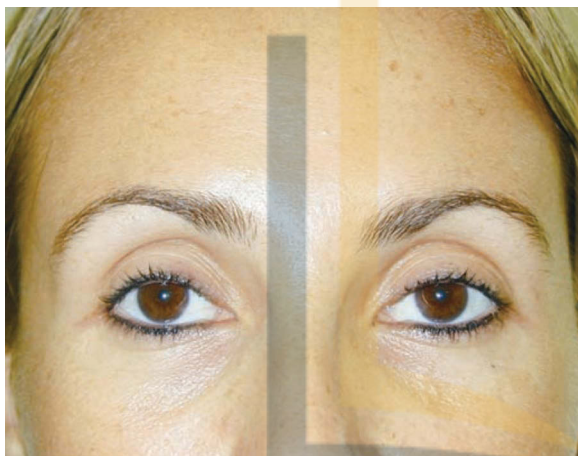
**Figure 50.1** Glabellar lines. Muscles involved: corrugators and procerus.



**Figure 50.2** The five-point technique for glabellar lines.



(a)

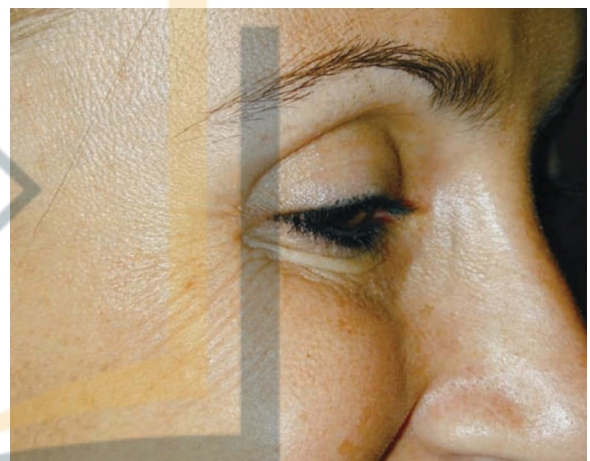


(b)

**Figure 50.3** (a) Original position of eyebrows, before treatment. (b) The same patient, showing changes in brow position and shape, after treatment.



(a)



(b)

**Figure 50.4** (a) Crow's feet wrinkles before treatment. (b) The same patient after treatment with botulinum toxin, showing residual wrinkles, which may be treated with adjunctive treatments.

varies from 8 to 25 of Botox (28) or Xeomin and 20 to 60 s.U of Dysport (26).

### Periorbital or Crow's Feet Lines

The wrinkles radiate from the lateral canthus outwardly and laterally, and are perpendicular to the direction of the muscle fibers of the orbicularis oculi m. (32) (Figure 50.4a).

The total doses used to treat crow's feet lines range from 6 to 15 U/side (28) of Botox or Xeomin and 15 to 30 s.U/side of Dysport (26) (Figure 50.4b). These doses are generally distributed over two or three injection sites (34), although sometimes four to five sites are needed (Figure 50.5). A study showed no difference in safety and efficacy when the same dose was injected in one or three points in periocular area (38). All the injections must be performed at least 1 cm lateral to the lateral orbital rim.

Infraorbital rhytides (Figure 50.6) can be treated with intradermal injections of 0.5 to 2 U/side of Botox (28) or Xeomin and 1 to 2.5 s.U/side of Dysport (27), 3–4 mm below the eyelid. It increases the palpebral aperture and thus widens the eyes. It is not recommended for patients having dry eyes, prominent eye bags, scleral show, or morning eyelid edema (27).



**Figure 50.5** Superficial injections of botulinum toxin type A for the treatment of crow's feet wrinkles.





**Figure 50.6** Injection sites for the treatment of infraorbital rhytides and bunny lines.

## MIDDLE FACE

Whereas the duration of the effects of BoNT-A injections in the upper face is about 3 to 4 months or longer, sometimes 6 to 8 months (3) the duration in the middle and lower face is about 2 to 3 months. Technique and doses, individual differences, and previous treatment with BoNT-A may lead to a decreased duration (28).

### Bunny Lines

This is the most common indication of the middle face. The levator labii superioris m. and nasal m., as well the medial portion of the *orbicularis oculi* (39) are involved in these wrinkles. Nasal wrinkles, called “bunny lines” (Figure 50.6), are treated with low doses of BoNT-A (40). These lines may become more pronounced after BoNT-A injections for the treatment of glabellar and periorbital wrinkles. The injection must be applied in the high lateral nasal wall, below the angular vein, avoiding injections near the nasofacial groove in order to prevent relaxation of the levator labii superioris m. which may lead to upper lip ptosis (40,41). The recommended dose is 1 to 4 U of Botox (28) or Xeomin and 5 U to 10 s.U of Dysport (27) injected intradermally in just one point 1 cm above the upper lateral part of nostril on each side. The treatment may be less effective in patients who recruit these muscles excessively or have had prior rhinoplasty (40).

### Nasal Tip Droop

The depressor septi nasi m. is a small muscle located in the external inferior base of the nasal septum, which contributes to the nasal tip lowering, aggravating the nasal tip ptosis that usually occurs with aging. Injections are done at the base of the columella (40). Injection of 2 to 6 U of Botox (28) or Xeomin and 5 to 10 s.U of Dysport (27) perpendicular and deep in a single site at the junction of the columella and the upper lip can result in partial, very discrete lifting of the tip of the nose.

### Repeated Nasal Flare

Some people present dilate or rhythmic contractions of the nostrils on certain occasions, which may cause embarrassment. Injections of BoNT-A are indicated on each side in the lower nasal fibers above the lateral nasal ala (40).

## LOWER FACE

Lower doses of BoNT-A than those usually used in the upper face are recommended for the lower face. Such doses permit muscle relaxation instead of paralysis of target muscles, which is desirable for this area (43). A recent study showed the safety and efficacy of full-face injections of BoNT-A also for the lower face indications (3).

Due to the low doses used to treat the lower face, it is recommended to inject lower face together with upper face. Care should be taken not to inject all areas in the lower face because of the increased risk of the accumulated effects and doses in this area, increasing the risks of side effects.

Most of the muscles of the lower face are functionally related to the mouth and lips (43). In the lower face, BoNT-A is useful to treat a series of conditions, including perioral wrinkles, masseteric hypertrophy, “peau-d’orange” chin (mentalis m.), marionette lines (depressor angulis oris m.), as well as gingival and asymmetric smile.

### Perioral Area

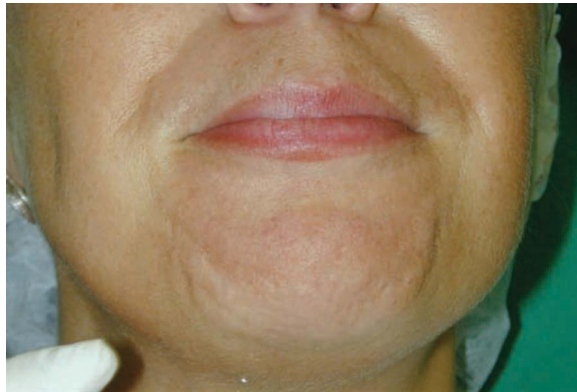
Photodamage, heredity, cigarette smoking, loss of deep structures and volume, sleep positions, orthodontic deformities, and dynamic components like playing a musical instrument that requires embouchure, or even whistling have been thought to cause this aesthetic problem (40,44). Fine vertical lip rhytids are also caused by repetitive action of the *orbicularis oris* m. Tiny doses of BoNT-A produce localized microparesis of the *orbicularis oris* m. reducing dramatically perioral lines and also giving a pleasant pseudoeverision of the lip, with enhancement of the vermilion contour (40).

To define the injection points, the patient is asked to pucker and the adjacent areas of muscle contractions are marked. It is recommended to inject low doses and superficially, above the vermilion ridge in the area of muscle contraction adjacent to the creases, away from the oral commissures and Cupid's bow. Injections of 1 to 5 U of Botox (28) or Xeomin and 4 to 12 s.U of Dysport (27) divided among 4 to 6 injection sites are recommended. Side effects for this area are dose-dependent (45), and higher doses are associated to higher frequency of side effects when treating this area (3).

### Mentalis and Depressor Angulis Oris

Mentalis m. cause wrinkles giving the chin a dimpled or “cellulitic” aspect (Figure 50.7a). These muscles can be treated with one injection in the midline or two injections in each side of the insertion of these muscles at the point of the prominence of the chin (Figure 50.7b), with good results (Figure 50.7c) (42). Consensus recommendations for Dysport suggest superficial injections be performed to obtain satisfactory results (27). Total doses of 4 to 10 U of Botox (28) or Xeomin and 10 to 20 s.U of Dysport (27) are recommended.

The depressor angulis oris m. is responsible for lowering the corner of the mouth; this worsens the nasolabial fold, producing the called “marionette” lines and thus giving a negative expression to the patient (Figures 50.7b and 50.7c). These muscles can be treated with injections of BoNT-A at the border of the jaw bone, at a point on an imaginary line descending from the nasolabial fold. The total dose ranges from 2 to 4 U/side of Botox (28,46) or Xeomin and from 5 to 10 s.U/side of Dysport (27). Lower lip dysfunction can be caused when the injection is too medial and high, reaching the depressor labii m. (22).



(a)



(b)



(c)

**Figure 50.7** (a) “Cellulitic” chin before treatment. (b) Treatment of mentalis m. with 3 U of BoNT-A on each side. (c) Same patient after treatment of the mentalis m.

### Gingival Smile

Gingival display is defined as the difference between the lower margin of the upper lip and the superior margin of the central incisors (47). Excessive upper gum exposure occurs when more than 3 mm of gingival is exposed when someone smiles and is called “gingival smile.”

Most of authors consider the gingival smile a consequence of excessive retraction of levator labii superioris alaeque nasi m., but levator labii superioris, zygomaticus major,

zygomaticus minor, levator anguli oris, orbicular oris, and risorius muscles are also involved. A classification based on area of gingival exposure—anterior, posterior, mixed and asymmetric—permits a better therapeutic approach (48).

BoNT-A injected into the levator labii superioris m. causes a slight to moderate drop of the upper lip, reducing the anterior gummy exposure. Doses should be adjusted according to the degree of gum exposure (48). The total dose suggested by the consensus for Botox use is 1 to 4U, but doses as high as 8U may be appropriate for some patients (28). A study reported the efficacy of 4 to 6 U of Botox to treat gummy smile (49). The consensus for the use of Dysport does not state doses for this indication (26). The total doses of 5 to 10 s.U of Dysport to treat anterior gummy smile and maximum dose of 2.5 s.U of Dysport on each side to treat posterior gummy smile are suggested (48). Duration of effects were reported to last from 3 to 5 months (48). Results are less satisfactory in middle-aged and older patients, as this causes slight vertical elongation of the upper lip (46).

### Masseteric Hypertrophy

The hypertrophy of masseter m. is a rare, asymptomatic problem of unknown cause. It can be uni- or bilateral and may also be related to bruxism. Usually it begins during infancy and may determine a more square shape of the face.

BoNT-A can safely be considered as a noninvasive drug treatment for patients with masseteric m. hypertrophy (50). Its effectiveness is noticed as early as 2 weeks after injections and reached a peak effect in 3 months. Injections are performed 1 cm below and above a reference line drawn from the tragus of the ear to the corner of the mouth. About 44% of patients would complain of reduction of mastication strength (51). Although a previous study (52) showed that 6 months after the injections the facial contour gradually returned to the original shape and volume, Lee and cols reported that masseters treated with BoNT-A maintained significant volume reduction 24 weeks after treatment (53). The patients can be treated again every 3 to 4 months, if needed. However, there are reports of retreatment each month until there is no palpable movement of the muscle on clenching the teeth (54). Doses can vary from 15 to 40U of Botox (28) or Xeomin, and up to 60 s.U of Dysport (27). Usually, higher doses are used in Asians (27,28).

### FACIAL ASYMMETRY

Facial asymmetry is a frequent complaint and can result from many different causes, which determine whether it will be a temporary or a permanent condition (55,56). Asymmetry occurs when one of the bilateral muscles is comparatively stronger or weaker than other. Three basic types of facial asymmetries have been described and result from different causes. The acquired facial asymmetry is the result of a medical or physical episode, for instance, a cerebral vascular accident. Facial asymmetries can also be the result of iatrogenic causes as in the case of certain types of surgery on the face or accidents. A third type of facial asymmetry can be idiosyncratic or familial, in which a muscle on one side of the face can be comparatively stronger or weaker than its partner muscle on the contralateral side of the face (55).

BoNT-A can also be used to treat lesions or muscle hypertrophy resulting from surgical procedures or trauma, in order to achieve better cosmetic and functional results. Usually BoNT-A injections are performed on the unaffected side of hemiparesis, treating the hyperkinetic muscles (25). Application of



BoNT-A in the healthy side of the face can improve its symmetry at rest and during facial motion, especially when smiling, speaking, or exposing the teeth (57). BoNT-A can provide a simple, noninvasive and safe way of correcting obtrusively distracting asymmetry (55).

### Asymmetric Smile

The most common cause of asymmetric smile is related to depressor labii inferioris m. hyperkinesis or weakness. The total doses are variable according to the patients and muscles to be treated. The toxin is injected into the belly of the hyperkinetic muscle. The results become evident in less than 5 days and the effects last 4 to 5 months after the first treatment. In subsequent treatments, it is recommended to reduce the doses and the results are usually longer (55).

### PLATYSMAL BANDS AND DÉCOLLETÉ WRINKLES

Platysmas m. are a pair of flat muscles that originate in the subcutaneous of the upper thorax, ascend laterally to the lower face, cross the neck, pass behind the mandible angle, and insert into the cutaneous muscles around the mouth (58). Platysmal bands are the result of the repeated and strong contractions of these muscles.

Platysmal bands are better visualized when the patient shows the lower teeth. In this indication, BoNT-A should be injected at various points along the length of the bands, at distances of 1–2 cm. The maximum suggested dose of Botox (28) or Xeomin for platysmal bands is 60 U and the consensus for Dysport establishes the maximum of 100 s.U (27). However, the risk of complications with high doses can exceed the potential benefits. Goldman and Wollina reported the injection of three to four sites with 5 U of Botox or Xeomin or 15 s.U of Dysport on both sides of the medial portion of the platysma m. for the attenuation of the platysmal bands and elevation and redefinition of the angle of the mouth (59).

The main platysmal bands are chosen to be treated in each session (usually two to four bands) (40). This results in improvement of the appearance of the bands and fine wrinkles. In older people, it can reduce wrinkles and bands in cases where surgical lifting is contraindicated or when there are postsurgical residual wrinkles (39). Greater benefits are seen in patients with apparent bands, good elasticity of the skin, and minimal fat deposition in this area (60).

Cutaneous wrinkles in the décolleté area can be classified as dynamic wrinkles, static wrinkles, and combined wrinkles. They result from several factors, such as photoaging (main cause), intrinsic aging, contraction of the pectoralis major m. and lower part of the platysmal bands as well as sleep position. Botulinum toxin relaxes the underlying muscles, improving these wrinkles (58). Doses of 2–5 U of Botox or Xeomin and 7.5–10 s.U of Dysport can be used at each injection site in a V-shaped technique, being 4 to 6 sites per side. The total dose of 50–100 U of Botox or Xeomin and 75–120 s.U of Dysport (27) is used, improving décolleté wrinkles in selected patients in about 2 weeks (61).

### ADJUNCTIVE TREATMENTS

Facial wrinkles are the result of a combination of many causes. Different therapeutic applications have been used throughout the years to give the face a youthful appearance. The combined use of BoNT-A with different cosmetic procedures such as fillers, lasers, light sources and Subcision® may improve the

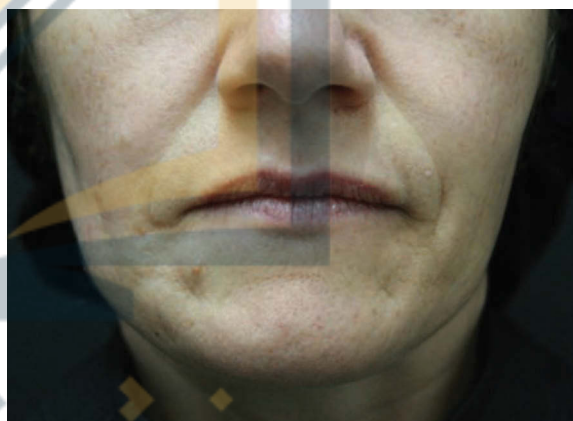
results and their duration, when compared to the same techniques used alone, producing a more polished and refined result (62) (Figures 50.8a and b).

The aesthetic improvement in moderate to severe glabellar and forehead lines can be effectively achieved with neurotoxin alone. However, the result is better when combining BoNT-A with hyaluronic acid fillers (63,64). Botulinum toxin minimizes muscle contraction, allowing the filler to remain in place longer than when injected alone. Both frontal and glabellar areas are considered dangerous areas for fillers injection.

In the perioral area, dermal fillers can be combined to BoNT-A to treat volume losses, a key component of perioral aging. Resurfacing techniques can also be used in combination with BoNT-A in this area (65). The adjunctive use of fillers into lip margin can be also useful in gingival smile treatment (40). Fractional therapies are also useful.

For décolleté wrinkles, BoNT-A can be combined with other techniques, such as peels, lasers, and surgical lift (58). A study showed that patients may be treated with several nonablative lasers immediately after BoNT-A injection without loss of efficacy or other apparent untoward effect (66).

Although BoNT-A can be used in combination to surgical procedures, it has been shown that the combination of non-surgical therapeutic modalities provides improvement of facial



(a)



(b)

**Figure 50.8** (a) before and (b) after lip augmentation with hyaluronic acid filler and botulinum toxin in the peri-oral area, including the orbicularis oris m. and depressor anguli oris m.



skin texture, pigmentation, tone, and wrinkles, as well as an improvement in facial contour (67), and significantly increases patient satisfaction (68,69).

# HYPERHIDROSIS

Hyperhidrosis (HH) is a condition characterized by intense sweating in one or more areas of the body (70), mainly affecting axilla, palms, soles, face, thigh, and inguinal area (70–72). HH may cause significant physical, emotional and/or social discomfort for patients, having a considerable impact in their quality of life (73).

HH occurs due to the exacerbated perspiration of the eccrine sweat glands, which are distributed over almost the entire body surface. There is a high density of these glands in some areas such as the soles of the feet and the forehead, followed by the palms and cheeks (74). The function of apocrine and apoecrine glands in HH is unknown, but they are believed to play only a minor role in the pathophysiology of the condition (72). Sweating is controlled by nerve fibers that, anatomically, are distributed through the sympathetic nervous system (75).

HH can be primary, which is idiopathic, or secondary to various causes. The main causes of secondary HH are high temperatures, physical exercise, fever, anxiety, fear, other psychological symptoms, thyrotoxicosis, lymphoma, cancer, hypoglycemia, nausea, neurological lesions, and some drugs, such as neuroleptics, antidepressant agents, and anxiolytic agents. In both cases, HH can be focal or generalized. Primary HH is a relatively common disorder, and it is usually localized and symmetrical (70).

The diagnosis is clinical and the criteria for focal forms of HH are (71): bilateral and relatively symmetric; impairs daily activities; frequency of at least one episode per week; age of onset less than 25 years; positive family history; cessation of focal sweating during sleep. Some tests are used to visualize (starch–iodine or Minor's test), and quantify (gravimetric test) the sweating. These tests are the most widely used for the visualization of the active sweat areas (76). Hexsel and cols. provided suggestions to perform the Minor's test and presented a new scale (Sweating Intensity Visual Scale) to better evaluate and interpret Minor's test results (76). Besides these methods, the Hyperhidrosis Area and Severity Index (HASI) has been developed to assess the HH taking into account not only the amount of secretion,

but also the size of the secreting area (77). Transepidermal water loss measurement has also been referred as a rapid, practical, and reliable technique for quantifying palmo-planar sweating (78).

Several treatments are used for HH and most of them cause significant adverse effects (64) or have limited efficacy in severe cases (75). Available treatments for HH include topical, medical, and surgical treatments. The Canadian Hyperhidrosis Committee developed guidelines that provide a recommended course of therapy for patients with different focal hyperhidrosis based on the severity of disease, which is evaluated through the Hyperhidrosis Disease Severity Scale (HDSS) (75).

Botulinum neurotoxin type A is an irreversible inhibitor of acetylcholine release from the presynaptic membranes of neuromuscular junctions, preventing the release of this neurotransmitter on the postganglionic sympathetic fibers that act on the sweat glands (71). Since apocrine, eccrine, and apoecrine glands respond to cholinergic stimuli, subcutaneous injections of BoNT-A into the sweating regions result in complete cessation of sweating from all gland types (79). So far, no anatomical differences in sweat glands have been demonstrated between hyperhidrotic patients and control groups. But after BoNT-A treatment morphological alterations in the glands ducts have been noticed (80).

Botulinum neurotoxin type A in the treatment of focal hyperhidrosis is considered fast, safe, and efficacious, and rarely produces significant side effects (81). The application is usually intradermal because it targets the sweat glands, which are located 2.5 mm below the skin (72). A recent study (82) showed that the field of anhidrotic effects (FAE) does not vary significantly when the same doses in different dilutions and depths are injected on the back of patients suffering from compensatory hyperhidrosis. This study also showed that areas of more intense sweating, like the midline of the back, needed twice the doses to achieve similar size of the FAE (82). These results supported the previous findings of the present author, who stated that brand, usual dilutions, and depths are less important in the size of the FAE or “diffusion” than dose and amount of sweating of the treated areas (83).

Ideal doses have been focus of discussion, aiming to obtain more efficacious and lasting results. Table 50.6 lists some of the published articles with the proposed doses for the treatment of axillary, inguinal, and palmar HH. The average duration of effects is also reported.

**Table 50.6** Proposed Doses for the Treatment of Axillary, Inguinal, and Palmar HH

Authors	HH Area	Study Design	Patients	Treatment	Results	Duration
Heckmann (71) (2001)	Axillary	R, DB, PC, multicenter	145	200U (ABO) unilaterally vs. placebo Placebo-treated side received 100 U after 2 weeks	Similar reduction in sweating with 200U and 100U doses.	26 weeks for both groups
Naumann and Lowe (84) (2001)	Axillary	R, DB, PC, parallel group	320	50 U (ONA) vs. placebo	Response at week 4: 94% (active group) vs. 36% (placebo) At week 16: 82% (active) vs. 21% (placebo)	16 weeks

(Continued)

**Table 50.6** Proposed Doses for the Treatment of Axillary, Inguinal, and Palmar HH (*Continued*)

Authors	HH Area	Study Design	Patients	Treatment	Results	Duration
Galadari (85) (2003)	Axillary	Case series	15	125U (ABO)	93% of the patients showed anidrosis after 1 week	1–6 months
Hexsel (72) (2004)	Inguinal	Case series	26	100 U (ONA) 60 and 80U can be used to treat less severe cases	Improvement showed for inguinal HH for first time	6–8 months
Lowe (86) (2007)	Axillary	R, DB, PC, multicenter, parallel-group	322	50 or 75 U (ONA) or placebo Retreatment if HDSS score of 3 or 4 and at least 50 mg of spontaneous resting axillary sweat over 5 minutes in each axilla	75% of the subjects with at least 2-point improvement in HDSS score at week 4 vs. 25% from placebo	6–7 months for the active group
Talarico-Filho (87) (2007)	Axillary	DB, R, prospective	10	50 U (ONA) on one side and 150U (ABO) on the other	Sweat rate decreased 97.7% (ONA) and 99.4% (ABO)	260 days for ONA and 290 days for ABO
Gregorious (88) (2010)	Palmar and plantar	Open label	36	100U (ONA) per palm	Significant improvement (assessed by gravimetry) Plantar HH: marginal improvement in 12 patients and worsening in 24	6.2 months
Frasson (89) (2011)	Axillary	R, SB, bilateral paired	10	50 U (ONA) vs. 2500 U of BoNT-B (contralaterally)	Both treatments were effective: reduction in sweat weight. BoNT-B more effective than ONA	6 months
Dressler and Adib Saberi (90) (2013)	Axillary	DB, intra-individual comparison	51	First: 100U (ONA) bilaterally Then: Direct comparison 100U unilaterally vs. 50U contralaterally; 50U bilaterally (extension period)	Both doses had similar effects	3–4 months

*Abbreviations:* R, randomized; DB, double blind; SB, single blind; PC, placebo-controlled.

Although some reports refer multiples treatments do not interfere in the duration of effects (91), Lecouflet and cols suggest an increase in the duration of efficacy of botulinum injections with the repetition of injections (92). Glogau have described the use of topical applications of BoNT-A in treatment of axillary hyperhidrosis. This technique appeared to be safe and showed statistically significant quantitative reduction of sweat production (93).

## COMPLICATIONS AND SIDE EFFECTS

The complications and adverse effects of the use BoNT-A are usually transitory and, in most cases, technique-dependent (35). There are isolated few reports of systemic adverse effects after BoNT-A injections with the use of doses larger than those usually recommended for cosmetic purposes (94).

The most common injection-related side effects are pain, transitory edema, erythema, hematomas, and ecchymoses (35,94). Common technique-dependent complications include eyelid ptosis, asymmetries, and excessive brow elevation.

In the lower face, the most common complications are related to high doses or erroneous application of BoNT-A. These can cause undesirable paralysis of the musculature, resulting in asymmetric smiling and complications due to the incompetence of the sphincter function of the mouth (94). Some symptoms appear as a consequence of lip movement difficulties, as in swallowing, speaking, smoking, whistling or playing wind instruments, involuntary biting of the tongue, lip parenthesis, filter disappearance, and difficulties in specific lip movements, such as spreading lipstick with the lips and involuntary dribbling during speaking (35). Thus, caution is required for musicians who play wind instruments, professional singers, speakers or actors, because of the chance to have difficulties with lip proprioception after treatment.

The most common complications in the treatment of the neck are dysphagia and difficulties in flexing the neck and nodding (95).

In some focal forms of hyperhidrosis, some transitory compromising of the adjacent musculature can occur (96). Patients who perform minutely detailed activities with the hands, such as artisans, pianists, and others, deserve special attention (96). Residual areas of hyperhidrosis and asymmetries are also reported with the use of BoNT-A.

## OTHER RECENT INDICATIONS

Recent studies showed effects of BoNT-A in other conditions or diseases, such as depression (97–100), rosacea (101–105), oily skin and associated conditions (106,107), Raynaud's phenomenon (108–110), and inverse psoriasis (111–112). Further studies are needed to establish effective doses and safety.

## CONCLUSION

Botulinum neurotoxin type A injections are safe and effective for a variety of therapeutic and cosmetic conditions. Facial wrinkles, especially those located in the upper face, and some asymmetries are mainly caused or worsened by the repeated contraction of facial muscles.

Knowledge of the anatomy of the facial muscles as well as the use of proper technique are mandatory to reach predictable results and avoid complications. Differences between products and patients must be considered in all BoNT-A treatments.

BoNT-A treatments have greatly developed and increased in the last few years with new potential uses. It is nowadays one of the most performed minimally invasive procedures for skin rejuvenation.

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# Soft Tissue Augmentation

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## INTRODUCTION

Throughout the past several decades, we have seen major changes and advancements in the injectable preparations used for soft tissue augmentation. Fillers have been used successfully for many years to improve the skin's contour and reduce depressions in the skin due to scars, injury, or facial aging. Examples of features that have been treated include static and dynamic lines of facial expression (e.g., glabellar lines, "smoker's" lines, "marionette" lines, forehead lines, "bunny" lines, "crow's feet," and nasolabial folds [NLFs]), cheek depressions, and acne scars. However, more recently, we have seen the transition from the two-dimensional to the three-dimensional approach for the correction of facial aging. What was started by Dr. Arnold Klein in the 1980s with lip augmentation and collagen has progressed to the broader concept of volumizing the face and the use of an arsenal of new filler materials and continuously evolving techniques to correct the subcutaneous atrophy and fat loss that are hallmarks of the aging face.

## EVOLUTION OF FILLERS AND SOFT TISSUE AUGMENTATION

Soft-tissue augmentation can be traced back to 1893, when Neuber described autologous fat transfer from the arms for facial depressions. In 1899, Gersuny first injected petrolatum into the scrotum for testicular prosthesis, and later Eckstein injected paraffin for fistulas, hernias, and cosmetic enhancement. With the discovery of the syringe, Bruning injected free fat in 1911. The use of liquid silicone for cosmetic purposes began in Germany, Switzerland, and Japan in the 1940s, and in the 1960s, the DOW Corporation developed a more purified medical grade silicone that gained popularity in the United States. However, despite initial success and reports of its safety in soft tissue augmentation by qualified medical professionals, illegal use of non-medical-grade silicone and improper injection volumes and technique continued to produce severe adverse reactions. In the 1990s the FDA made its position on injectable silicone for aesthetic purposes clear—injectable silicone is an unapproved medical device.

Following extensive clinical trials in the late 1970s, bovine collagen was first approved by the FDA in 1981. The approval of bovine collagen marked the beginning of a new era of soft-tissue augmentation. Over the past 10 years, the number of approved facial fillers in the United States and Europe has grown rapidly, and there is a constantly evolving and expanding assortment of filler materials and devices for soft tissue augmentation. Throughout the literature, these have been subdivided based on injection level (superficial dermis, mid-dermis, deep dermis, and subdermal), derivation (autologous, biologic, synthetic), and duration of effect, to name a few. For the purposes of this chapter, the soft tissue fillers are categorized as temporary

(~3–12 months), long-lasting/semipermanent (~12 months–5 years), and permanent (>5 years).

## TEMPORARY FILLERS

The most well established temporary fillers include bovine collagen, human collagen, porcine collagen and hyaluronic acid (HA) fillers. We only briefly overview the former three, as they are either no longer commercially available or archaic. The bulk of this section rightfully focuses on HA acid fillers.

Bovine collagen fillers, Zyderm I®, Zyderm II®, and Zypplast® (Allergan/Inamed, Irvine, CA), were FDA approved in 1981 and have traditionally been considered the "gold standard" for fillers. Bovine collagen is composed of 95% type I collagen and 5% type III collagen suspended in buffered saline and 0.3% lidocaine. Because 2%–3% of treated patients developed localized allergic reactions associated with injections, pretreatment skin testing was recommended.

The risk of hypersensitivity to bovine collagen led to the development of human collagen as a dermal filler, which eliminated the allergic reactions seen with the bovine products. Human collagen, Cosmoderm 1® and 2® and Cosmoplast® (Allergan/Inamed, Irvine, CA), is bioengineered human collagen grown from noncadaveric human tissue culture lines and have the same concentrations and consistency as their bovine counterparts. However, human-derived collagen exhibited a limited duration of effect (~3–4 months) and different flow characteristics. Their place in the market was short-lived with the introduction of HA fillers only 9 months later. However, from 1981 to 2003, collagen was the only commercially available FDA-approved product in the U.S. market.

Porcine collagen, Evolence® (Colbar, Herzliya Israel), was approved by the FDA in 2008. It is composed of type I collagen derived from porcine tendons with ribose as a cross-linker. In contrast to bovine collagen, hypersensitivity reactions were rare (skin testing was not required) and initial findings suggested a longer duration of effect of up to 1 year. However, the manufacturer (Johnson and Johnson) discontinued production and marketing in 2009.

## Hyaluronic Acid

HA is a member of the glycosaminoglycan family and a natural component of human connective tissue. The HA molecule is identical across all species and lacks a protein component, thus it has little to no potential for immunologic reaction in humans. It is composed of repeating disaccharide units stabilized with cross-linked hydroxyl groups that bind water to create volume and plump the skin. With age and sun exposure, the amount of HA in the skin decreases, reducing the skin's water-binding capacity and turgor, ultimately leading to skin

wrinkling and sagging. HA can absorb up to 1000 times its molecular weight in water, and HA fillers volumize the face by replacing HA and restoring hydration. HA gels come in prepackaged syringes and do not require refrigeration.

Initial studies establishing the biologic compatibility and stability of HA as a filler material were pioneered in both a guinea pig model and in a multicenter clinical study. By varying the type of cross-linking material and its amount, the characteristics of the gel can vary in the degree of hardness, amount of lift, duration of effect, and resistance to degradation by heat or enzymes. Two types of HA filler substances have been FDA approved: streptococcal-derived fillers or NASHA (non-animal-sourced HA) gel and rooster comb-derived fillers (Hylaform® and Hylaform Plus® [Allergan/Inamed, Irvine, CA]), with the latter being withdrawn from the market shortly after introduction in 2004. The first NASHA filler, Restylane® (Medicis, Scottsdale AZ), was approved by the FDA in 2003. The new NASHA formulation, produced from bacterial fermentation, was safe and effective and could be reversed with hyaluronidase injections if necessary. The following NASHA fillers are currently FDA-approved: Juvederm® Ultra, Ultra Plus, and Voluma® (Allergan Inc, Irvine, CA), Restylane/Perlane® (Medicis, Scottsdale, AZ), Prevelle® Silk (Mentor, Irving, TX), Hydrelle® (formerly Eleveess®; Anika Therapeutics, Woburn, MA), and Belotero® (Merz, San Mateo, CA).

#### Indications

HA fillers are FDA approved for correction of “moderate to severe facial wrinkles and folds such as the nasolabial folds” (NLFs). However, they have been widely and successfully used for off-label volume enhancement of the vermilion lip, perioral area, suprabrow region, earlobes, back of hands, prejowl sulcus, and tear troughs. Most recently in 2013, Juvederm Voluma became the first HA filler FDA-approved to temporarily correct age-related volume loss in the mid-face in adults over the age of 21. Dermatologists Alam et al, Goldman et al, and Carruthers et al have also explored the safety and synergistic effects of combining HAs with other treatment modalities such as radiofrequency, intense pulsed light, and BoNT.

#### Application and Technique

As listed above, there are a plethora of HA fillers currently on the market from which to choose. The main difference among the various HA fillers is viscosity of the product and concentration of (cross-linked) HA. (Not all of the HA in a given filler is cross-linked, and this varies among the products. Non-cross-linked HA lubricates flow through the needle but does not contribute to the final correction.) In general, higher concentrations of HA and larger particle sizes are associated with stiffer products,

greater capacity to lift, and longer duration of effect. However, multiple variables affect the performance of individual fillers and ultimately, clinical efficacy is most important. For instance, Juvederm Voluma consists of lower molecular weight HA, but this allows for more effective cross-linking, thus resulting in a more viscous product and greater lift capacity than all other HA fillers available currently (Table 51.1).

It is also important to keep in mind that the relative hydration of each prepackaged product varies. For instance, Juvederm and Restylane are not fully saturated, while Prevelle Silk is maximally saturated with water in the syringe. Due to the hydrophilic nature of HA, more concentrated and less saturated products will absorb more water initially, resulting in more swelling after injection, and maintain more hydration after an equilibrium is reached with surrounding tissue, resulting in more sustained fullness in the treated area. Thus, overcorrection is not recommended or necessary with HA fillers.

Combining HA fillers with lidocaine has helped improve tolerability. However, patient comfort is often optimized with local anesthesia or nerve blocks with injections. Injection through a blunt tipped cannula also can increase tolerability and minimize pain, bruising, and edema. Numerous injection techniques have been described when using HA fillers, such as threading, fanning, serial puncture, cross-hatching, depot, layering, and the tower technique, to name a few. Further, the injection vehicle (cannulas and needle gauges), depth of injection, and particular brand of HA filler that is selected, as well as the personal preference of the physician, determine the area of the face to be treated.

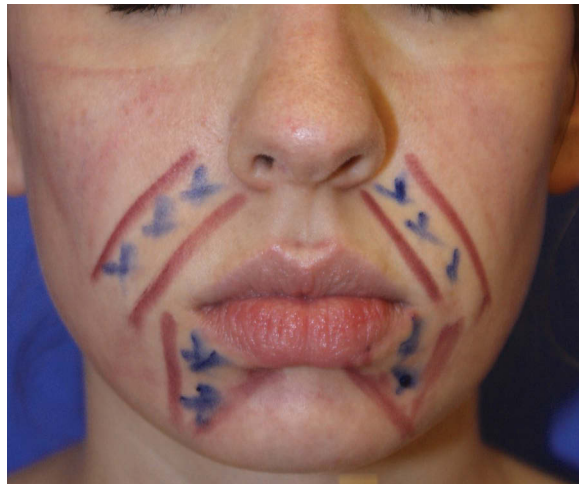
Most commonly, HA fillers are injected into the subcutaneous plane. However, two newly approved NASHA fillers, Belotero and Juvederm Voluma, deserve special mention given their unique injection depths, intradermal and supraperiosteal, respectively. Advocates of Belotero argue that it distributes more evenly within the dermis and optimally treats fine lines without producing the Tyndall effect. And as noted above, Juvederm Voluma is an FDA approved injectable HA filler indicated for deep (subcutaneous and/or supraperiosteal) injection for midface volumizing. Figures 51.1 and 51.2 illustrate some key injection strategies for HA fillers.

#### Safety and Efficacy

The safety and efficacy of currently FDA-approved HA fillers have been well reported in the literature and are very good. Notably, a pivotal randomized double-blind multicenter split-face study in 138 patients first demonstrated superior efficacy and comparable safety of NASHA (Restylane) compared with collagen (Zyplast®) over 6 months. HA fillers have also shown

**Table 51.1** Total HA Concentrations and Notable Gel Properties/Particle Sizes for the Most Commonly Used, Commercially Available NASHA Fillers

NASHA Filler	HA Concentration	Gel Property/Particle Size
Prevelle® Silk	5.5 mg/mL	~250 µm, maximally saturated
Restylane®	20 mg/mL	~260 µm
Belotero®	22 mg/mL	Continuous gel
Juvederm® Ultra and Ultra Plus	24 mg/mL	Homogenized gel (variably sized particles, highly cohesive)
Juvederm® Voluma®	20 mg/mL	Lower molecular weight and smaller chain HA, highly cross-linked
Perlane®	20 mg/mL	~1000 µm
Hydrelle®	28 mg/mL	Contraindicated if sulfite allergy



(a)

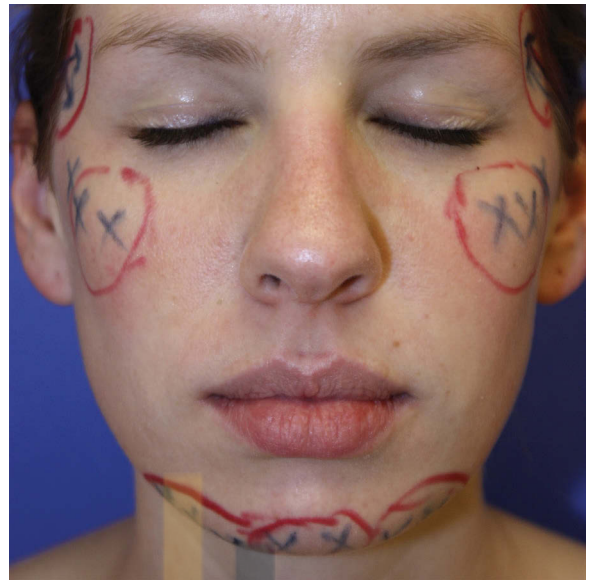


(b)

**Figure 51.1** (a,b). Hyaluronic acid (HA), tower technique. Possible treatment areas (marionette and nasolabial) for HA fillers are outlined in red. Blue X's indicate injection sites. HA filler is injected in the subcutaneous plane using the tower technique in this example.

a longer duration than collagen, often lasting 6–12 months. Further studies have shown that correction persists even longer than the expected 6–12 months when retreatment is performed before dissolution of the initial treatment product. (Initial studies of the newest HA filler, Juvederm Voluma, have suggested an even longer persistence of 1 to 3 years.) Additionally, Dover et al compared a large-particle NASHA-based filler with a small-particle NASHA filler and found similar efficacy, durability, and safety profiles. Finally, Taylor et al and Grimes et al have established safety and efficacy of HA filler injections in over 300 patients with skin of color in two prospective randomized clinical trials.

As reviewed above, HA fillers avidly bind water, and adverse events are most commonly limited to redness, swelling, and bruising in the days following treatment, which can be mitigated by immediate pressure following injection and periodic post-op icing. To alleviate tenderness associated with swelling in the days that follow treatment, acetaminophen is preferred over NSAIDs. Arnica forte has also been reported to



(a)



(b)

**Figure 51.2** (a,b). Hyaluronic acid (HA), suprapreriosteal. Potential treatment areas for suprapreriosteal placement of HA (Juvederm®, Voluma®, or Perlane®) are outlined in red. Suprapreriosteal injection entry points are indicated by the blue X's and blue arrow tips. The blue arrows illustrate the recommended direction of injection.

prevent bruising anecdotally, but no definitive evidence exists to support its efficacy.

While HA is a clear substance, blue discoloration, also known as the Tyndall effect, as well as lumps and nodules are possible if the product is placed too superficially. This may be corrected by dissolving the product with hyaluronidase injection. In addition, an acute angioedema-type hypersensitivity reaction has been reported with Restylane injection into the lip; however, the incidence of hypersensitivity reactions has declined dramatically over the years due to availability of more purified product. Intra-arterial injection is perhaps the



most feared and serious complication of HA fillers; however, occurrences are rare. Extra care should be taken when injecting in the glabella and periorbitally, as risk of intra-arterial injection is higher and carries greater morbidity. Moreover, Glogau and Kane demonstrated a direct correlation between injector techniques such as rapid injection and higher volumes with the rate of local adverse events in a prospective, blinded, controlled study of 283 patients undergoing HA injection.

### LONG-LASTING/SEMIPERMANENT FILLERS: 12 MONTHS TO 5 YEARS Poly-L-Lactic Acid (Sculptra®)

Injectable poly-L-lactic acid (PLLA) has been used worldwide for more than a decade to treat soft-tissue lipoatrophy related to aging and HIV-related lipoatrophy. Injectable PLLA is composed of microparticles of poly-L-lactic acid (polyglactin 910), the same substance used for absorbable sutures (e.g., Vicryl®). PLLA was first approved in 1999 in Europe (New-Fill®, Biotech Industry SA, Luxembourg) for soft tissue augmentation of scars and wrinkles. In August 2004, injectable PLLA was approved in the United States (Sculptra, Dermik Laboratories, Bridgewater, NJ) for the treatment of HIV-associated lipoatrophy. It has been described in the literature as an “injectable implant” and is effective in replacing diffuse volume loss. Injectable PLLA is biocompatible, biodegradable, and immunologically inert, thus no skin testing is required.

#### Indications

Sculptra is currently approved in the United States for correcting HIV-associated facial lipoatrophy (2004) and for aesthetic treatment of lines and contour deficiencies (2009). Injectable PLLA differs from other facial fillers such as HA in that it does not directly fill in lines or depressions. It creates the appearance of immediate volumization due to the mechanical effects of the injected suspension, but this disappears within a few days as the suspension fluid is absorbed. The long-lasting effects of injectable PLLA are seen in the months that follow as the PLLA microparticles degrade, inducing a fibroblastic host response and de novo collagen synthesis, resulting in gradual correction of the volume-depleted areas caused by lipoatrophy or age-related changes.

#### Application and Technique

To safely and effectively use injectable PLLA, it is imperative to understand facial anatomy, including location of major nerves, vessels, and fat pads, as well as to understand the changes that occur with aging. Moreover, awareness of the varying characteristics of facial skin and soft tissue in different cosmetic units is necessary for proper injection. Previous literature on this topic has highlighted the superficial and deep fat compartments and associated retaining ligaments that serve as boundaries. Atrophy due to the loss of fat compartments contributes to the exaggerated appearance of folds and lines as well as contour changes in the aging face. Volumization of these compartments restores facial contour and creates a fuller, more-youthful appearance.

Currently, the commercially available form of PLLA consists of microparticles measuring 40–63 µm in diameter. The practical reconstitution recommendations for Sculptra differ from the reconstitution instructions outlined in the Sculptra Aesthetic and Sculptra for HIV lipoatrophy package inserts. Reconstitution with sterile water +/- lidocaine with epinephrine

should be done at least 2 hours prior to injection and ideally 24 hours prior to injection at a dilution of no less than 5 mL and preferably 9 mL per vial of injectable PLLA. If lidocaine with epinephrine is included in the reconstitution, we recommend adding 6 mL of sterile saline at least 24 hours in advance and 3 mL of lidocaine with epinephrine prior to injection. (In our practice, we have found that lidocaine with epinephrine leads to less post-injection bruising and minimizes discomfort during injection.) After thorough hydration by reconstitution, the injectable PLLA solution may be vigorously mixed using a laboratory vortex to more evenly distribute the product and create a uniform suspension, decreasing the chance of nodule formation. With the more-dilute suspension, vigorous mixing using a laboratory vortex may be less important, but this has not been studied. Just before injection, the mixed product is drawn into 3-mL syringes using an 18-G needle, and a 25-G or 26-G needle (of length varying with personal preference) or 25-G blunt tip cannula are often used for all injection locations and planes. As smaller-bore needles tend to clog easily, a minimum 25-G bore size is recommended to allow adequate flow of product while optimizing patient comfort.

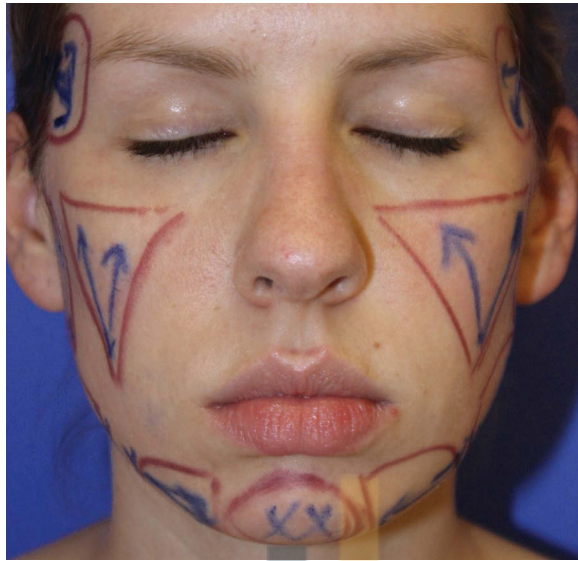
Early work by Vleggaar and Lowe helped establish the injection techniques and demonstrated efficacy of PLLA for sculpting and rejuvenating the aging face. Notably, one should avoid intramuscular or intradermal injection, and we recommend the fanning technique for deep subcutaneous injection (cheeks, preauricular areas) and fanning, depot, or retrograde linear threading technique for suprapariosteal injection (medial maxillary area, zygomatic area, temples, prejowl sulcus, chin). After injection, manual massage is performed for 5 minutes to further ensure even distribution of the product. Figure 51.3 (a, b) illustrates common areas of volume loss and associated site-specific injection techniques recommended by the authors when treating with injectable PLLA.

It is important to counsel patients that the immediately observed effects will fade over the following days as the injection fluid is absorbed. Moreover, overcorrection must be avoided, because as outlined above, injectable PLLA is a progressive collagen-stimulating product that will gradually volumize over the following weeks to months. Injectable PLLA requires multiple treatment sessions (generally 3–4) with 1–2 vials at 4–6 week intervals to restore volume.

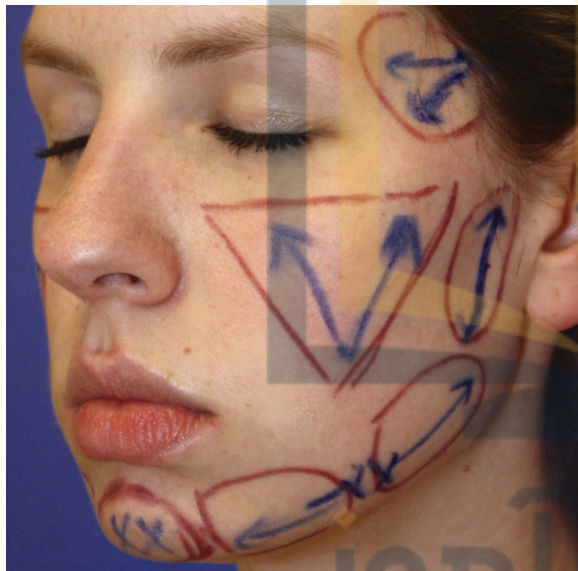
#### Safety and Efficacy

Akin to all soft tissue fillers, adverse events, such as bruising, edema, and erythema, may be seen. Recent retrospective and prospective studies have further examined the safety and efficacy of injectable PLLA, including an extensive cohort study published by Hanke, Redbord, and Levy which explored the safety and efficacy of injectable PLLA for treating HIV lipoatrophy. Importantly, given that injectable PLLA relies on neocollagenesis to produce a clinical effect, results are less predictable. However, once effects are realized, they are long lasting, most often 12–24 months. Some reports suggest that PLLA may have a duration period of 3 years or more.

One of the frequent side effects of injectable PLLA has been product clumping and formation of subcutaneous nodules. Refinements in technique have led to greater safety and efficacy, primarily by avoiding intradermal injections, by paying careful attention to correct injection depth and technique according to anatomic location, particularly periorbitally and periorally, by thoroughly mixing the PLLA suspension before injection, and by injecting only highly diluted



(a)



(b)

**Figure 51.3** (a,b). Poly-L-lactic acid (PLLA). Recommended treatment areas for suprapariosteal (chin, jaw, and temple) and deep subcutaneous (submalar cheek and pre-auricular) placement of PLLA are outlined in red. Blue X's and blue arrow tips indicate needle or cannula injection points, and blue arrows indicate direction of injection.

PLLA suspensions. Potential treatments reported to expedite nodule resolution in some cases include intralesional triamcinolone (~40 mg/mL) and/or intralesional 5-FU (50 mg/mL). Fortunately, if encountered, most nodules or product clumping resolve spontaneously with time.

### Calcium Hydroxylapatite (Radiesse®)

Injectable calcium hydroxylapatite (CaHA), also known as Radiesse (BioForm Medical Inc., San Mateo, CA) was first used in 2002 and initially approved by the FDA for vocal fold

insufficiency, oral/maxillofacial defects, and radiographic tissue marking. It is a non-allergenic bioceramic that is identical to the primary mineral constituents found in bone and teeth. CaHA has several properties that make it an important tool in facial recontouring, including lack of allergenicity, long-lasting effects, and high elasticity. Radiesse contains microspheres of CaHA, 25 to 45  $\mu\text{m}$  in diameter, in a soluble carboxymethylcellulose gel carrier.

After the CaHA is injected, the gel carrier is phagocytized, and the CaHA microspheres act as a scaffold for autologous collagen synthesis. As time passes, the CaHA microspheres are gradually broken down via normal metabolic processes into calcium and phosphate ions and are removed by the renal system. New tissue deposition and collagen proliferation in combination with the slow breakdown of the CaHA are responsible for the prolonged effects of Radiesse, often 12–18 months.

#### Indications

Radiesse is FDA-approved for subdermal implantation to correct moderate to severe folds/wrinkles (e.g., NLFs) and for HIV facial lipodystrophy. Currently, it is also widely used for volumization of facial soft tissue, including hand rejuvenation, correction of dorsal nasal deformities, chin and cheek augmentation, jaw recontouring, and for volumization of the prejowl sulcus, among other uses. It is not advisable to use CaHA in the lips. Skin testing is not required prior to injection.

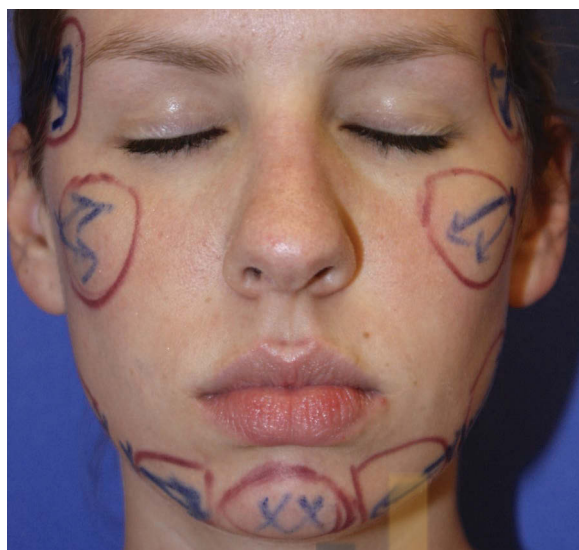
#### Application and Technique

In 2002, Dr. Mariano Busso became the first dermatologist in the United States to explore the use of CaHA for aesthetic enhancement. Busso described a unique 3-dimensional vectoring technique to be used for filler injections. Moreover, Busso developed a series of safe and cosmetically effective CaHA injection techniques for the malar, zygomatic, temporal, and supraciliary areas, as well as the lower face and hands. Notably, Busso's hand injection technique using CaHA represents the first clinical trial to evaluate the efficacy of a soft tissue filler for hand rejuvenation.

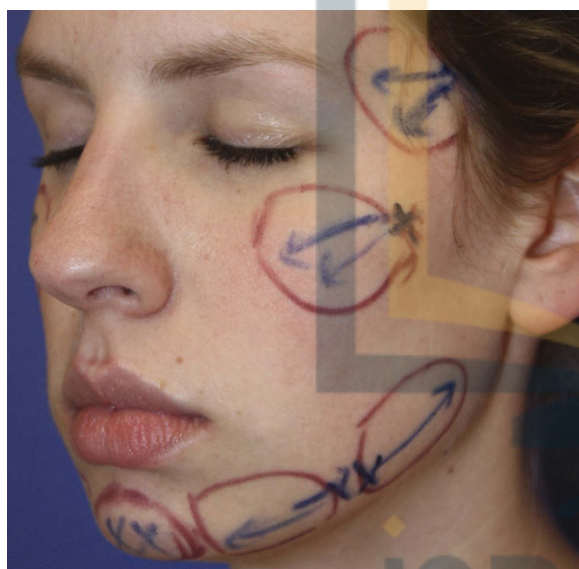
Commercially available CaHA, Radiesse, is sold as a white, sterile, semisolid gel in a 1.3-mL disposable syringe and can be stored at room temperature for up to 2 years. CaHA premixed with lidocaine is not available. In the past, pain during injection of CaHA was a significant limitation. However, a study by Busso and Voigts in 2008 showed that lidocaine can be added to CaHA safely without harmful changes in its physical properties or significant effects on the product longevity and clinical benefits. In 2009, a novel technique for mixing the product received FDA approval. The technique involves drawing up 0.2 mL of 2% plain lidocaine into a 3-mL syringe and then mixing this with the commercially available 1.3-mL syringe of Radiesse via a Luer-lock connector at least 10 times to create a homogeneous mixture. Further, as discussed in later commentary by Busso, the addition of lidocaine to CaHA by definition alters the rheological properties of the filler (i.e. decreased viscosity, elasticity, and extrusion force). This creates more possibilities for injectors to tailor treatments, as the rheological properties of the filler can be matched to the area being treated by adding varying amounts of a diluent such as lidocaine.

As a general rule for soft tissue fillers, the depth of the defect guides the depth of injection, and deeper defects are better filled with more viscous fillers. Radiesse should be injected slowly in either an antegrade or retrograde fashion into the deep subcutaneous or suprapariosteal planes using a 25- to 27-gauge needle or cannula. Importantly, injectors should be





(a)



(b)

**Figure 51.4** (a,b). Calcium hydroxylapatite (CaHA). Possible treatment areas for supraperiosteal (chin, jaw, and temple) and deep subcutaneous (malar cheek) placement of CaHA are outlined in red. Blue X's and blue arrow tips illustrate needle or cannula injection entry sites. The blue arrows indicate the direction of injection.

cognizant of danger zones and use extreme caution, particularly if injecting the glabella (supratrochlear artery) and superior NLFs (angular artery and its branches/anastomoses), given the increased risk of embolization in these areas. As with other soft tissue fillers, a plethora of techniques have been described including linear retrograde, tunneling, threading, serial puncture, fanning, and cross-hatching, and often the technique utilized is guided by personal preference and location being treated. Overcorrection is not recommended. Areas of volume loss well suited for treatment with Radiesse and their associated injection techniques are shown in Figures 51.4 and 51.5



**Figure 51.5** Calcium hydroxylapatite (CaHA), hand rejuvenation. Treatment areas for hand volume loss are outlined in red. Blue X's indicate entry points for cannula or needle, and blue arrows indicate recommended direction of injection in subcutaneous plane.

#### *Safety and Efficacy*

In general, complications after treatment with injectable CaHA are mild and often related to technique. As CaHA requires a fibrotic reaction in order to be effective (collagen synthesis), granulomatous reactions and nodules can be seen. Nodules are most frequently observed after lip augmentation, and thus CaHA is generally discouraged for correcting hypolabium. If encountered, nodules may be treated with massage, needle disruption, or excision. Many also discourage the use of CaHA near the infraorbital rim, as injuries to the infraorbital nerve may cause prolonged anesthesia and paresthesia.

The pivotal trial evaluating safety and efficacy by Smith, Busso, McClaren, and Bass compared CaHA with human-based collagen for correction of NLFs. The randomized controlled prospective study included 117 subjects who were treated with CaHA on one side and with collagen on the opposite side. In this initial study, the subjects were followed up to 6 months: 79% had superior improvement on the CaHA side ( $P < 0.0001$ ) using the Global Aesthetic Improvement Scale and significantly less volume was needed for optimal correction in the fold treated with CaHA. The adverse events were limited to erythema, edema, and bruising and comparable between human-based collagen and CaHA; however, bruising and edema were more documented on the side treated with CaHA. One nongranulomatous nodule was observed after treatment with CaHA compared with three nodules seen after treatment with human-based collagen. Subsequent results from this same trial, including data from 99 of the original 117 subjects, were reported after more than 3 years, and at 30 months, 40% of the CaHA-treated folds were rated as "improved" or better. No long-term or delayed-onset adverse events were observed. Two additional blinded, split-face, randomized controlled trials by Moers-Carpi and colleagues in Europe found longer-lasting results and increased satisfaction with CaHA compared with HA fillers in the treatment of NLFs.

Busso et al. published a multicenter, blinded, randomized clinical trial in which patients were treated with either CaHA or placebo for hand rejuvenation. They found statistically significant improvement in the treatment group, and no adverse effects on hand function were noted in the study. Finally, as CaHA is a normal constituent of bone, there have been concerns that it may interfere with radiological imaging.



Reassuringly, a study by Carruthers and co-workers found that while CaHA is usually visible (on CT scans more consistently than on x-rays), it does not obscure underlying structures, appears distinct from surrounding bone, and does not interfere with normal analysis. In addition, the study did not find any evidence supporting migration of injected CaHA or evidence of osteogenesis associated with deep dermal and subcutaneous injection.

### PERMANENT FILLERS: >5 YEARS

The comprehensive list of commercially available injectable fillers, including those available outside of the United States, is not only too vast for the confines of this chapter, it is also a continuously moving target. Further, as the demand for facial volumization and rejuvenation continues to grow, the search for a permanent, safe, effective, and cost-friendly soft tissue filler is particularly acute. An array of new classes of permanent fillers such as polyacrylamide gels, carboxymethylcellulose, and acrylic acid-derived fillers—to name a few—are being explored throughout other parts of the world, but are not FDA approved for use in the United States at this time. For completeness, two permanent soft tissue fillers with particular significance in the United States deserve brief mention: injectable silicone and polymethylmethacrylate (PMMA). However, in the opinion of the authors, in-depth knowledge of the temporary and long-lasting/semipermanent soft tissue fillers discussed above is most clinically relevant and necessary for safe and effective facial volumization currently.

PMMA (Artefill®; Artes Medical, San Diego, CA), is a suspension containing 20% PMMA suspended in 80% bovine collagen. It was FDA approved in the United States for the correction of NLFs in October 2006, following earlier use in Europe and other parts of the world as Arteplast® and later Artecoll® in the 1990s. The carrier, bovine collagen, provides initial correction and is degraded over several months, leaving behind the PMMA microspheres. Given the bovine component, pretreatment skin testing is necessary. Sensitivity to bovine collagen and animal derivation as well as granulomatous reactions remain issues with injectable PMMA; however, the latter has declined with newer generation product, Artefill.

Injectable silicone is a synthetic, viscous compound composed of long polymers of dimethylsiloxanes with terminal trimethylsiloxane ends. As reviewed previously in this chapter, injectable silicone has been used in the United States since the 1960s but was never FDA-approved for soft tissue augmentation due to problems with product purity, misuse, and tainted public perception (at least partially attributable to controversy surrounding silicone breast implants in the 1990s). Silikon-1000® (Alcon, Fort Worth, TX) is a highly purified 1000 centistoke liquid silicone oil FDA approved in 1994 for treatment of retinal detachment. Under the FDA's modernization act of 1997, injectable silicon such as Silikon-1000 can legally be used "off-label," including usage for soft tissue augmentation and other aesthetic purposes. Some dermatologists use Silikon-1000 for the treatment of HIV-associated facial lipoatrophy, acne scars, and to correct age-related subcutaneous atrophy and fat loss. The safety and efficacy of highly purified injectable silicone, such as Silikon-1000, is a point of controversy between its critics and advocates and remains highly debated. More objective data and long-term studies are needed to elucidate the proper space injectable silicone should occupy in the expanding world of soft tissue fillers.

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# Bioelectricity and Its Application in Cosmetic Dermatology

Ying Sun and Jue-Chen Liu

## INTRODUCTION

The interest in using electricity to treat ailment has a very long history. The electric torpedo fish was used to shock people to treat headache and gout over two centuries ago (1). The importance of the electrical control of cell physiology became apparent from the famous experiments of Galvani. His epic work on frog nerve-muscle preparations included the use of lightning rods connected to nerves via wires resulting in leg muscles twitching during a lightning storm. Similarly, static electricity generators creating sparks that activated nerve conduction also caused muscle to twitch. Equally important was his observation during a public experiment in Bologna in 1794 that the cut end of a frog sciatic nerve from one leg stimulated contractions when it touched the muscles of the opposite leg.

Collectively, these experiments provided definitive evidence for “animal electricity” or bioelectricity, i.e., the electricity generated by biological systems. In addition, with this last experiment, Galvani had demonstrated the existence of the injury potential. An injury potential is a steady, long-lasting direct current voltage gradient induced within the extracellular and intracellular spaces by current flowing into and around an injured nerve. This discovery predated the finding of the better known action potential, which is a rapid, self-regenerating voltage change localized across the cell membrane (2). In parallel with Galvani's work, Volta was developing these ideas to create the first battery. Recognizing the parallel with animal electricity, Volta used batteries therapeutically to treat deafness. Others, however, were less rigorous scientifically in the promotion of electrical-based therapies.

For more than a century there was widespread and irrational use of galvanism and static electricity. Static electricity generators were in common use and were promoted and sold because they created an allegedly beneficial “electric air bath” or a “negative breeze.” The electric air bath involved charging the patient and using a grounded electrode to draw sparks from a chosen part of the body. The negative breeze allegedly was helpful in treating insomnia, migraine, and baldness. With the electrode polarity reversed, a “positive breeze” was used to treat kidney disease (2).

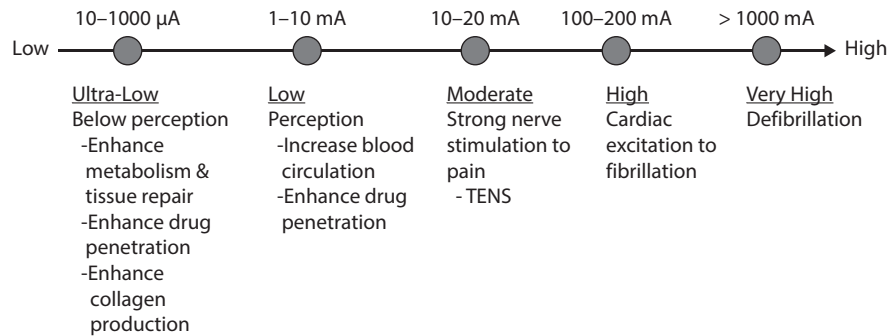
Naturally occurring electric currents at human skin wounds were measured by the German physiologist Emil Du Bois-Reymond over 160 years ago. As a founder of modern electrophysiology, he documented in detail the electric activities associated with nerve excitation, muscle contraction, and wounds. While electric activities in the nervous system and muscles are prevailing concepts in science, however, the wound electric fields have remained very poorly understood and largely ignored until recently. Modern technologies, such as vibrating probe,

glass and platinum microelectrodes have been used to confirm and provide significant understanding of the wound electric fields.

Therapeutic applications of electrotherapy may be generally grouped into two areas: electric neurostimulation and biomimetic electric stimulation. Electric neurostimulation applications are well known and relatively well studied. Electric neurostimulation devices can either be implanted inside the body such as pacemaker, or be applied to the skin surface such as transcutaneous electric nerve stimulation (TENS) devices. While better mechanistic understanding has been achieved with electric neurostimulation applications, much less well understood is the biomimetic electric stimulation that utilizes very low electric potential and current intensity for tissue healing and regeneration. In contrast to electric neurostimulation, the micro-amperage of electricity used in biomimetic electric stimulation is typically below the threshold of human sensory detection, which is in the similar magnitude of body's own bioelectricity, hence the terminology biomimetic electric stimulation. While electric neurostimulation offers a surprisingly wide range of therapeutic applications and possibilities, the potential of electric biomimetic stimulation is equally promising due to its tissue regenerative ability. Historically, there has been periodic interest in the bioelectricity and use of biomimetic electricity for therapeutic applications. However, due to the separate research paths between biophysics and modern cellular biology and molecular biology, a knowledge gap exists between the biophysical and biochemical research.

It is well known that the levels of electric current intensities affects induced biological responses (3). There are some general thresholds (as approximate reference points) corresponding to different tissue responses to electrical current. The passage of a high electrical current ( $> 100$  mA– $200$  mA) through the human body may result in serious injury or tissue damage such as cardiac excitation, fibrillation, or electrical burns. If greater than  $1000$  mA, the electric current may cause defibrillation or even fatality. Low to moderate electrical currents ( $1$ – $20$  mA) are generally considered safe and have been applied to patients for neuron stimulation for a wide variety of therapeutic applications (Figure 52.1) (3–5). When an ultra-low electrical current ( $10$ – $1000$   $\mu$ A) is applied to a patient, the electrical stimulation of the skin tissue cannot be perceived by the patient because it is below the sensory detection threshold of the skin. Nevertheless, the biological responses of the tissue under the treatment are as profound as, if not more than, the neurostimulation by commonly known electrotherapeutic modalities. Therefore, the focus of this chapter is on less commonly known ultra-low microampere electrical stimulation of tissues and its potential applications to cosmetic dermatology.





**Figure 52.1** Different electrical current intensities produce different biological responses. Ultralow electric current intensity in the range of 10 to 500 mA is known to have regenerative activity, whereas higher current intensities in 1 to 20 mA have neuron-stimulation activity. Above certain thresholds of electric current intensities, adverse effects such as cardiac excitation, fibrillation, or defibrillation may occur. (Note: these electric current intensities only serve as approximate reference points rather than precise threshold values.)

It should be noted that bioelectricity is low-level electric potential or current generated by the biological systems, whereas biomimetic electricity is a similar magnitude of current generated externally to mimic the levels of bioelectricity. Scientists have devoted significant effort to investigating the role of bioelectricity in healing, regeneration, and other effects as well as to develop devices/delivery systems to generate biomimetic electricity for biological applications.

### TRANSCUTANEOUS ELECTROTHERAPY AND DELIVERY ENHANCEMENT

Transcutaneous electrotherapy is one of the fundamental elements of physiotherapy practice and sports medicine with widespread applications including TENS, and interferential current, as well as microcurrent therapy for pain control and wound healing. Electrical devices and battery-powered patches are used for topical or transdermal drug administration via iontophoresis, and electrical stimulation in various medical practices such as pain and chronic wound management (6,7) and dermatology (8,9).

#### Ultra-Low Intensity Electricity Therapy

It has been reported that direct electric currents ranging from 10  $\mu$ A to 1000  $\mu$ A increase ATP concentrations in the tissue and stimulate amino acid incorporation into the proteins of rat skin. Minimum current intensities of approximately 50  $\mu$ A are necessary to obtain a maximal stimulatory effect on protein synthesis. When higher currents at a range above 1000  $\mu$ A are applied, the current failed to increase ATP levels significantly. These stimulatory effects are maintained to a level of approximately 1000  $\mu$ A (10). The application of specific low intensity currents for the metabolic effects imply a new area for exploration. The amino acid transport through the cell membrane, followed by the  $\alpha$ -aminoisobutyric acid uptake, is stimulated between 100  $\mu$ A and 750  $\mu$ A. The stimulatory effects on ATP production and on amino acid transport, apparently mediated by different mechanisms, contribute to the final increased protein synthesizing activity, DNA metabolism followed by thymidine incorporation remains unaffected during the course of current application. The effects on ATP production can be explained by proton movements on the basis

of the chemiosmotic theory of Mitchell, while the transport functions are controlled by modifications in the electrical gradients across the membranes.

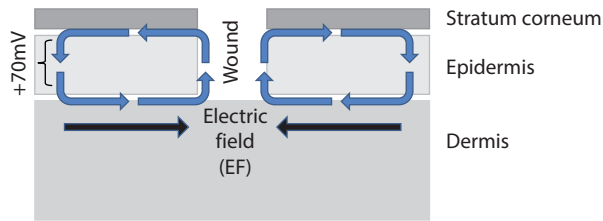
Microcurrent treatment was reported to be more effective than conventional therapy for chronic Achilles tendinopathy in a randomized comparative trial (11). The healing processes of tendon tissue remain to be explored.

### BIOELECTRICITY

Biological systems including human body has its own innate electrical system. For example, the innate electrical system in the human body regulates the body's functions via communication among organs through the well-known neural system, and some less understood cellular activities such as the bioelectricity associated with tissue regeneration (12). When injured, the body generates a low level wounding electricity to facilitate the healing process, e.g., human skin generates up to 10  $\mu$ A/cm<sup>2</sup> of outward bioelectric current during healing, and common signaling pathways are able to steer cell movement in both electrical and chemical gradients (13,14).

Most recently developed microneedle arrays are able to measure the transdermal skin potentials at multiple sites simultaneously. Bio-Electric Imager® detects and visualize electric potential at skin surface without direct skin contact, which will be discussed in detail in a later section.

The measurable bioelectricity during the wound healing process has been described as the endogenous "skin battery" that pumps sodium ions from the exterior the epithelium to the interior using the Na/K-ATPase located on the basal surface of epidermal cells together with Na<sup>+</sup> channels on the pical surface with an outward electric current of about 10–100  $\mu$ A/cm and an electric potential gradient about 60 mV/mm around the wound (1). The bioelectric field and current can be detected within about 0.5–1 mm from the edge of the wound and last until the wound is re-epithelialized (2). For an intact epithelium (or skin), because of the Na<sup>+</sup> transported inwards is not completed balanced out by anion movement, an excess of positive charge accumulated results in positive potential of the epithelium membrane, i.e, transepithelial potential as shown in Figure 52.2. However, if the epithelium is perforated by a wound, as shown in Figure 52.2, the potential drives the



**Figure 52.2** Ion transport (predominantly inward transport of Na) properties of mammalian skin result in a substantial transepithelial electric potential (TEP) of about 70mV, which establishes an injury current (curved arrows) upon wounding and an electric field within the subepithelial tissues (horizontal arrow). In this case, the return path for the current is in the layer between the dead, cornified stratum corneum and the living epidermis. (Modified from McCaig CD et al., *Physiol Rev*; 85:943–78, 2005.)

current flow through the newly formed low resistance path, generating an electric field with the negative pole at the wound and the positive under the unbroken epithelium or skin surrounding the wound which behaves just like a battery. Hence the term “skin battery” which is sometimes used to describe the bioelectricity phenomena influencing healing of a skin wound.

As mentioned earlier, endogenous DC electric fields occurring naturally during skin injury was first demonstrated in wounds by Emil DuBois-Reymond over 150 years ago. He measured electric currents flowing out of a cut he made in his own finger. In recent studies with various modern techniques such as micro-glass electrodes and vibrating probes, scientists confirmed a similar electric current flow in wounds in both the skin and cornea of several species, including human skin (14). In cornea and skin, a laterally oriented, wound-induced electric field is generated instantaneously when the epithelium is damaged, and it persists until re-epithelialization restores the electrical resistance barrier function of the epithelium. These electric fields are estimated to be at least 40–50 mV mm<sup>-1</sup> at cornea wounds and 100–150 mV mm<sup>-1</sup> at skin wounds (2). Growing experimental evidence suggests an important role for such electric signals in directing cell migration in wound healing.

Endogenous DC electric fields have also been measured during development and regeneration and after damage to non-epithelial tissues. These EFs arise because of spatial and temporal variations in epithelial transport of charged ions such as Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup>, and spatial variations in the electrical resistance of epithelial sheets. Disruption of the endogenous electrical gradients during development induces skeletal and neural abnormalities. For example, it has been reported that the spinal cord responds to damage by generating large and persistent electrical signals. Consequently, externally applied electric stimulation of physiological magnitude can promote spinal cord repair in human and other mammals (21,23).

It is now generally accepted that there are endogenous electric fields, and disruption of these electric fields disrupts wound healing. Research has been conducted into cellular response to electric fields for several decades. Among the various signals hypothesized to guide cell migration and

division in development and wound healing, electric signals have not been well studied. The biological and medical research community generally is not familiar with the possible roles of electric fields as a directional signal in guiding cell migration to heal a wound. The lack of rational explanation for the mechanism of bioelectricity on tissue regeneration has led to skepticism, especially when modern molecular biology and electrophysiology has taken different approaches to the phenomenon, and few researchers are really at home in both fields (1).

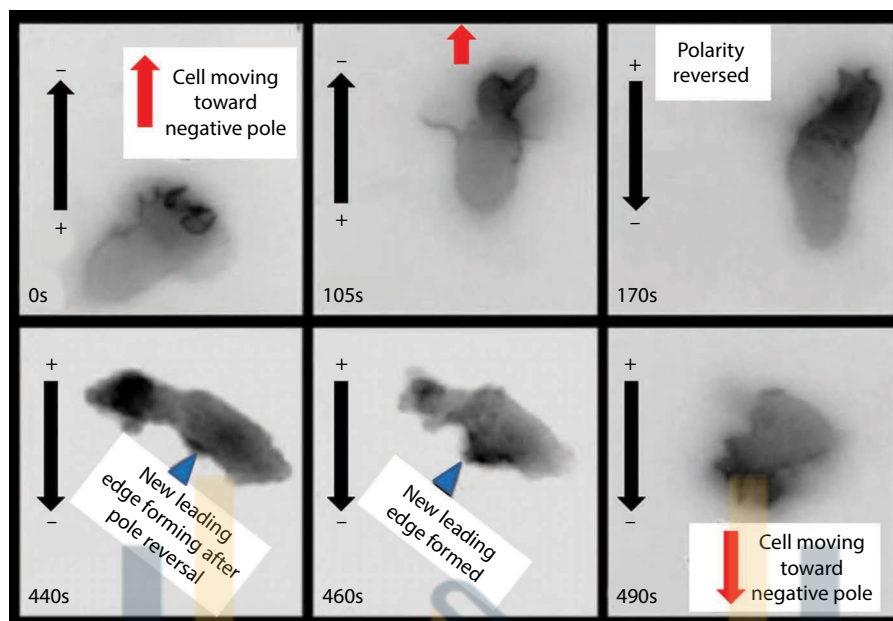
It has been repeatedly demonstrated by in vitro experiments that electric fields of strength similar to those measured in vivo dictate important cell behaviors such as directional cell migration and cell division orientation. Zhao et al. (13) provides further experimental evidence that the electric signal as a directional cue probably plays a far more important role in directing cell migration in wound healing of epithelium than previously believed, and two genes are important for electric field-induced cellular response. It was shown that polarized phosphatidylinositol 3-kinase (PI3K) signaling steers the migration of human cells across a gradient of electric potential, a process called electrotaxis. The lipid phosphatidylinositol 3,4,5-triphosphate (PIP3) appears to be a pivotal molecule. It is concentrated at the leading edge of the cell, where signaling components bind to it. These signaling components, in turn, lead to the localized polymerization of actin and the formation of a protrusion in the direction of migration (14). Figure 52.3 shows a promyelocytic cell with its leading edge labeled with a probe that detects PIP3 production. The narrow arrows at the top left show the gradient of electric potential in which the cells are migrating. The wide arrows show the direction of cellular movement. Reversal of the polarity of the electric field is followed by a change in locale of PIP3 to a region that becomes the new leading edge, shown as arrowheads.

## RECENT SCIENTIFIC ADVANCES IN APPLICATIONS OF BIOELECTRICITY/BIOMIMETIC ELECTRICITY

The application of biomimetic electrical stimulation similar to the body's own bioelectricity has been used to achieve clinical efficacy, particularly in healing enhancement of chronic wounds (15–17), as well as to perform various in vitro and in vivo investigation on cellular activities in order to result in the effects of physiological level of electricity on tissues (18–26) or to utilize its powder for tissue engineering (27). It was reported that the biomimetic electric field appeared to play an important role in controlling human fibroblast activity by either significantly increasing or decreasing gene expression of over 400 transcripts investigated, including activity within specific cellular signaling pathways such as TGF-β, G-proteins, and inhibition of apoptosis.

## BIOELECTRICITY OF THE SKIN WOUNDS AND ITS CONNECTION TO SKIN AGING

The epidermis generates a transepithelial potential (TEP) of 20–50 mV across itself, inside positive. Any wound or break in the epidermis creates a low resistance pathway and the TEP at the wound site is 0 mV. However the TEP of the intact epidermis around the wound is still present, resulting in a lateral voltage gradient or electric field along the skin surrounding the wound. There is evidence that this lateral electric field stimulates



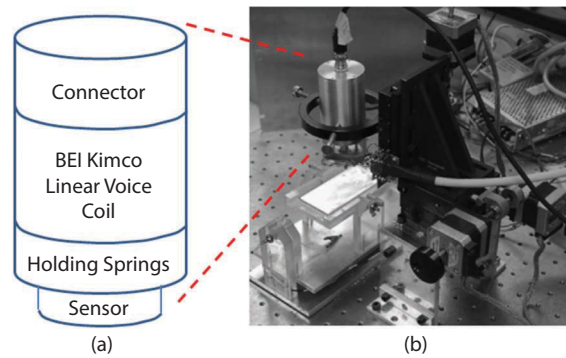
**Figure 52.3** The effect of electric signal in directing the migration of human keratinocyte cell in wound healing. It shows a promyelocytic cell with its leading edge labeled with a probe that detects PIP3 production. The narrow arrows at the top left show the gradient of electric potential in which the cells are migrating. The wide red arrows show the direction of cellular movement. Reversal of the polarity of the electric field is followed by a change in locale of PIP3 to a region that becomes the new leading edge shown by blue arrowheads. (Modified from Huttenlocher A, Horwitz AR, *N Engl J Med*; 356:303–4, 2007.)

keratinocytes in the area to migrate toward the wound, with the optimal response occurring at field strength of 100 mV/mm. To determine the actual fields strength at the edge of human wounds, Nuccitelli et al. (27,28) developed a new instrument that vibrates a small sensor perpendicular to the skin about 100 micrometers above the surface and uses the oscillating capacitance signal to determine the surface potential of the epidermis just beneath the stratum corneum. By measuring this surface potential in many positions around the wound, a spatial map of the surrounding electric field was generated. Based on an ultrasensitive vibrating probe technique for measuring extracellular currents (27), a sensitive noninvasive bioelectric field imager developed by Nuccitelli et al. (28) as shown in (Figure 52.4), and was used to measure the bioelectric wound current in vivo in mice. A more detailed description of the vibrating probe equipment setup and technique was given by Reid et al. (29). A noninvasive instrument based on the principle of vibrating probe (bioelectric field imager [BFI]) was used for mapping the electric field between the epidermis and the stratum corneum near wounds in both mouse and human skin (28). Rather than touching the skin, the vibrating probe vibrates a small metal probe with a displacement of 180 micrometers in air above the skin to detect the surface potential of the epidermis through capacitive coupling. The application of the vibrating probe device was demonstrated by measuring the electric field between the stratum corneum and epidermis at the margin of skin wounds in mice (Figure 52.5). An electric field of  $177 \pm 14$  mV/mm was measured immediately upon wounding and the field lines pointed away from the wound in all directions around it. Because the wound current flows immediately upon wounding, this is the first signal indicating skin damage. This electric field is generated at the

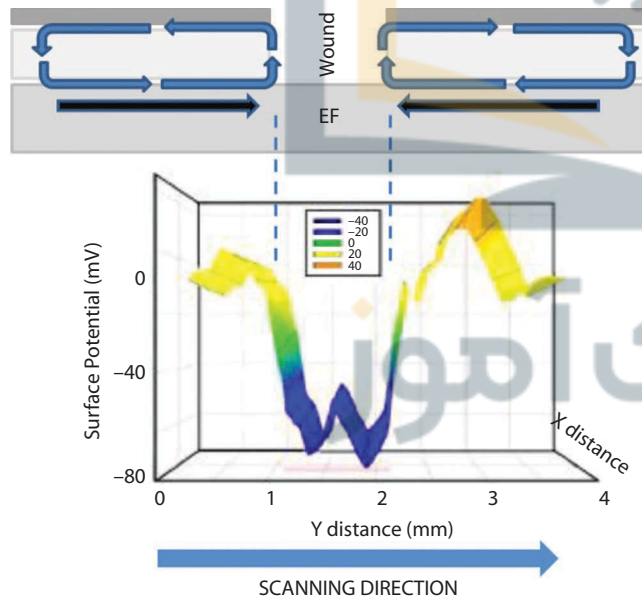
outer surface of the epidermis by the outward flow of the current of injury. An equal and opposite current must flow within the multilayered epidermis to generate an intra-epidermal field with the negative pole at the wound site. Because the current flowing within the multilayered epidermis is spread over a larger area, the current density and subsequent E field generated in that region is expected to be smaller than that measured by the BFI beneath the stratum corneum. The field beneath the stratum corneum typically remained in the 150–200 mV/mm range for 3 days and then began to decline over the next few days, falling to zero once wound healing was complete. Figure 52.6 shows the bioelectric field imager scans of the mouse skin wounds over time. It can be seen that wounding resulted in the development of wound electric potential, which persisted until the wound closed at day 3. Nuccitelli et al. (30) also demonstrated that the wounding electric field can be modified by topically applied pharmacological agents. The mean wound field strength decreased by  $64 \pm 7\%$  following the application of the sodium channel blocker, amiloride, to the skin near the wound and increased by  $82 \pm 21\%$  following the application of the  $\text{Cl}^-$  channel activator, prostaglandin E2. This experimental evidence suggests that both sodium ion influx and chloride ion efflux are maintaining the TEP of the skin and are carrying the wound current.

Recently, a similar bioelectric field imager, a commercially available DermaCorder™ (BioElectroMed, Berlingame, CA) was used to measure human skin's wound current in two test subject groups to investigate the role of endogenous electric fields in wound healing of young and old human subjects (30). The lateral surface wound field was measured between the stratum corneum and epidermis near a lancet wound on the arm and leg in 40 adults by DermaCorder as shown in Figure 52.7. Ten



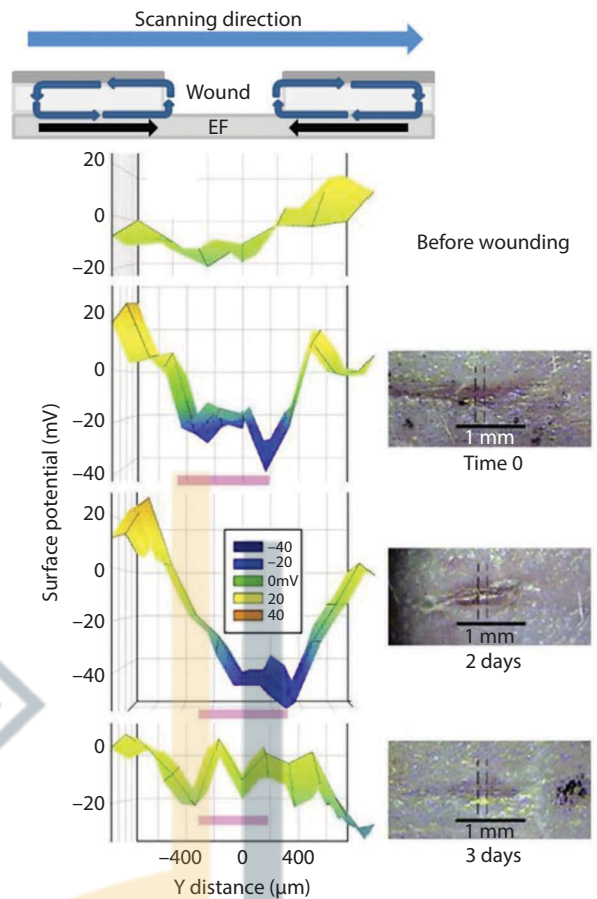


**Figure 52.4** Bioelectric field imager (BFI) vibrator and motorized manipulator. (a) Diagram of the vibrator using a voice coil (BEI Kimco LA 16-27-000A, Vista, California). The vibrator assembly incorporates two springs to hold the probe in place when the voice coil is not powered. (b) Photograph of motorized manipulator used to position BFI above a wound in mouse skin. An adjustable mouse platform maintained at 378C is shown on the lower left and the cylinder above it is the vibrator. (Modified from Nuccitelli R et al., *Wound Rep Reg*; 14:432–41, 2008.)



**Figure 52.5** Common field profile for wounds with a significant break in the epidermis. (Modified from Nuccitelli R et al., *Wound Rep Reg*; 14:432–41, 2008.)

women and ten men in the 18–29-year-old age group exhibited a mean electric field of 16359 mV/mm. Ten women and ten men in the 65–80 age range exhibited a mean field of 7815 mV/mm. Therefore the mean electric field of individuals in the older age group is only half that of the younger group (Figure 52.8). Since the wound electric potential is linked with the healing, the reduced wound electric potential may be a contributing factor to the well-known decreased healing rate associated



**Figure 52.6** Bioelectric field imager (BFI) scans of skin wounds over time. Mouse skin wound scanned before and at the indicated times after wounding. The precise region that was scanned is indicated by the dotted lines on the micrographs. Pink bar on the BFI scan indicates the location of the wound. (Modified from Nuccitelli R et al., *Wound Rep Reg*; 14:432–41, 2008.)

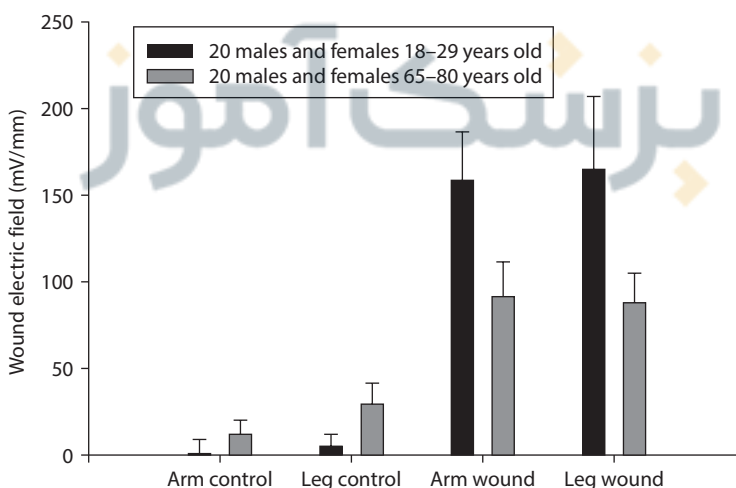
with wound healing among elderly. On the other hand, it is conceivable that if an external electric potential of physiological magnitude of electric would to apply on the wounds of these elderly to bring it to the level of the wound electric potentials among the younger subjects, the wound healing rates of these elderly might be enhanced.

### CELLULAR EFFECTS OF ELECTRICITY OF PHYSIOLOGICAL MAGNITUDE

In a well-cited study, Cheng et al. (10) reported an in vitro study to examine the cellular effects of low intensity direct electric current on freshly excised rat skin. Direct electric currents ranging from 10  $\mu$ A to 1000  $\mu$ A increased ATP concentrations about 500% in the tissue and stimulated amino acid incorporation into the proteins of skin tissues. Minimum current intensities of approximately 50  $\mu$ A were necessary to obtain a maximal stimulatory effect on protein synthesis. The amino acid transport through the cell membrane, followed by the  $\alpha$ -aminoisobutyric acid uptake, was stimulated between 100  $\mu$ A and 750  $\mu$ A. The stimulatory effects on ATP production and on amino acid transport, apparently mediated



**Figure 52.7** The DermaCorder™, a commercially available vibrating probe device, was used to measure wound electric potential by Nuccitelli et al. to investigate the effect of age on wound electric potentials in human subjects. (From Nuccitelli R et al., 18th Annual Meeting of the Wound Healing Society, San Diego, CA, April 24–27, 2008. With permission.)



**Figure 52.8** Ten women and ten men in the young group (18–29 years) exhibited a mean electric field of 16359 mV/mm, which is twice as much as the mean field of 7815 mV/mm exhibited by the 10 women and 10 men in the old group (65–80 years). (From Nuccitelli R et al., 18th Annual Meeting of the Wound Healing Society, San Diego, CA, April 24–27, 2008. With permission.)

by different mechanisms, contributed to the final increased protein synthesizing activity, DNA metabolism followed by thymidine incorporation remained unaffected during the course of current application. The highest stimulatory effects

were obtained with 50  $\mu\text{A}$  to 1000  $\mu\text{A}$ , with glycine incorporation increased by as much as 75% compared with nontreated controls. Higher current intensities, exceeding 1000  $\mu\text{A}$ , inhibited the protein synthesis by as much as 50% with currents of

15,000  $\mu\text{A}$ . Constant currents from 100  $\mu\text{A}$  to 500  $\mu\text{A}$  increased the transported amino acid analog by 30%–40% above control levels. Stimulation with higher intensities reduced the  $\alpha$ -aminoisobutyric acid uptake. The effects on ATP production can be explained by proton movements on the basis of the chemi-osmotic theory of Mitchell, while the transport functions are controlled by modifications in the electrical gradients across the membranes. During the course of normal wound healing, fibroblasts at the wound edge are exposed to electric fields ranging from 40 to 200 mV/mm. Various forms of electric fields influence fibroblast migration, proliferation, and protein synthesis. Thus, electric fields may contribute to fibroblast activation during wound repair. To elucidate the role of electric fields during the normal progression of healing, Jennings et al. (20) compared gene expression in normal adult dermal fibroblasts exposed to a 100 mV/mm electric field for 1 hour to non-stimulated controls. Significantly increased expression of 162 transcripts and decreased expression of 302 transcripts was detected using microarrays, with 126 transcripts above the level of 1.4-fold increases or decreases compared to the controls. Above the level of twofold, only 11 genes were significantly increased or decreased compared to controls. Many of these significantly regulated genes are associated with wound repair through the processes of matrix production, cellular signaling, and growth. Activity within specific cellular signaling pathways is noted, including TGF- $\beta$ , G-proteins, and inhibition of apoptosis. In addition, RT-PCR analysis of the expression of KLF6, FN1, RGS2, and JMJD1C over continued stimulation and at different field strengths suggests that there are specific windows of field characteristics for maximum induction of these genes. EFs thus appear to have an important role in controlling fibroblast activity in the process of wound healing.

Endogenous electric fields are generated lateral to skin wounds, with the cathodal pole of the field residing in the center of the wound. These fields are thought to be an important mechanism in guiding the migration of keratinocytes and other cells into wounds to effect healing. Sillman et al. (25) studied human dermal fibroblasts exposed to direct current electric fields of physiological strength, and quantified their migrational behavior. It was observed that human dermal fibroblasts only moved randomly, not directionally, in direct-current electric fields under conditions that support the directional migration of human epidermal keratinocytes. Additionally, neither the presence of the serum nor the serum plus additional  $\text{Mg}^{++}$  in the experimental medium supported the directional migration. Migratory rates of fibroblasts varied depending on the experimental medium used: in serum-containing medium the average velocity was as low as 0.23  $\mu\text{m}/\text{min}$ , while in serum-free keratinocyte medium the average velocity was as high as 0.36  $\mu\text{m}/\text{min}$ . These studies suggest that dermal fibroblasts do not respond to the endogenous electric field of a wound, and use other migratory cues to direct their movement into the wound bed.

Directional cellular locomotion is thought to involve localized intracellular calcium changes and the lateral transport of cell surface molecules. Brown et al. (26) examined the roles of both calcium and cell surface glycoprotein redistribution in the directional migration of two murine fibroblastic cell lines, in contrast to human dermal fibroblast result reported by Sillman et al. (25) and directional migration of NIH 3T3 and SV101. These cell types exhibit persistent, cathode-directed motility when exposed to direct current electric fields. Using time lapse phase contrast microscopy and image analysis, we have determined that electric field-directed locomotion in each cell type is a calcium

independent process. Both exhibit cathode-directed motility in the absence of extracellular calcium, and electric fields cause no detectable elevations or gradients of cytosolic free calcium. Based on the evidence, the authors suggested that galvanotaxis in these cells involves the lateral redistribution of plasma membrane glycoproteins. Electric fields cause the lateral migration of plasma membrane concanavalin A receptors toward the cathode in both NIH 3T3 and SV101 fibroblasts. Exposure of directionally migrating cells to Con A inhibits the normal change of cell direction following a reversal of electric field polarity. Additionally, when cells are plated on Con A-coated substrata so that Con A receptors mediate cell-substratum adhesion, cathode-directed locomotion and a cathodal accumulation of Con A receptors are observed. Immunofluorescent labeling of the fibronectin receptor in NIH 3T3 fibroblasts suggests the recruitment of integrins from large clusters to form a more diffuse distribution toward the cathode in field-treated cells. It was concluded that the mechanism of electric field directed locomotion in NIH 3T3 and SV101 fibroblasts involves the lateral redistribution of plasma membrane glycoproteins involved in cell substratum adhesion.

Sun et al. (31) reported keratocyte fragments and cells utilizes competing pathways to move in opposite directions in an electric field. Sensing of an electric field by cells—galvanotaxis—is important in wound healing, development, cell division, nerve growth, and angiogenesis. Different cell types migrate in opposite directions in electric fields, and the same cell can switch the directionality depending on conditions. A tug-of-war mechanism between multiple signaling pathways can direct *Dictyostelium* cells to either cathode or anode. Mechanics of motility is simplest in fish keratocytes. Keratocyte fragments are the simplest motile units. Cell fragments from leukocytes are able to respond to chemotactic signals, but whether cell fragments are galvanotactic was unknown. It was found that keratocyte fragments are the smallest motile electric field-sensing unit: they migrate to the anode, in the opposite direction of whole cells. Myosin II was essential for the direction sensing of fragments but not for parental cells, while PI3 kinase was essential for the direction sensing of whole cells but not for fragments. Thus, two signal transduction pathways, one depending on PI3K, another on myosin, compete to orient motile cells in the electric field. Galvanotaxis is not due to electric field force and does not depend on cell or fragment size. It was proposed that a “compass” model according to which protrusive and contractile actomyosin networks self-polarize to the front and rear of the motile cell, respectively, and the electric signal orients both networks toward cathode with different strengths.

Allen et al. (32) reported the electrophoresis of cellular membrane components creates the directional cue guiding keratocyte galvanotaxis. The key findings of their study include: (a) no evidence of asymmetric ion flow into the cell as the galvanotactic sensor, (b) cells move in the direction of fluid flow, but this is not required in galvanotaxis, (c) the charge and mobility of membrane components are critical to galvanotaxis, and (d) a PI3K-dependent pathway exists that transduces this polarization to the cytoskeleton.

## BONE REGENERATION INFLUENCED BY ELECTRIC FIELDS

Sundelacruz et al. (33) investigated bioelectric modulation of wound healing in a three-dimensional (3D) in vitro model of tissue-engineered bone. Long-standing interest in bioelectric



regulation of bone fracture healing has primarily focused on exogenous stimulation of bone using applied electromagnetic fields. Endogenous electric signals, such as spatial gradients of resting potential among non-excitabile cells in vivo, have also been shown to be important in cell proliferation, differentiation, migration, and tissue regeneration, and may therefore have as yet unexplored therapeutic potential for regulating wound healing in bone tissue. To study this form of bioelectric regulation, there is a need for 3D in vitro wound tissue models that can overcome limitations of current in vivo models. We present a 3D wound healing model in engineered bone tissue that serves as a preclinical experimental platform for studying electrophysiological regulation of wound healing. Using this system, we identified two electrophysiology-modulating compounds, glibenclamide and monensin, that augmented osteoblast mineralization. Of particular interest, these compounds displayed differential effects in the wound area compared to the surrounding tissue. Several hypotheses are proposed to account for these observations, including the existence of heterogeneous subpopulations of osteoblasts that respond differently to bioelectric signals, or the capacity of the wound-specific biochemical and biomechanical environment to alter cell responses to electrophysiological treatments. These data indicate that a comprehensive characterization of the cellular, biochemical, biomechanical, and bioelectrical components of in vitro wound models is needed to develop bioelectric strategies to control cell functions for improved bone regeneration.

### OTHER TISSUES/ORGANS AFFECTED BY ELECTRIC FIELDS

Cao et al. (34) studied polarizing intestinal epithelial cell electrically through the tyrosine protein kinase transmembrane receptor Cor2, and found that the endogenous electric field created by the transepithelial potential difference might act as an essential coordinating signal for apical membrane formation at a tissue level, through activation of the serine/threonine kinase LKB1 (also known as STK11) mediated by Ror2-ERK signaling.

Microorganisms also response to electric fields. Rudell et al. (35) reported migration of *Acanthamoeba*, a parasite, in an electric field. *Acanthamoeba* keratitis is a serious and debilitating, sight-threatening infection of the cornea. *Acanthamoeba* organisms have been found widely in our environment, including contaminated contact lens solutions, bottled water, public water supplies, freshwater lakes, and air, and the prevalence of *Acanthamoeba* infections has risen rapidly in the past few decades. The authors found that *Acanthamoeba* trophozoites move directionally in response to an EF in a 2D and 3D culture system. *Acanthamoeba* trophozoite migration is also voltage-dependent, with increased directionality with increasing voltage. This may provide new treatment modalities for *Acanthamoeba* keratitis.

### BIOELECTRICITY AND SKIN PIGMENTATION

Wounding skin generates endogenous electric fields of 100–200 mV/mm in the immediate vicinity of the wound. When keratinocytes are exposed to direct current electric fields of this magnitude, they exhibit galvanotaxis, or directional migration toward the cathode, suggesting that wound-generated electric fields provide migrational cues that contribute to wound healing. Because melanocytes must also migrate into the healing wound

to repigment it, their motility in response to electric fields of physiologic magnitude of was examined. Human skin-derived melanocytes, either exposed to 100 mV/mm direction current electric fields or non-exposed controls, both exhibited motility rates of 9  $\mu\text{m}/\text{hour}$ , significantly (three- to five-fold) lower than the motility rates of keratinocytes under identical conditions. However, Grahn et al. (36) reported that in sharp contrast to keratinocytes, melanocytes exhibited no directional migration in the electric field. Additionally, neither the number of primary dendrites per cell, nor the orientation of the dendrites with respect to the field vector, nor the average length of the dendrites was significantly different in melanocytes exposed to the electric field as compared to non-exposed controls. Thus, in marked contrast to keratinocytes, human skin-derived melanocytes do not respond to direct current electric fields of physiologic magnitude with either directional migration or reorientation of dendrites. This may account for the delay in repigmentation that often accompanies wound re-epithelialization.

### BIOELECTRICITY RANGES AND CELL PROLIFERATION

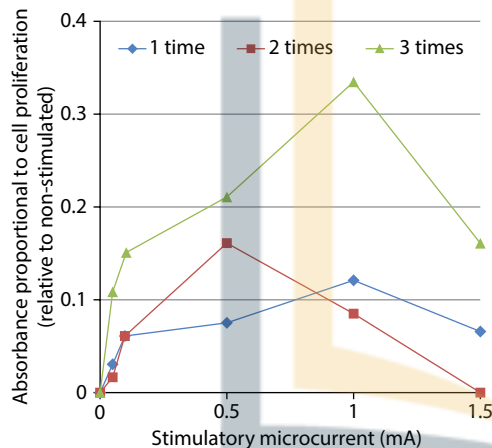
Yi-lo Lin et al. (37) used tissue cultures of tendon fibroblasts or tenocytes taken from 20 horses. Cells from these cultures were used for microcurrent stimulation (METS) experiments. The METS device delivered waveform consisting of a brief monophasic square pulse, duration 0.8 milliseconds. The pulse frequency was 150 Hz. Electrical current consisted of 0, 0.05, 0.1, 0.5, and 1.5 mA. To assess the effects of METS on tenocyte proliferation, viable cells were quantified by use of a non-radioactive, colorimetric cell proliferation kit (Cell Proliferation Kit II XTT, Roche Molecular Biochemicals, Basel, Switzerland). The spectrophotometric absorbency measured with the Kit was proportional to the cell proliferation. The investigators found that application of microcurrent had a stimulatory effect on cell proliferation which was significantly increased with repeated microcurrent applications (Figure 52.9). The same results were observed for DNA content, except that a single application of microcurrent did not lead to a significant DNA content increase in comparison with the control sample. However, repeated microcurrent application significantly increased DNA content (Figure 52.10). Protein content significantly increased after one application of 0.5 and 1 mA, and after two applications of 0.1, 0.5, and 1 mA of microcurrent. However, application of microcurrent three times significantly decreased protein content. Apoptosis rate did not alter after the first application. However after the third microcurrent application apoptosis rate significantly increased with increasing current intensity, so that the highest rate of apoptosis occurred at 1.5 mA. The results of this study provide some evidence for the positive effects of microcurrent on cell proliferation, DNA, and protein content, however, it raises questions as to the specifications and specific ranges of microcurrent necessary to produce optimum cell proliferation, DNA, and protein content, while minimizing apoptosis. Cheng et al. (10) who studied the effects of milliamperes (mA) and microcurrent on ATP generation protein synthesis and membrane transport, showed that although low microcurrent stimulated physiologic activity of damaged cells and increased ATP up to 500%, ATP progressively decreased at mA ranges and dived down to 0 around 1.5 mA. Therefore, the cell apoptosis observed in Yi-lo Lin et al. study (37) cited above, that appeared to be maximum with repeated applications of 1.5 mA, may well be the result of mA ranges depleting ATP.

## WOUND HEALING ENHANCEMENT

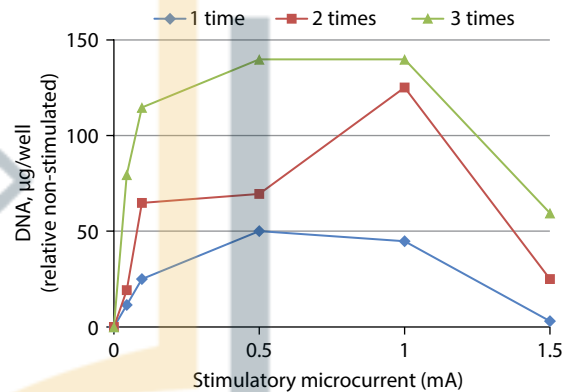
Application of electrical stimulation for wound healing enhancement is the subject of recent review papers (15,34–36). As mentioned above, that low-intensity electrical stimulation electricity has a positive impact on wound healing including increased both DNA and collagen synthesis, directed epithelial, fibroblast, and endothelial cell migration into wound sites, inhibited growth of certain wound pathogens, and increased tensile strength of wound scar. Clinical reports are heavily dominated by case studies and case series. A number of randomized controlled trials have demonstrated efficacy of electrical stimulation for enhancing chronic wounds, with the strongest evidence on treating pressure ulcers, but inconsistencies in the protocols by different investigators make it difficult to choose one regimen or electrical stimulation modality over another. Among the electrical stimulation modalities or

electricity waveforms reported in the literature (Figure 52.11), both electrical stimulation from direct current (DC, monopolar, or monophasic) or alternating current (AC, bipolar, or biphasic) have shown efficacy for wound healing enhancement, reflecting the complex nature of the wound healing process that requires the collaborative efforts of many different tissues and cell lineages and different electrical waveforms may affect different cellular pathways or cell responses (15).

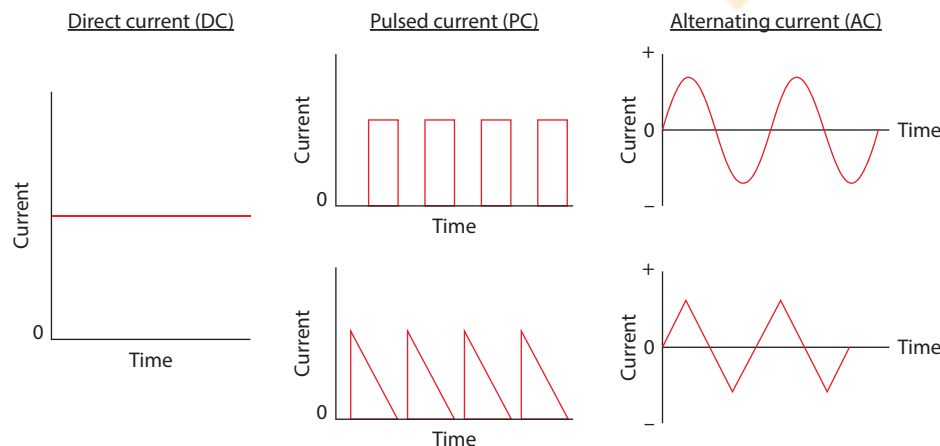
Mehmandoust et. al. (38) investigated the effects of anodal and cathodal electrical stimulation on wound healing. The results indicated that both cathodal and anodal stimulations increased the rate of wound closure. Beginning with day 12, a significant difference was observed in the percentage of the decrease in wound surface between all treatment and control groups ( $p < 0.05$ ). Ultimate tensile strength and stress increased in the anodal compared with the cathodal and control groups.



**Figure 52.9** Effect of METS on cell proliferation of tenocytes in culture. Repeated application of microcurrent resulted in significantly increased cell proliferation. (Modified from Allen GM et al., *Curr Biol*; 23(7):560–8, 2013.)



**Figure 52.10** Effect of METS on DNA content of tenocytes in culture. Repeated application of microcurrent resulted in significantly increased DNA content, whereas a single application of METS did not cause increased DNA in comparison to the control. (Modified from Allen GM et al., *Curr Biol*; 23(7):560–8, 2013.)



**Figure 52.11** Electric stimulation modalities (electricity waveforms) for wound healing. There are reported evidences that both DC and AC electric stimulation enhance wound healing.

The authors concluded that electrical stimulation, regardless of polarity regimen, benefits wound healing, but anodal stimulation the first 3 days and cathodal stimulation the remaining days can lead to stronger repaired tissue.

Lee et al. (16) conducted a study to investigate the efficacy of ultra-low microcurrent delivered by the Electro Pressure Regeneration Therapy (EPRT) device for the management of chronic wounds. In this study, 23 patients with chronic skin ulcers and two with abdominal dehiscence that was present for an average of 16.5 months, who were not responsive to standard conservative treatment in a hospital setting, were treated with the EPRT device. Wounds were treated with direct current (maximum of 3 mA) of 1 polarity for 11.5 min and then with a current of the opposite polarity for another 11.5 min. Treatment was applied through ultra-low microcurrents (in the mA to nA range) conducted through special wraps applied above and below the wound. The results revealed that 34.8% of cases achieved complete wound healing after an average of 45.6 h of treatment, and 39.1% achieved  $\geq 50\%$  healing after an average of 39.7 h of treatment. Several patients achieved significant results after 1 to 2 treatments. The EPRT device not only accelerated healing but also appeared to negate the effect of a person's age on wound healing.

Driban (39) conducted a clinical study to measure the transient electric changes in skin and muscle tissue immediately after trauma, with 11 participants (8 females, 3 males) with a mean age of  $65.18 \pm 11.36$  years undergoing total hip arthroplasty.

An incision approximately 10 cm distal to the posterior superior iliac spine extended distally over the greater trochanter and along the lateral limb. The incision was completed in 2 cuts: (1) skin and subcutaneous fat and (2) muscle tissue. Three measurement sessions were performed with an electrometer before and after a skin incision and after a muscle incision. Potential differences and current intensity were measured immediately after acute trauma to determine the transient electric changes associated with soft tissue injury. The electric potentials were significantly more negative after the skin incision and skin plus muscle incision. Current intensity changed significantly after the skin plus muscle incision. It was concluded that soft tissue trauma generated negative transient electric changes.

## TISSUE ENGINEERING

Low intensity biomimetic electricity has been shown to improve tissue regeneration. In a recent review on regenerative medicine, Levin (40) pointed out that endogenous ion flows serve as key epigenetic regulators of cell behavior. Bioelectric signaling involves feedback loops, long-range communication, polarity, and information transfer over multiple size scales. Understanding the roles of endogenous voltage gradients, ion flows, and electric fields will contribute to the basic understanding of numerous morphogenetic processes and the means by which they can robustly restore pattern after perturbation. Many researchers have investigated the use of low level electricity to mimic bioelectricity to regenerate human tissues for tissue engineering applications (41–44). Tandon et al. (45) reported an experimental methodology for tissue engineering of synchronously contractile cardiac constructs by culturing cardiac cells with the application of pulsatile electrical fields designed to mimic those present in the native heart. Tissue culture is conducted in a customized chamber built to allow for cultivation of (i) engineered 3D cardiac tissue constructs, (ii) cell monolayers on flat

substrates, or (iii) cells on patterned substrates. This approach also allowed for analysis of the individual and interactive effects of pulsatile electrical field stimulation and substrate topography on cell differentiation and assembly. The experimental protocol was designed to allow for delivery of predictable electrical field stimuli to cells, monitoring environmental parameters, and assessment of cell and tissue responses. Engineered constructs that were electrically stimulated during culture demonstrate a remarkable level of ultra-structural differentiation, comparable in several respects with that of native myocardium.

Collagen synthesis in heart tissue was reported to increase with 50 micro A, but not with 100 micro A electric current. Mueller et al. (50) investigated the improvement of cardiac function by unloading with a cardiac assist device, and found it mainly depends on the duration of heart failure. Patients with a short history of heart failure ( $< 5$  years) show improvement due to reverse remodeling of the cardiomyocytes, and in particular of the collagen composition of the extracellular matrix. One reason why patients with long-term heart failure ( $> 5$  years) do not show significant cardiac function improvement is that the collagen composition of the extracellular matrix is then insensitive to mechanical unloading.

It is successful clinical practice to apply microcurrent in patients with bone fractures and wound healing disturbances to improve the healing process by modulation of the collagen synthesis.

To examine whether microcurrent can also influence the collagen synthesis in the myocardium, the effect of microcurrent application on collagen synthesis of adult cardiomyocytes was investigated (46). The results suggest that microcurrent is able to modulate the synthesis of collagen. In dependency of the current magnitude collagen I can be up- or down-regulated. Collagen I is responsible for the stiffness and the degree of dilatation of the heart.

## MICROCURRENT THERAPY FOR PAIN AND OTHER TREATMENTS

Microcurrent electrical therapy represents a significant improvement in rapid pain control and acceleration of healing (47,48). It uses current in the microampere range, 1000 times less than that of TENS and below sensation threshold. The pulse width, or length of time that the current is delivered with a microcurrent device is much longer than previous technologies. A typical microcurrent pulse is about 0.5 seconds, which is 2500 times longer than the pulse in a typical TENS unit and a good microcurrent unit has approximately ten times the electronic circuitry of a TENS unit. Unlike TENS, MET is usually administered through hand-held probes positioned so that current flows between them, through the painful area, for ten seconds. Pain control following painful orthopedic procedures such as total knee arthroplasty is an ongoing challenge, as current pain management techniques often result in undermedication and/or complications.

Microampere current provides physiologic current flow and has been used in the treatment of some pain syndromes. McMakin (49) reported an uncontrolled retrospective analysis of patients receiving microcurrent treatment for fibromyalgia following cervical spine trauma, subjective pain scores are utilized as a primary outcomes measure. Accompanying changes in inflammatory cytokines are examined in a subgroup of the same patient population to test the hypothesis that microcurrent treatment produces substantial measurable objective and subjective outcomes supporting the efficacy of this treatment.



In this retrospective study based on analysis of subjective VAS pain scores for 54 patients, symptoms of fibromyalgia following cervical spine trauma were successfully treated with microamperage current. In a subgroup of the same patients, subjective pain improvement scores were accompanied by substantial reduction in serum levels of the inflammatory cytokines IL-1, IL-6, and TNF- $\alpha$ , and the neuropeptide substance P. Beta-endorphin release and increases in serum cortisol were also observed in these patients during the same treatment period. The subjective outcomes scores in conjunction with biological markers for pain and pro-inflammatory cytokines observed in response to this treatment protocol are important preliminary findings. Based on the observations reported in this analysis, controlled prospective clinical studies to evaluate the clinical efficacy of microcurrent treatment of FMS associated with cervical spine trauma are warranted.

El-Husseini et al. (50) reported a study designed to test the effect of the microcurrent skin patch on pain relief in patients following total knee arthroplasty. Wound healing was better with the application of the microcurrent skin patch: grade 1 wounds were observed in 50% of the patients of the microcurrent skin patch group as compared to 8.3% in control group. The total drain volume was lower in patients of the microcurrent skin patch group compared to the controls. None of the patients indicated that they wished to discontinue microcurrent skin patch therapy. This pilot study shows that microcurrent skin patch therapy led to better pain control with a markedly lower need for tramadol as compared to the control group. This better pain control was accompanied by a better healing of the wound and a lower drain volume.

Cranial electrotherapy stimulation (CES), a noninvasive technique that delivers a microcurrent to the brain via ear clip electrodes, has been shown to effectively treat several neurological and psychiatric disorders. Tan et al. (51) reported a clinical study using cranial electrotherapy stimulation to treat pain associated with spinal cord injury. Treatments for chronic pain in persons with spinal cord injury (SCI) have been less than effective. This study examined the effects of daily 1-hour active CES or sham CES treatment (randomly assigned) for 21 days on pain intensity and interference with activities in 38 males with SCI. The active CES group (adjustable electric current: 100–500 micro A) reported significantly decreased daily pain intensity compared with the sham CES group. Additionally, the active CES group reported significantly decreased pain interference in contrast to the non-significant decrease in the sham CES group. These results suggest that CES can effectively treat chronic pain in persons with SCI.

Chronic low back pain associated with myofascial trigger point activity has been historically refractory to conventional treatment. McMakin (52) reported a case review on microcurrent therapy for 22 patients with chronic low back myofascial pain, of 8.8 years average duration, is presented. Following treatment with frequency-specific microcurrent, a statistically significant 3.8-fold reduction in pain intensity was observed using a visual analog scale. This outcome was achieved over an average treatment period of 5.6 weeks and a visit frequency of one treatment per week. When pain chronicity exceeded 5 years, there was a trend toward increasing frequency of treatment required to achieve the same magnitude of pain relief. In 90% of these patients, other treatment modalities including drug therapy, chiropractic manipulation, physical therapy, naturopathic treatment, and acupuncture had failed to produce equivalent benefits. The microcurrent treatment was the

single factor contributing the most consistent difference in patient-reported pain relief.

## ANTIMICROBIAL ACTIVITY

Low intensity electricity was known to have inhibitive or killing activity on microorganisms, and was also known to prevent attachment of bacterial biofilm to medical implants or to cause detachment of bacterial biofilm.

The bioelectric effect, in which electric fields are used to enhance the efficacy of biocides and antibiotics in killing biofilm bacteria, has been shown to reduce significantly the amount of antibacterial agents needed to kill biofilm bacteria to levels very close to that needed to kill planktonic (free floating) bacteria of the same species. Costerton et al. (53) reported that biofilm bacteria are readily killed by an antibiotic on all areas of the active electrodes and on the surfaces of conductive elements that lie within the electric field but do not themselves function as electrodes. Considerations of electrode geometry indicate that very low (<100 microA/cm<sup>2</sup>) current densities may be effective in this electrical enhancement of antibiotic efficacy against biofilm bacteria, and flow experiments indicate that this bioelectric effect does not appear to depend entirely on the possible local electrochemical generation of antibacterial molecules or ions. These data are expected to facilitate the use of the bioelectric effect in the prevention and treatment of device-related bacterial infections that are caused by bacteria that grow in biofilms and thereby frustrate antibiotic chemotherapy.

Del Pozo (54) recently reviewed the bioelectric effect and bacterial biofilms. Bacteria growing in biofilms cause a wide range of human infections. Biofilm bacteria are resistant to antimicrobials at levels 500 to 5000 times higher than those needed to kill non-biofilm bacteria. In vitro experiments have shown that electric current can enhance the activity of some antimicrobial agents against certain bacteria in biofilms; this has been termed the "bioelectric effect." Direct electrical current has already been safely used in humans for fracture healing. Application of direct electric current with antimicrobial chemotherapy in humans could theoretically abrogate the need to remove the device in device-related infections, a procedure associated with substantial morbidity and cost. In this article, we review what has been described in the literature with regard to the bioelectric effect.

Bacterial biofilms are believed to be a major factor in problems of ineffective sterilization often encountered in clinics, hospitals, and industrial processes. There have been indications that the addition of a relatively low intensity direct current electric field with the sterilant used to combat the biofilm greatly increases the efficacy of the sterilization process. The results of the experiments reported (53) support the concept of the bioelectric effect as reported by Costerton's work (53). With a current of 1 mA flowing through the chamber containing bacterial biofilm, an increase in the killing of the bacteria of about 8 log orders was observed at the end of 24 h (compared with the control with the same amount of antibacterial agent but no current). It was also confirmed that the current alone does not affect the biofilm and that there appear to be optimum levels of both the current and the sterilant that are needed to obtain the maximum effect.

Kalinowski et al. (55) described the use of low voltage direct current as a fungicidal agent for treating onychomycosis. Onychomycosis, most commonly caused by two species of dermatophyte fungi—*Trichophyton rubrum* and

*T mentagrophytes*—is primarily treated with regimens of topical and systemic antifungal medications. This study was undertaken to evaluate *in vitro* the efficacy of low-voltage direct current as an antifungal agent for treating onychomycosis. Agar plate cultures of *T rubrum* and *T mentagrophytes* were subjected to low-voltage direct current electrostimulation, and antifungal effects were observed as zones in the agar around the electrodes lacking fungal growth. Zones devoid of fungal growth were observed for *T rubrum* and *T mentagrophytes* around anodes and cathodes in a dose-dependent manner in the current range of 500  $\mu$ A to 3 mA. Low-voltage direct current electrostimulation has great clinical potential for the treatment of onychomycosis and perhaps other superficial maladies of fungal etiology.

Bolton et al (56) reported that positive carbon-containing electrodes conveying 5 or more  $\mu$ A of constant direct current per  $\text{cm}^2$  showed bactericidal activity on intact back skin of 13 human subjects. This effect increased with the duration of stimulation up to a total surface bacterial kill at 20 h. When total current and current density were varied independently on 16 sites on the backs of eight subjects, the effect was dependent on current density, not on total current. Electrodes driven by similar voltages but which removed the electrochemical reaction from inoculated sites on the backs of three subjects failed to reduce the numbers of colony-forming units as compared with those sampled from control sites. This showed the bactericidal effect to be electrochemical in origin, probably mediated by local acidity generated at the surface of the positive carbon-containing electrodes. With an adhesive tape stripping technique on three sites on each of six subjects, it was determined that the effect extended into the epidermis of the human back. No effect was observed beneath negative or control electrodes under the same conditions.

## BIOMIMETIC ELECTRICITY FOR DERMATOLOGICAL APPLICATIONS

Electrical stimulation of the body used in electrotherapy, and iontophoretic drug delivery are typically achieved by the use of battery-powered electronic medical devices, which are often complicated and cumbersome to use. Other means of applying biomimetic electricity to the body conveniently and effectively are therefore highly desirable. Physiological level of biomimetic electricity may be derived from the electrochemical reactions of a galvanic couple, a pair of dissimilar metals electrically connected together in contact with a conductive electrolyte medium. Galvanic current is the electron flow that runs between the two dissimilar metals of a galvanic couple and the surrounding electrolyte solution. The theory of galvanic couple's electrochemical reactions is well known, and is the underlying principle for batteries/electrochemical cells. Galvanic electrochemical reactions have been used as the electrical power source in commercial medical devices based on a zinc-silver/silver chloride galvanic couple for transdermal iontophoretic drug delivery (57,58). Electrochemical reactions on the electrode surfaces of the galvanic couple concurrently generate zinc ions on zinc anode via oxidation of metallic zinc, and hydrogen gas on the cathode via reduction of hydrogen ions in the water (59). We are reporting here a new method of delivering biomimetic electricity for anti-inflammatory effects by using fine particles of zinc-copper galvanic couple made of pure metallic zinc metal partially coated with metallic copper with a coplanar electrode configuration for the zinc anode and copper cathode similar to that reported by Doig and Flewitt (60).

## Biomimetic-Electricity Delivery Systems Containing Biomineral Complex

Based upon the insights of bioelectricity for wound healing, a micro electricity delivery system was reported using a unique combination of elemental zinc and copper to generate biological levels of electricity when in contact with conducted media such as physiological fluid, moisturizer, etc. The titration results demonstrated that the intensity and duration of the electricity can be adjusted through bi-mineral complex combination, ratio, and particle size.

Tandan et al. (61) described characterization and modeling of the zinc-copper galvanic microparticles, and the observation that the galvanic microparticles increased migration of human dermal fibroblasts in a wound-healing model via reactive oxygen species. One method to generate electrical signals similar to those naturally occurring in wounds is by supplementation of galvanic particles dispersed in a cream or gel. We constructed a three-layered model of skin consisting of human dermal fibroblasts in hydrogel (mimic of dermis), a hydrogel barrier layer (mimic of epidermis), and galvanic microparticles in hydrogel (mimic of a cream containing galvanic particles applied to skin). Using this model, we investigated the effects of the properties and amounts of Cu/Zn galvanic particles on adult human dermal fibroblasts in terms of the speed of wound closing and gene expression. The collected data suggest that the effects on wound closing are due to the ROS-mediated enhancement of fibroblast migration, which is in turn mediated by the BMP/SMAD signaling pathway. These results imply that topical low-grade electric currents via microparticles could enhance wound healing.

## In Vitro Biological Responses of Biomineral Complex

### Anti-Inflammatory Activity

In several *in vitro* cytokine studies, treatment with the bi-mineral complex significantly reduced release of pro-inflammatory cytokines from activated human T-cells and inhibited release of cytokines from keratinocytes and macrophages exposed to bacteria (62). Furthermore, topical application of a lotion containing the bi-mineral complex reduced the UV-induced damage to human skin equivalents. Taken together these results demonstrate that biomimetic electricity reduces inflammatory responses and therefore may protect skin from the numerous external aggressions encountered daily by skin.

Kaur et al. (63) reported that galvanic zinc-copper microparticles produce electric stimulation that reduces the inflammatory and immune responses in skin. A galvanic couple comprised of elemental zinc and copper was used to determine the effects of low-level electrical stimulation on intact skin physiology using a Dermacorder device. Zn-Cu induced the electrical potential recorded on intact skin, enhanced  $\text{H}_2\text{O}_2$  production and activated p38 MAPK and Hsp27 in primary keratinocytes. Treatment with Zn-Cu was also found to reduce pro-inflammatory cytokines such as IL-1 $\alpha$ , IL-2, NO and TNF- $\alpha$  in multiple cell types after stimulation with PHA or *Propionibacterium acnes* bacteria. The Zn-Cu complex led to a dose-dependent inhibition of TNF- $\alpha$ -induced NF- $\kappa$ B levels in keratinocytes as measured by a dual-luciferase promoter assay and prevented p65 translocation to the nucleus observed via immunofluorescence. Suppression of NF- $\kappa$ B activity via crosstalk with p38 MAPK might be one of the potential pathways by which Zn-Cu exerted its inflammatory effects.

Topical application of Zn-Cu successfully mitigated TPA-induced dermatitis and oxazolone-induced hypersensitivity in mice models of ear edema. Anti-inflammatory activity induced by the Zn-Cu galvanic couple appears to be mediated, at least in part, by production of a low level of hydrogen peroxide since this activity is reversed by the addition of catalase enzyme. Collectively, these results show that a galvanic couple containing Zn-Cu strongly reduces the inflammatory and immune responses in intact skin, providing evidence for the role of electric stimulation in non-wounded skin.

#### Dermal Extracellular Matrix Production

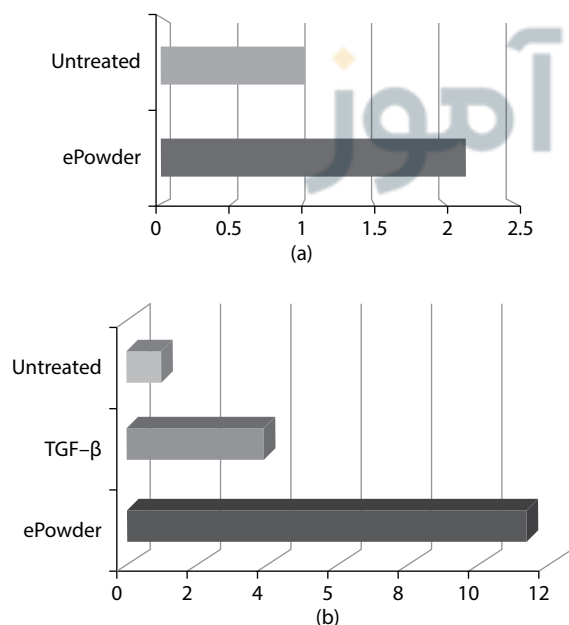
The elemental bi-mineral complex, which produces physiological levels of electricity, was also evaluated for its possible effects on dermal extracellular matrix (64). Fresh human abdominal skin samples were obtained from surgical procedures and tested as human skin explants maintained in a culture medium. Human skin explants were topically treated with this bi-mineral complex once daily for 7 days, and the effect on elastin and collagen was evaluated. LUNA elastin staining showed that treatment with the bi-mineral complex increased the elastin fiber network, as compared to untreated controls. QPCR analysis documented an increase in elastin and collagen expression in the bi-mineral complex-treated skin samples (Figure 52.12). The results suggest that this bi-mineral complex may be effective in restoring the integrity and functionality of dermal extracellular matrix, and in particular of elastic fibers, suggesting its cosmetic use in the aged skin.

#### Melanogenesis Inhibition

To examine the effect of electric field modulation on melanogenesis, pigmented epidermal equivalents were topically treated with a proprietary elemental bi-mineral complex that produces biomimetic electricity, once daily for 7 days (65). F&M staining showed a significant decrease in melanin

deposition in epidermal equivalents treated with the bi-mineral complex, compared to untreated control. This reduction in melanin deposition was also observed in epidermal equivalents treated with a cosmetic formula containing the bi-mineral complex. Exploring the mechanism of this biomimetic electricity-induced depigmentation, it was shown that there was no direct inhibitory activity against phagocytic activity of keratinocytes. Interestingly, the bi-mineral complex inhibited tyrosinase and tyrosinase related protein 1 (TRP-1) expression using mouse melanoma B 16 cells, as analyzed by TYR and TRP-1 promoter-luciferase reporter assays. Using cultured human skin explants, it was confirmed that melanin content and TYR mRNA are reduced by the exposure to the bi-mineral complex. These data support the potential of using biomimetic electricity generated by an elemental bi-mineral complex for skin lightening applications.

Won et al. (66) reported that galvanic zinc-copper microparticles inhibit melanogenesis via multiple pigmentary pathways. The findings showed that galvanic zinc-copper microparticles inhibited melanogenesis in a human melanoma cell line (MNT-1), human keratinocytes and melanoma cell co-cultures, and in pigmented epidermal equivalents. Treatment of galvanic zinc-copper microparticles inhibited melanogenesis by reducing the promoter transactivation of TYR and TRP-1 in human melanoma cells. In a co-culture Transwell system of keratinocytes and melanoma cells, galvanic zinc-copper microparticles reduced melanin production via downregulation of endothelin-1 secretion from keratinocytes and reduced tyrosinase gene expression in melanoma cells. In addition, exposure of pigmented epidermal equivalents to galvanic zinc-copper microparticles resulted in reduced melanin deposition. In conclusion, our data demonstrated for the first time that galvanic zinc-copper microparticles reduced melanogenesis in melanoma cells and melanin deposition in pigmented epidermal equivalents by affecting multiple pigmentary pathways.



**Figure 52.12** Effect of bimineral complex on upregulation of collagen and elastin expressions.

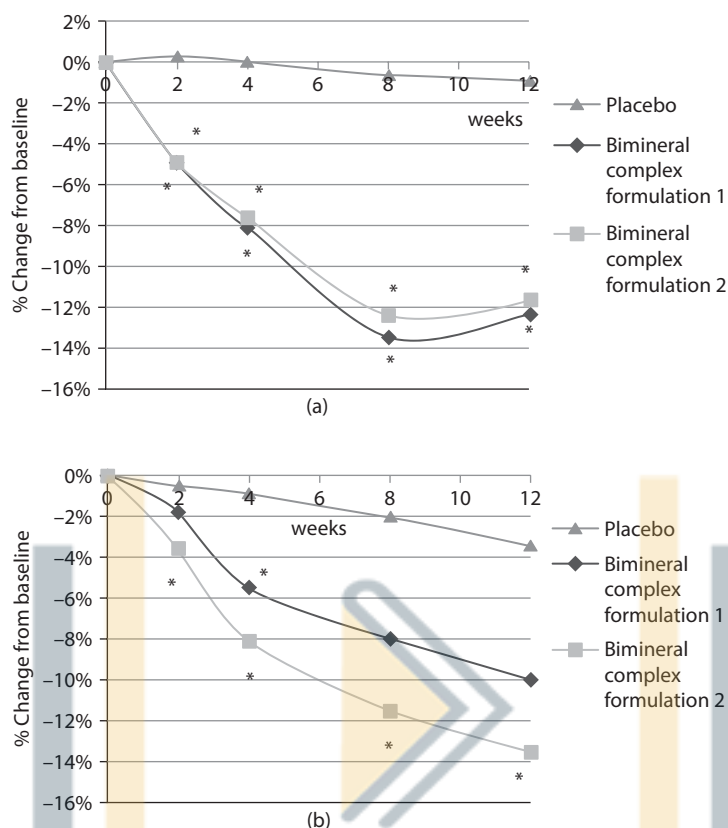
#### Clinical Safety and Tolerability of Biomineral Complex

Several clinical and preclinical safety studies were conducted (67). Clinical safety was assessed through human repeat insult patch tests (RIPT). Five RIPT studies were performed on topical compositions containing various concentrations of the bi-mineral complex. Four RIPT studies were conducted in a predominately Caucasian population in the United States, and one study was conducted in an Asian population in Thailand. Combined, over 800 healthy subjects completed the clinical safety studies. Results show that none of the topical compositions induced dermal sensitization.

In addition, a topical composition containing the bi-mineral complex was evaluated versus placebo for its potential to induce dermal and ocular irritation in a human skin model (EpiDerm™) and human corneal model (EpiOcular™). Results show that the bi-mineral complex has low potential for skin and eye irritation.

Topical compositions containing the bi-mineral complex have been evaluated for their tolerability and efficacy in reducing the signs of facial and periorbital photo-aging in over eight clinical studies spanning populations in three countries—the United States, France, and Singapore. The bi-mineral compositions were shown to be mild and well tolerated in all populations studied.





**Figure 52.13** Twelve-week randomized placebo-controlled clinical results of eye conditions after topical administration of bimineral complex.

### Clinical Efficacy for Skin Anti-Aging of Bimineral Complex

#### *Twelve-Week Placebo-Controlled Anti-Aging Study*

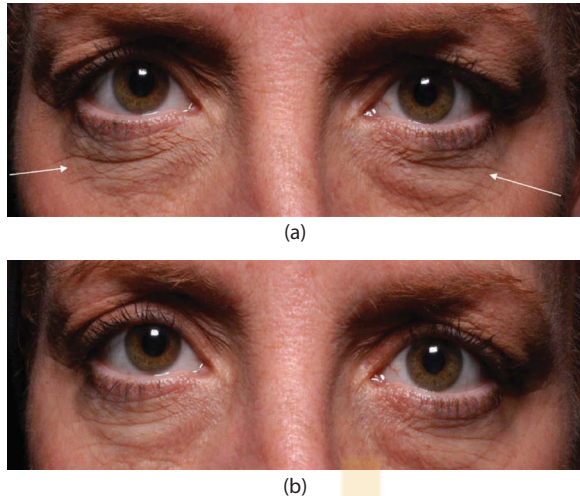
The clinical signs of photo-aging are a persistent concern for many patients. Intrinsic factors and cumulative exposure to extrinsic factors such as UV lead to the development of skin laxity, fine lines, wrinkles, hyperpigmentation, and sallowness. A 12-week, double-blind, placebo-controlled clinical study was performed to evaluate the efficacy and tolerability of a topical composition containing the bi-mineral complex in reducing the clinical signs of facial photo-aging, including the delicate periorbital area (68). The study consisted of three treatment groups: 1) placebo moisturizer, 2) bi-mineral complex moisturizer, and 3) bi-mineral complex moisturizer with activator. The study population consisted of 94 healthy women, ages 40–65, with Fitzpatrick skin type II–IV and mild to moderate photo-aging. Subjects applied the bi-mineral complex twice daily, in the morning and evening. Evaluations included clinical grading of safety and efficacy parameters, high-resolution digital imaging, and subject self-assessments. All measures were taken at baseline and weeks 2, 4, 8, and 12.

Both treatment groups using the bi-mineral complex showed statistically significant ( $p < 0.05$ ) clinical improvement versus placebo and versus baseline starting as early as week 2 in several parameters and continued to improve versus placebo through week 12. The bi-mineral complex performed significantly ( $p < 0.05$ ) better than placebo and baseline in overall appearance, drooping eyelids, under-eye bags, under-eye

wrinkles, cheek wrinkles, pigmentation, radiance, fine lines, and global lifting/firming. Figure 52.13 shows significant improvement of 12-week clinical grades for under-eye bag and drooping eyelids. Figure 52.14 shows a pair of sample clinical images after 2 weeks of topical treatment of a formulation containing bi-mineral complex. There were no adverse events related to the treatments in this clinical study, and the bi-mineral complex and placebo were all shown to be well tolerated throughout the 12-week study.

#### *Four-Week Anti-Aging Study Combining Bi-Mineral Complex with Natural Extracts*

Natural ingredients are increasingly being recognized for their skincare benefits as they continue to be shown to have various in vitro and in vivo activities. Preclinical and initial clinical data have shown the potential of a bi-mineral complex consisting of a proprietary blend of elemental zinc and copper that generates biomimetic signals for improving photo-aging, and in vitro data has also suggested the anti-aging benefits of a combination of dill and blackberry leaf extract. A clinical study was performed using a regimen of a bi-mineral complex gel and a dill-blackberry leaf extract lotion with SPF 30 to evaluate its ability to rapidly improve multiple signs of facial photo-aging, while being mild to the skin (69). Thirty healthy female subjects, between the ages of 30–55, with moderate facial photo-aging, exhibiting mottled hyperpigmentation, skin roughness, laxity, and fine wrinkling completed this 4-week study. Patients applied the two-product system once



**Figure 52.14** Sample images of clinical results (a) before and (b) after 2 weeks of twice-daily topical treatment of a bimineral complex topical formulation.

per day in the morning. Clinical evaluations, self-assessments, and instrumental analysis demonstrated this two-product regimen's multiple skin benefits throughout the study. Clinical evaluations indicated significant improvements ( $p < 0.05$ ) in facial skin clarity, smoothness, and overall photo-aging after 2 weeks of use. Significant improvements ( $p < 0.05$ ) in the appearance of mottled hyperpigmentation, firmness, and fine wrinkling were observed by the 4-week time point. Patients also perceived significant ( $p < 0.05$ ) improvements in skin tone, brightness, and textural parameters as early as after 2 weeks of use. Digital photographs also confirmed improvements in various overall photo-aging parameters. In conclusion, this clinical study demonstrated that a regimen of a bi-mineral complex gel and a dill-blackberry leaf extract lotion with SPF 30 was effective in improving the overall signs of facial photo-damage including improvements in fine lines and wrinkles, tone, pigmentation, and texture, while being mild and gentle to the skin.

#### *Four-Week Laxity Clinical Study*

We have evaluated the clinical efficacy of a facial treatment regimen consisting of this bi-mineral complex plus a facial moisturizer in a 4-week double-blinded controlled study (70). Thirty subjects with mild to moderate signs of photodamage and moderate skin laxity completed the study. The patients applied the regimen once per day in the morning. Clinical evaluations, self-assessments, and instrumental analysis demonstrated this two-product regimen's multiple skin benefits throughout the study. The facial treatment was particularly effective in improving skin firmness and laxity ( $p < 0.05$ ) while also providing significant improvements in overall photodamage. Patients also perceived significant ( $p < 0.05$ ) improvements in overall signs of photodamage and lifting. The regimen was well tolerated.

#### *Anti-Aging Focusing on Eye Areas*

The first signs of skin aging often occur in the delicate skin of the periorbital area. Characteristic signs associated with chronological and photo-aging in the eye area include fine

lines and wrinkles, dark circles and bags under the eye, and puffiness and laxity in both the upper and lower eyelids. Three clinical studies were conducted to evaluate efficacy and tolerability in reducing signs of photo-aging in the periorbital area for immediate and continuous benefits.

The first study focused on immediate benefits after a single application of the bi-mineral complex (71). Twenty-two females ages 25–45 completed this double-blind, benchmark controlled study. To enroll at baseline, subjects must experience at least one of the following conditions of mild to moderate severity based on expert grading: under-eye bags, puffiness, dark circles, lines, and wrinkles. Clinical imaging and subject self-assessments were taken at baseline, 20–30 minutes post-product application, and 3 hours post-product application. Study results show that the bi-mineral complex demonstrated visible, measureable improvement ( $p < 0.05$ ) at 20–30 minutes post application and continued to show improvement ( $p < 0.05$ ) at 3 hours in parameters such as under-eye puffiness, bags, and fine lines.

The second clinical study was a placebo-controlled, double-blind study to evaluate the immediate and continuous effects of the bi-mineral complex over an 8-week period (71). 120 women, ages 40–65, with mild to moderate photo-aging at baseline completed this study. Clinical grading, clinical imaging, and subject self-assessments were performed at baseline, immediately after product application, and after 1, 2, 4, and 8 weeks of product use. Clinical grading immediately after the first product application shows that the treatments containing the bi-mineral complex have significant ( $p < 0.05$ ) improvement versus placebo in under-eye dark circles, under-eye bags, skin radiance, and overall photodamage. In both studies, the bi-mineral complex showed immediate and lasting improvement versus baseline, and all compositions were well tolerated.

A third study was performed on 34 healthy Caucasian women volunteers aged between 35 and 60 years old with wrinkles and/or fine lines at the eye contour area, dark circles, and puffiness (72). All volunteers signed an informed consent form. Product was applied for 8 weeks, once a day, in the morning. Clinical grading of the signs of ageing was done at baseline, immediately after the first application, then after 1 week, 4 weeks, and 8 weeks of application. Wrinkles, tone, and sagging were assessed by an expert grader around the eyes using visual analog scales. A paired student t-test was performed versus baseline and the confidence level considered was  $p < 0.05$ .

Immediately after the first application, all the parameters assessed were significantly improved versus baseline ( $p < 0.05$ ). The product improved bags and dark circles (respectively +32% and +21%) as well as wrinkles (crow's feet: +24%, under eye: +22%), sagging (sloping eyelids: +20.29%, folds on the eyelids: 17%), and skin complexion (radiance: +17%, yellowish complexion: +52%). Some of the changes were still increasing after 1 week of application. The tested product containing the new biomimetic signaling technology was demonstrated to be efficacious from the first week of use and delivered instant benefits to the eye area.

Nollent et al. (73) reported clinical evaluation of zinc- and copper-based eye area anti-aging complex. A zinc and copper complex generating biomimetic electricity on a physiological level has shown to improve signs of photo-aging in clinical studies. This zinc-copper complex was applied to the eye area to confirm its efficacy as antiaging treatment option with good skin tolerance. The study was a non-interventional, open-labeled baseline-controlled clinical study, including a 30-day washout period and 8-week treatment period. The results of this

study showed that both subjective and objective assessments demonstrated significant decreases in facial photodamage signs during the 8-week treatment with the zinc-copper complex. Clinical grading and self-assessments indicated statistically significant improvements in crow's feet, fine lines and wrinkles, under-eye fine lines and bags, eyelid puffiness and dark circles, and skin texture (i.e., skin radiance and tone). Objective measurement methods showed a reduction in total wrinkle surface and mean length in skin replicas. In this sensitive and fragile skin area, the treatment was also well tolerated throughout the study period, with no adverse events.

## SUMMARY

Bioelectricity is an emerging science presenting a new opportunity for medical therapies. Basic research suggests that ultra low electrical fields can enhance tissue healing, ATP production, antimicrobial activities, inflammation reduction, pain relief, and improving dermatological conditions. However, specific mechanisms of action remain unclear. The application of a specific low intensity electricity or biomimetic electricity for the medical applications warrants a new area for exploration.

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# Chemical Peels

Philippe Deprez

## INTRODUCTION

Various peeling formulas are currently available, many of them resulting of a more or less adequate combination of chemicals whose aim is usually to peel the skin and induce a skin rebuilding, an architectural **re**structuration. Some peels consist of strongly destructive **solutions**, able to dissolve the full skin thickness; others are simply stimulating solutions with little destructive power; their application can induce a nearly invisible skin desquamation. The difficulties of peels procedure, the results, the inconveniences, and the side effects are often directly linked to the depth reached by the acids.

Not much will be said about pre-peel skin conditioning in this chapter, breaking a quasi-dogma. Indeed, there is a possible way to do peels that makes this pre-peel strict conditioning not often necessary. I just use peeling formulas that usually penetrate evenly and that dramatically **s**low down the immediate post-peel inflammatory reaction, mainly responsible for postinflammatory hyperpigmentation (PIH). The rest is a question of good indication (don't go too deep if not **n**ecessary) and the practitioner's ability or experience.

Pre-peel conditioning is principally used when a quite deep chemical peel has to be done on a phototype IV to VI: in this case, molecules are used to equalize the acid penetration (glycolic acid, tretinoin), to speed up epidermal regeneration (pre-peel Tretinoin does that but immediate post-peel tretinoin has a contrary effect; vitamins). Pre-peel conditioning is also necessary to induce melanocyte sedation and lower the risk of post-inflammatory hyperpigmentation. Many other molecules can be used during pre- and post-peel periods.

## DEPTHS OF PEELS

I usually consider seven depths of peels, as seen in Figure 53.1.

### Depth 1—Exfoliation: VHS

The most superficial peel consists of simple exfoliation of stratum corneum dead cells: it gives a good skin cleansing, a touch of better hydration. This "hydration touch" results in reality of skin damage: the peel has removed the protective stratum corneum layer and the fingers are now directly in contact with superficial keratinocytes. Keratinocytes are living cells (only the most superficial layers are near to death), containing more water than stratum corneum cells.

*Main types of peels:* Alpha hydroxy acid peels are mostly used for this exfoliation purpose.

*Action mode:* The activity of AHA on the corneocytes seems to be secondary to an action on ionic charges,

to the inhibition of enzymes involved in the formation of ionic links. For example, AHAs could compete with sulfates and phosphates at the level of sulfotransferases, phosphotransferases, or kinases, involved in the formation of sulfated or **phosphorylated** mucopolysaccharides, glycoproteins, sterols, and lipids. This could produce a lower quantity of **electrically** negative groups on the surface of keratinocytes and corneocytes and lower the adhesion forces with amines or basic aminated acids (electrically positive).

Basically, AHAs penetrate between cells, unsticking the proteins responsible for corneo-desmosome adhesivity (it is a non-covalent, electric link) and allowing the cells to separate from each other, inducing desquamation. Since there is **n**o strict chemical reaction during this process, AHAs are not much consumed and have to be neutralized. Rinsing with a basic solution stops their action.

*Clinical signs:* Irritative erythema is usually the only visible sign.

*Desquamation:* Roughly, no desquamation is clinically seen

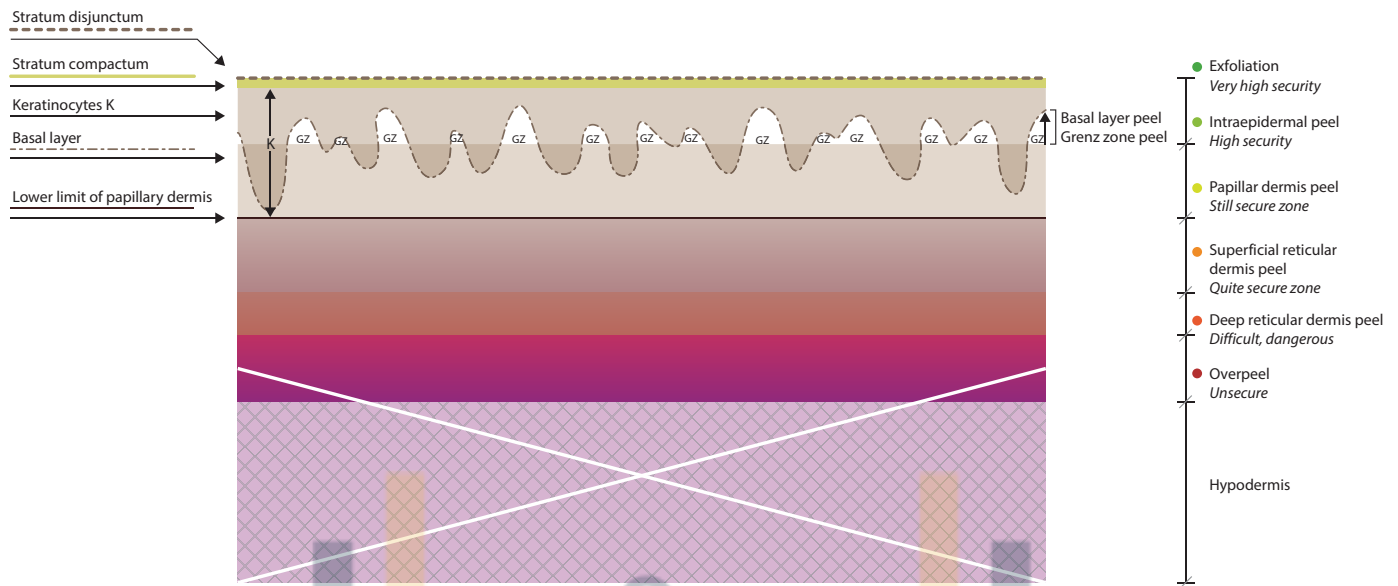
*Risks and Problems:* Globally, this is not a risky depth. Main risks are a higher sensitivity to sun during some days and a **h**igher risk of skin infections. Neutralization is the main **p**roblem: if too early, it gives no result; if too late, it could induce more side effects. We will see later that this problem can be avoided by using specific slow-release and self-neutralizing AHA mixtures.\*

### Depth 2—Intraepidermic Peel: VHS

The peel solution penetrates deeply into the epidermis, removing more cells; nevertheless, it does not touch any part of dermis or the basal layer. The final touch of the skin is still more hydrated than in the case of simple exfoliation (depth 1). After the peel, living keratinocytes are suddenly directly exposed to air, sun, pollution, and dryness. They react, synthetizing more TNFa (inducing a faster transformation of keratinocytes into corneocytes) and sending a message to the basal layer in order to stimulate basal layer turnover and substitute the removed cells with new ones. At the same time another message reaches the fibroblasts, responding by stronger synthesis of all the dermal intercellular matrix.

Intraepidermic peels (depth 2) give better results than depth 1 peels and can be used for treating superficial epidermal melasma and many keratinization problems.

\* Easy Phytic Solution.



**Figure 53.1** Graph showing the various possible depths of peel.



**Figure 53.2** Erythema: intraepidermal peeling.

*Main types of peels:* Alpha hydroxy acid peels, alpha ceto acids, trichloracetic acid (TCA), resorcine peels, and salicylic acid can be used.

*Action mode:* We have roughly seen how AHAs work. TCA is a proteocoagulant chemical. When in contact with proteins, it coagulates them, modifying their

tridimensional structures in a way that does not allow their normal function. All membrane proteins are therefore damaged, making the keratinocytes unable to survive. In the same time, intercellular proteins are also coagulated.

*Clinical signs:* More clearly visible erythema can be seen, but no white pinpoints appear yet (Figure 53.2).

*Desquamation:* Can look like very thin dandruff.

*Risks and problems:* Intra-epidermic peels are usually not dangerous. Nevertheless, cases of post inflammatory hyperpigmentation (PIH) have been seen, making prevention of this side effect necessary when the skin is known to be sensitive. Neutralization of AHAs remains the main problem since it must be done following strict rules (neutralization has to be done when an erythema appears—it is always irregular erythema—and in any case, before any sign of protein coagulation that would appear as skin frosting points). It is difficult to foresee the right moment for an ideal neutralization.

With TCA, no neutralization can reverse the proteinic coagulation; therefore the total amount of TCA applied on the skin has to be perfectly calculated, in relation with the skin permeability. The TCA application technique has to be perfect for even penetration. Resorcine and salicylic acid are phenol derivatives, not widely used out of the United States. Treatments of large surfaces using phenol derivatives are suspected to potentially induce toxic reactions. TCA and AHAs, on the other hand, are not toxic products.

### Depth 3—Basal Layer Peel: HS

This is a very interesting peel level, since it is easily reached and gives good results. Stratum corneum cells are completely removed; keratinocytes are largely damaged up to the level of basal layer keratinocytes (depth 3 in the graph) (Figure 53.3).





**Figure 53.3** Before and after four sessions of Easy TCA (Skin Tech), one peel every week, basal layer depths.

Epidermis is nevertheless not completely destroyed, since many keratinocytes—less damaged by UVs—are living in deep epidermal papillae; skin regeneration is fast and easy.

Basal layer peels can be used as serial peels for treating skin aging (Glogau 1–2), fine lines, epidermal melasma, keratoses, and acne (from black dots up to papule-pustule acne). Together with a good control of melanin synthesis (blending bleaching cream\*), a TCA basal layer peel can treat many cases of melasma. If attention is paid to adequately clean the skin (remove black dots, open microcysts etc.) and if the patient applies disinfecting creams and creams limiting sebum production, than we can treat active acne, usually without antibiotics.<sup>†</sup>

*Main types of peels:* AHAs should not be used at this depth since this is the border after which AHA's side effects are prone to appear, are difficult to treat, and are inconvenience for the patient's social life. TCA represents the best choice for this depth, if the right concentration, formula, and post-peel care are selected.

*Action mode:* We have seen (see depth 2) the TCA action mode: keratinocyte destruction induces a basal layer strong reaction, dramatically stimulating the turnover of basal cells.

*Clinical signs:* TCA coagulates proteins and its entry through the domes of papillae induces a specific proteinic coagulation; dermal protein coagulation points occur, clinically appearing as little white marks (white pinpoints) called “frosting points.”

*Desquamation:* Looks like a sunburn desquamation, allowing a social life in the majority of cases.

*Risks and problems:* Basal layer peels are usually not dangerous peels. The application technique is important since a perfect protocol is very secure. If the peel application is a little bit too strong, basal layer peeling can nevertheless give the start to a vicious cycle of inflammation based on free radicals liberation that induces cells damage, etc. If we allow this inflammatory vicious cycle to begin, melanocytes could react and cause post-inflammatory hyperpigmentation (PIH). Cases of PIH have been seen, making the control of this post-peel inflammatory vicious cycle necessary. No herpes prevention is necessary. Problems are mainly linked to too-strong melanocyte stimulation, without adequate treatment, or to an irregular application, too-deep application, or infectious rebounds in cases of acne. Pre-peel skin conditioning can be used from this point if the peel used does not penetrate evenly or does not control the post-peel inflammatory reaction. Using Easy TCA peel, no pre-peel conditioning is necessary. Except in cases of deep scratching and/or strong local infection, no scar is to be anticipated from this peel depth.

#### Depth 4—Grenz Zone Peel: HS

This is a very interesting peel level, easy to perform, not very painful for the patient, with a low level of risk and pretty good results. Stratum corneum and a large part of keratinocytes are destroyed. Acids penetrate slightly into the more superficial layers of papillary dermis, eliminating abnormal cells from epidermis (treatment of lentigines, keratoses), eliminating many keratinocytes excessively charged in melanin and melanocytes producing the melanine (melasma). Grenz zone (German, meaning *border area*) peel also directly stimulates the superficial coats of papillary dermis, allowing a strong collagen and elastin deposit into the Grenz zone. These Grenz zone peels, together with basal layer peels, are the depths of peel I use the most frequently.

*Main types of peels:* TCA is the must for these depths. AHAs are not used because of their irregular penetration, making their use risky for Grenz zone peels. Resorcine and salicylic acids are difficult to use for reaching exactly the Grenz zone. Phenol should be kept for other indications.

*Action mode:* We have seen the TCA action mode. TCA coagulates keratinocyte proteins and also dermal proteins, inducing a wider skin “frosting.”

*Clinical signs:* No more pinpoints of frosting, but “frosting clouds” are seen, together with a diffuse erythema (Figure 53.4).

*Desquamation:* Desquamation looks like a strong sunburn, easy to live with if the skin is fair. Nevertheless, dead skin becomes dark brown on darker phototypes (Figure 53.5).

*Risks and problems:* The patient's social life, especially dark phototype patients, can be difficult for a few days. At the same time, the risk of PIH becomes higher, making “pigment synthesis sedation” quite interesting before and after this peel depth. PIH prevention is mandatory if the

\* Skin Rebirth®

† See [www.estetik.com](http://www.estetik.com), peelings, treatment tips.



**Figure 53.4** Basal layer peeling: frosting points.



**Figure 53.5** Desquamation after Easy TCA up to Grenz zone.

patient is phototype Fitzpatrick 4 or more, or works outside in a sunny environment. Infections are uncommon at this depth, since the immune system is still widely valid. Herpes prevention is not yet obligatory, except in special cases of frequent, recurrent herpes attacks. Other problems are the same as for basal layer peels.

### Depth 5—Papillary Dermis Peel: S

Depth 5 peel is the dividing line between secure and insecure depths. Here we reach positive limits in the sense that a depth 5 peel is able to treat many skin defects such as lentigines, solar keratoses, melasma, freckles, and fine lines. Negative limits are that since a papillary dermis peel is not able to treat real

wrinkles or skin sagging. When strictly respected, this depth is safe regarding scarring: scars should never appear when a peel is strictly limited to the level of papillary dermis.

*Main types of peels:* AHAs, Salicylic acid peels, alpha keto acids, and resorcinol are not good choices. Phenol sometimes has been used to perform a depth 5 peel, but the related (cardiac, renal, hepatic) toxicity makes me avoid its use at this depth. TCA peel, if correctly applied, remains the master choice for a safe and efficient papillary dermis peel.

*Action mode:* The TCA action mode has been described above.

*Clinical signs:* After application, frosting clouds progressively or rapidly become a pink-white uniform frosting that can progressively or rapidly turn into a pure white frosting (Figure 53.6).

Why progressively or rapidly? Passing from clouds to even frosting is progressive when we use relatively low concentrations, as Unideep\* (23% w/w) (Figure 53.7). This concentration allows the doctor to stop his (very safe) application as soon as the desired frosting appears. Conversely, an even pure white frosting rapidly appears when using higher TCA concentrations, like 35% or 40% (w/w). In this case, it is not possible to stop the TCA action. It can be compared to initiating an artificial fire: we know how big the firecracker is, but we are not sure about the final result of the spectacle. Nevertheless, when the fuse is inflamed, it is impossible to stop it or modify the course of future events. Identically, to believe that it is possible to undo (by neutralization) what TCA has done is a deep misunderstanding. Frosting appears as pink-white as long as the acids did not coagulate the blood vessel proteins. When acids have been strong enough to coagulate the well-defended perivascular area, blood cannot pass beyond the top of the dermal papillae, close to the epidermis basal layer, and frosting tonality passes from pink-white to pure-white.

At the same time as the acids penetrate dermis, they coagulate proteins, sticking epidermis to dermis, and the sign of "epidermal sliding" appears. This sign will last for a while and disappear when dermal edema is strong enough for tenting the epidermis over it.

*Desquamation:* Is quite important, as it looks like a snake changing its skin and lasts from about 6 days to 8–12 days (usual TCA in water solution, pharmacy made) (Unideep peel, SkinTech) (Figure 53.8). Social life is usually possible during the first evening, but not from the



**Figure 53.6** "Frosting Clouds": Easy Tca Grenz Zone Peel.

\* See [www.skintech.info](http://www.skintech.info)





**Figure 53.7** Frosting of Unideep Papillary Dermis Peel.



**Figure 53.8** Desquamation Day 4 After Unideep Papillary Dermis.

next morning. Days 2 to 6 are days of reclusion, during which skin will largely peel in more or less dark plaques, in relation with the phototype.

**Risks and problems:** The most immediate risk consists of infections: viruses, bacteria, and mycoses find the skin totally open and without defenses. It is easy for them to penetrate and locally proliferate. It is therefore mandatory to prevent viral infections using herpes prevention (valacyclovir i.e., 3–4 days before and 4–5 days after peel). Bacterial and mycotic infections can be avoided by strict control of medical hands and material cleanliness as well as by avoiding any other source of iatrogenic infections. The patient has to be aware to wash his hands before any contact with his skin (like scratching), to avoid direct contact with mammal or non-mammal pets, and to call the doctor for any question or inconvenience that could arise. Scratching the skin after papillary dermis peel usually induces infection. Non- or badly treated infections could induce PIH, depigmentation, or scarring.

Another risk comes from the fact that not every peeling solution is able to evenly penetrate the skin. Simple TCA-in-water solutions irregularly penetrate the tissues and can give uneven results: some areas are too deeply treated (causing local erythema, pigmentations, depigmentations, infections, scarring.)

The real action of TCA being largely hidden during the application process, damage can occur that the practitioner cannot see immediately and hence cannot correct.

Therefore, the concentration of the peeling solution and, more important, the total amount of TCA applied on the skin during a period of time, has to be strictly calculated before application. No neutralization of TCA is possible, as will be explained later.

When using a simple TCA-in-water solution, skin conditioning is mandatory for four main reasons: to allow a more even penetration, to allow a deeper penetration and hence a better result, to keep melanocytes in rest and limit the occurrence of PIH, and to stimulate the basal layer turnover and facilitate post-peel skin regeneration. Mixtures of AHAs, tretinoine, and hydroquinone are often used for this purpose.

### Depths 6–7—Reticular Dermis Peel: QS–D

(Range is from “QS”—the risks largely depend on many parameters, controllable or not, to “D”—it is always dangerous to perform such a deep peel, but it can be done in some cases.) Reticular dermis peel is a kind of Holy Grail of peeling: this depth of peel allows treating nearly every pigment problem: it tenses the skin and removes wrinkles. Nevertheless, thick and oily skins are not the best candidates for a reticular dermis peel since these skins resist very well to the action of acids. Unfortunately folds usually resist the action of deep peels. Very often, we have to discuss with the patient the choice between two therapeutic options: surgical lifting or deep peeling. At Hera Clinic (Empuriabrava, Spain), our first guide is a simple decision table (Table 53.1). Naturally, this has to be adapted to the specific skin and situation. The global meaning is that we will not recommend a phenol peel to a patient with thick sagging skin and no sun aging problem. Conversely, we will not recommend surgery to a patient with thin skin, without sagging but with important pigment or sun aging problems. The quest for the Holy Grail is a risky trip, as also is deep reticular peeling. Not only does the selected peeling solution have to be perfectly adapted to the doctor's and the patient's aims, but also the application technique and post-peel care have to be totally professionally done. Full-face reticular peel is a very aggressive treatment that leaves no room for improvisation. Any mistake can cause scarring, and pigmentary problems are frequent. Nevertheless, reticular peels are pearls in the right hands and the right circumstances.

**Main types of peels:** Two main molecules are used for reaching this depth: TCA and phenol. I really appreciate concentrated TCA for performing focal deep peels, for deeply treating lentigine and keratoses less than 1 cm in diameter (Only Touch, for example, is a 45% w/w TCA), but I would not be keen to use it for large areas, as a full face peel. In this case, phenol seems to be better; its activity depth can be better kept under control and the results are definitively better, even at similar depth as TCA. Phenol seems to have a better “rebuilding effect” on the skin than TCA. My best phenol peel is actually

**Table 53.1** Problems and Preferred Treatments

Problem	Preferred Treatment
Pigmentary problems	Peelings
Sagging skin	Surgery
Pigmentary and sagging	1/ surgery wait 6 months 2/ phenol peel
Thick skin	Surgery
Thin skin	Peeling



Lip and Eyelid Formula (SkinTech), which is an oil of phenol, penetrating slowly into the skin, which limits its general toxicity (giving the liver, lungs, and kidneys more time for detoxifying it) and allows a longer contact time between phenol and the skin proteins, inducing a potential larger protein coagulation and hence a better result (Figure 53.9).

**Action mode:** Phenol is proteolytic or proteocoagulant, depending on the concentration. Higher concentrations are proteocoagulant and lower concentrations are proteolytic. For peeling purposes, the best concentration range is 40%–60% (w/w). Nevertheless, many substances can interfere with its action and speed up or slow down its penetration. These substances are described at greater length in (1).

**Clinical signs:** Acids reach reticular dermis after having largely coagulated papillary dermis, showing a pink- or pure-white frosting (depending of the concentration, an aggressive peel will develop an immediate pure-white frosting, without passing through a pink-white one). After the typical papillary dermis frosting, the tonality quickly will shift to a grey-white or grey frosting.

It is possible to reach this depth by applying various acids concentrations; nevertheless, I have always felt more comfortable using less concentrated products, but applying more coats. Indeed, when we are using proteocoagulant products, the final result depends on the total amount of active acid molecules that have been able to interact with skin proteins. A very strong and aggressive peeling solution could induce a superficial thick coagulation, only letting pass the acids at the level of higher skin permeability, which could induce irregular results and local overpeeling. When using a progressive application technique, we can always decide to apply no more acid on the higher permeability areas, and keep applying

on the areas where we did not see the desired frosting appear. Doing that has allowed me to have zero over-peels in the last 20 years.

**Desquamation:** Desquamation is huge, always forcing a social retirement of 7–8 days. Dead skin layers should be left in place as a natural protection and only extracted at around day 6 or 7, as long as this extraction is easy and atraumatic. Phenol peel can be used under a complete 24-hour occlusion, inducing a maceration of the upper coats of the skin. During occlusion, skin melts (in open techniques, skin usually dries) and has to be protected by using, i.e. Bismuth subgallate powder. From day 5 or 6, sterile petrolatum jelly can be applied on the dead skin to help with desquamation. Occlusion causes the phenol peel to be deeper and more efficient. TCA occlusion does not have the same result.

**Risks and problems:** Histologically, there is only one reticular dermis, situated between papillary dermis and subcutaneous tissues. However, with peels, we have to consider that we face two different depths.

Into the more superficial reticular dermis, overall at the face level, we still can find keratinocytes (mainly at the level of hair roots and sebaceous glands—sebocytes are phenotypically differentiated keratinocytes, able to undifferentiate into normal keratinocytes when necessary to repair the skin). Superficial reticular dermis still has material to rebuild the skin. Deep reticular dermis is empty of this reservoir but contains big fibroblasts, also differentiated to be able to synthesize a thick bundle of collagen that will stick to the neighbor fibroblast. In addition, they are considered contractile nonmuscular cells, able to contract when necessary. They are one of the main things responsible of the scarring process: when these cells are strongly stimulated during a self-maintained inflammatory vicious cycle, scarring can appear.

**Problems:** All possible side affects can appear when using deep peeling. Pigment problems are frequent, since deep TCA kills melanocytes and phenol can make them impotent, unable to synthesize melanin. As a result, many cases of unaesthetic depigmentation have been seen in the past. A new formulation (Lip and Eyelid Formula, SkinTech) seems to be much safer in this regard, since it induces mainly PIH, which is easy to treat, other than “porcelain skin,” which cannot be treated. Table 53.2 summarizes many of the possible side effects.



**Figure 53.9** Frosting of Otp +/- Desquamation Secondary to Intraepidermic Peel. Abbreviation: Otp, Only Touch Peel.

## ABOUT ACID NEUTRALIZATION

Acid neutralization is a recurrent problem. Why neutralize? What to neutralize? How to do it? When to do it? Together with dermatological use of AHAs appeared the notion of neutralization; pre-existing peels (i.e. phenol derivatives and TCA) had not been and could not be neutralized, since proteocoagulant molecules definitively interact with skin's proteins and combine with them, forming a kind of conglomerate that cannot be separated. As a result of this interaction, non-AHA acids are largely and automatically neutralized and trapped into destroyed proteins in which tridimensional structures have changed, inducing the well-known sign of “frosting.” Neutralization of proteocoagulant acids is therefore impossible after their action has begun. At a maximum we could neutralize an eventual floating excess over the skin, before this excess can penetrate the skin.

**Table 53.2** Possible Side Effects

Locoregional side effects	Regional side effects	General side effects (phenol)
Insufficient results	Larynx oedema	Symptoms occurring rapidly
Post inflammatory hyperpigmentations	Long lasting face oedema	<b>Neurological problems:</b> headaches, acouphens, hypoacusy, paresthesies, muscle hypotony, stupor
Melanotoxicity (up to porcelaine skin)	Dynamic wrinkles resurgence	<b>Digestive problems:</b> Nauseas, Vomit, Pain in belly, diarrhoeas
Demarcation line		<b>Cardio vascular problems:</b> Arrhythmias, Asystoly
Erythema		<b>Symptoms occurring later</b>
Télangiectasies		Nephropathy
Not even complexion		Hepatopathy
Scars ( + ectropion or entropion )		Hemoglobinury, Methemoglobinury
Prurit		
Scratching lesions		
Bactérien, viral, mycotic surinfections		
Acné, milium grains		
Conjunctivitis		
Iritis, opacification of cornea		
Post peel pain		
Sun sensibilité		
Dilation of pores		
Petechies, purpura		

Is this even really possible? This idea of neutralizing proteocoagulant acids (as is TCA) is in reality extremely tricky since we have to introduce a time scale in our discussion: the appearance of a frosting is a signature of past events. The frosting that we see right now does not represent what is actually happening, but what happened into the skin some time ago\*. Frosting events have some kind of invisible inertia; they are not immediately-appearing phenomenons; proteocoagulation will continue going on during the neutralization process. It is therefore very risky to apply too much of the phenol derivative or TCA acid on the skin, thinking that it will be possible to neutralize it after seeing a frosting to appear. That is the best way to cause overpeeling. To try to neutralize TCA after seeing a frosting could be compared to trying to stop an arrow just before it touches the target. In addition, neutralizing a proteocoagulant acid will never reverse the potential damage caused to cellular proteins or matrix proteins. When an acid combines with amino acids into a protein, it is transformed to a salt that sticks to this amino acid and modifies the volumetric, tridimensional, proteinic appearance.

This is why a safe behavior is to rub on the skin the right amount of acids to get the desired frosting, without any neutralization. Remember that only a few seconds are necessary for proteocoagulant acids to pass through epidermis: that is

why phenol induces a skin anaesthesia only 12 seconds after its application!†

We have also seen that AHAs have a very low proteocoagulant power; they do not easily combine with proteins. Their natural neutralization by the skin can only be done by using the skin buffer potential, which is too slow-acting. Without neutralization, pure, non-partially neutralized AHAs would eventually burn the skin.

We cannot neutralize an acid by adding water to it. When we pour water in a recipient containing an acid solution, we only dilute the acid; a huge dilution is necessary in order to lower the resulting pH. Pouring water on the face after peeling, cannot therefore be considered as neutralization. Pouring a basic solution in a recipient containing acid will induce chemical acid-base reactions; the acid will become a salt that has no more protons to liberate in solution and therefore is no more acid. Neutralization changes the chemical structure of acids. Simple sodium bicarbonate in a saturated solution can be used to neutralize AHAs.

Finally, the answers to the questions at the beginning of this section are simple: we neutralize AHAs, but we cannot neutralize proteocoagulant molecules. A slightly basic solution, in good volume, will be poured on the skin until the end of the chemical reaction (usually seen as little bubbles). Neutralization of AHAs should begin as soon as an erythema can be seen and before any frosting appears.

We touch here the big question: when to neutralize AHAs? Too-early neutralization does not allow enough time for acids to interact with the skin, and the result will be very poor. A too-late neutralization allows acids to burn the skin and induces many side effects. That is why the industry

\* The frosting we see in T(0) results from a past action of the acids into the skin. It can be compared to the fact that the starlight that we see has been emitted millions years ago (the age of universe being supposed to be at least 17,300,000,000 years). What we see in the night sky can have been emitted in the past by a star that is now dead and maybe now transformed into a black hole after a last fantastic explosion followed by a contraction to infinite levels of energy. The frosting we see in T(0) results from a past action of the acids into the skin.

† That fast, phenol coagulates sensitive nerve sensors, inducing local anesthesia.

proposes partially neutralized AHAs (pH 2.5, 3, 3.5) which are less dangerous and can be washed with simple water, since they are between 10 and 1000\* times less aggressive (efficient) than pure AHA solution, without partial pre-neutralization.

There is one exception, called Easy Phytic Solution (EPS). In spite of the solution's very acid pH (0.5–1) and a total concentration of acids of average 60%, this AHA medical device does not need to be neutralized. The time-controlled technology permits slow release, complete progressive penetration, and full action of all acids. Absence of neutralization = absence of problems and greater efficacy. The best indications are acne and photoaging prevention and treatment. EPS is made up of three AHAs: glycolic acid, lactic acid, and mandelic acid. AHA is adsorbed on the polar groups of keratin chains and inhibits the reactions between these groups. This improves elasticity of the skin, and alpha hydroxy acids are better for that than beta hydroxyl acids because the alpha position of the hydroxyl group allows a better penetration between keratin chains than the beta position. The three AHAs show different velocities of penetration through the skin. The smallest one, glycolic acid, penetrates first, followed by the lactic, followed by the mandelic. These acids begin their action at the level of the upper layers of the epidermis. Because there is no neutralization, they continue their action, going down inside the epidermis and reaching the dermis, slowly and without ever passing the capacity of natural neutralization by the skin itself, so, the acids of EPS progressively lose their aggressiveness into the skin, producing their full activity. Phytic acid is not an AHA, but a big molecule of inositol hexaphosphoric acid considered as an excellent antioxidant and an antityrosinase. It binds out iron. In our point of view, phytic acid is unable to produce any peeling effect, so why do we find phytic acid in this solution? Actually, every peel produces an inflammation; this inflammation produces free radicals (FR) and vasodilatation. Vasodilatation brings more oxygen in situ allowing the formation of more FR. FR binds immediately with the closest structure, damaging it. So a peel always promotes the skin regeneration but induces a lot of FR that can damage the structures that are supposed to regenerate the skin during the post-peel period. The actual scientific understanding of the aging processes generally blames FR as one of the major causes of cell degeneration. It is important to fight these FR during the post-peel period. Phytic acid slowly penetrates the skin, after the three AHAs of the EPS solution have opened it, and can be present in the skin when FR are produced in parallel to the inflammation. Scavenging FR cuts the vicious cycle of inflammation-vasodilatation-FR and scavenges the FR produced post-peel.

It is known that AHAs make a thicker epidermis and produce more polymuccosaccharids in the dermis and a better quality elastin. The density of papillary dermis collagen is better, and patients under EPS describe a tightening sensation after peeling and a visible difference of the aspect of the skin.

How to use it? Cleanse the skin twice with SkinTech's cleanser foam, rinse and dry. Apply a maximum total volume of 2.5 cc of EPS on the face, using a little cotton ball, poured once only into the peeling solution. Apply it in successive coats, massaging the face between every coat for uniform coverage.

When the patient says he feels a tingling sensation, apply the last coat, using the same cotton ball. No frosting at all should occur. Two or three coats are usually sufficient. In the event of accidental frosting, neutralize immediately (sodium

bicarbonate solution). DO NOT NEUTRALIZE. For more information about details of the application technique, please refer to the packaging insert or to [www.skintech.info](http://www.skintech.info).

Another way to manage the peeling neutralization is to do it with a neutralizer that modifies its color, depending on the skin pH. This is what has been recently done with Easy Droxy versicolor peel, a medical device patented by SkinTech Pharma Group (Spain). A neutralizing blue cream is applied to the skin at the end of the peeling procedure. This cream neutralizes the skin and becomes yellow as long as the skin is acid. When the skin is at pH 7, the cream passes from blue to green. When the skin pH is over 7, the cream simply stays blue. The endpoint of the neutralization is achieved when the cream becomes green or stays blue, and this allows control of the uniformity of the neutralization process.

## ABOUT TCA

Many books have been written about TCA-in-water application techniques so I will not go into great detail here. Nevertheless, I would like to point out few important things.

The main questions of beginners are: What is the best TCA concentration? How many coats? How often? How and when to neutralize? These are not the good questions since the answers would give an appearance of an easy cooking recipe to be inflexibly respected.

The easiest of these questions to answer is about when to neutralize. TCA action cannot really be neutralized. See above.

The best TCA concentration is the one we selected, that simple. The point is to know how to select a TCA concentration and how to calculate it. I largely explained in my *Textbook of Chemical Peels* (1) why I prefer to use a weight by weight (w/w)<sup>†</sup> calculation and not a weight by volume (w/v) or a volume by volume (v/v) one. In short, we are using chemical products and we should keep our calculations scientifically reproducible and totally correct. Only the w/w calculation makes sense from this point of view, even if the w/v or the v/v is more common in the United States.

The second point is to select the right concentration for the patient. Remember two points: first, a thick-skinned patient will need more acid than a thin-skinned patient; second, the most important is the total quantity of acid that is able to interact with proteins. Too-concentrated acid applied on a thin skin will burn it immediately but will do a great job on a thick, oily skin. Looking at the thickness of the skinfold on the area of the malar bone gives us a simple clinical appreciation of skin thickness: if the fold is one cm thick, the skin should be "normal"; less than 1 cm, the skin is thin, more than 1 cm, the skin is thick. Thin skin is more sensitive to acids than thick skin, so this can help us to select the concentration.

Transepidermal acid penetration does not only depend on skin thickness, it also depends on the type of acid itself (a little acid will penetrate faster than a big one—long fatty acids for example, penetrate more slowly through the skin than

\* Logarithmic relation between pH and aggressivity: pH 3 is 10 times less aggressive than pH 2, 100 times less than pH 1.

<sup>†</sup> Indeed, a 40% TCA solution could be calculated in many ways: 40 gr TCA + 60 gr water or 40 gr TCA + 100 mL water or 40 gr TCA + the necessary quantity of water for obtaining a final 100 mL solution or even, 100 gr—or 100 mL—of any of the above described solutions, diluted with water in w/w, w/v or w+v. This makes too many possibilities, too many ways to create errors. For more reproducible peelings, I definitively selected the w/w concentration. For me, 40% TCA is always 40 gr of fresh TCA crystals mixed with 60 gr of water to do a final weight of 100 gr. This is a true 40%.



the little lactic acid). Transepidermal penetration also depends on the skin permeability, on the pre-peel skin conditioning. A thick oily skin\*, after skin conditioning† and acetone‡ degreasing can become as permeable as a thin skin. A skin dermabrasion (preferently using 3M wet or dry sandpaper 200) easily removes the stratum corneum and greatly deepens the action of the acids. I use this quite difficult technique that I call “anterior chemabrasion” together with Easy TCA peel in the treatment of acne scars and old atrophic and deep stretch marks, with splendid results.

We can see that many events are able to modify the skin permeability: did the patient do a scrub to have clean skin before seeing you? The skin will be more permeable. Did the patient wax the face before peeling for depilation? More permeability! Did the patient apply an oily moisturizing, a sunscreen, a gel containing hyaluronic acid before coming? These will reduce skin permeability.

How many coats to apply? Every peeler would like to have a clear and simple answer to this question. Unfortunately, the number of coats depends on what is described above about skin permeability. It also depends on the TCA concentration of the solution. Note also that considering concentration alone is tricky because there are so many variants that the problem of concentration is only a small part of the global decision.

Globally, we can go two different ways. The first one consists of guessing what will be the right concentration for a single patient's skin ... and praying for days, hoping that there was no mistake and that the acid will stop its action at the right depth. The second one, that I naturally prefer, is to use very few different concentrations (three different concentrations) and progressively apply several coats until the desired kind of frosting appears. This way always brings my peel exactly to the desired level, without possibility of mistake.

Let's take an example: Patient with normal thickness and permeability skin, phototype 2, pre-peel classical conditioning, and we would like to reach the Grenz zone: the very good and easy case! I can decide to apply a 30% TCA (yes, but w/w? w/v? etc.). What I cannot decide is about how deep this acid will penetrate by itself: I just can rub the product on the skin and see the result at the end, knowing that more than one coat of a 30% w/w TCA solution on such a skin can be dangerous, or conversely, it can be insufficient. We will see the action of the acid solution, but only when the eventual damage is done and irreversible. This is why there are so many reports of skin damage after TCA peel, and this is why some authors erroneously claim that TCA is not adapted to melasma treatment. It is nevertheless only a question of application technique and pre-peel decisions. A 30% w/w TCA solution rubbed once on a normal thickness skin after pre-peel conditioning usually gives a pink-white uniform frosting, showing a papillary dermis penetration. We overpassed our target that was Grenz zone.

Another possibility for the same patient is to use a lower concentration TCA solution, let's say a 15% w/w solution. A first coat induced only erythema: we understand from this that the skin was less permeable than guessed and that we reached the epidermal level only. When the skin will be dried

by evaporation, we will apply another coat of the same acid solution: the skin will show little “frosting points” and we will deduce that it has reached the basal layer depth. A next coat would induce a cloudy frosting, sign of the penetration of the acids into the Grenz zone. When we see these frosting clouds, we stop the TCA solution application and we know that the peel has reached exactly the desired depth. Easy, isn't it?

Usually, peelers focus their attention on good pre-peel conditioning for better and more even penetration and faster regeneration. Nevertheless, closer attention should be paid to the immediate post-peel events, to the inflammatory reaction that began immediately after the first contact with the first drop of acid applied on the skin.

This inflammatory reaction is necessary but dangerous. A peel that would induce no inflammatory reaction would also not be efficient, since inflammation is the real skin rebuilding source. At the same time, if this inflammation is uncontrolled, if it is self maintained, it enters into a vicious cycle in which the free radicals and the pro-inflammatory components liberated from cell destruction induce more cell damage and more inflammation. This inflammatory vicious cycle is responsible, i.e, for a long stimulation of melanocytes that will respond by synthesising more melanin and inducing PIH.

It is easily understandable that a peel can be a booby trap: you think that your chemist prepared a 30% mass by volume but he did it w/w (which is stronger); or your patient had a virtual mesotherapy§ or a depilation face wax the day before the peel: the patient's skin will be permeabilized, acids will penetrate faster and deeper and you will get (not surprisingly), an overpeel and side effects. I have progressively solved this problem by using a safer and easier formula: Easy TCA peel¶. This formula allows me to avoid the long and uncomfortable pre-peel conditioning phase in the great majority of cases\*\* since it can be applied using the progressive technique explained above; since it uses a specific post-peel mask, able to control the post-peel inflammatory reaction; since it will be repeated once a week for 4 weeks. Every peel, done up to frosting points or maximum local frosting clouds, treats an eventual pigment rebound induced by the preceding peel.

The Easy TCA post-peel mask is a quite complex formula, containing vitamins, trace elements, a lot of strong antioxidants, tretinoine precursors, selenio-methionin, anti-tyrosinases, etc. This cream is applied once by the practitioner immediately after the desired frosting has been seen. It immediately scavenges FRs, stopping the excess of immediate inflammatory reaction at the same time as the burning sensation induced by the easy TCA peel solution. It brings into the skin elements that can help it to regenerate faster, and lowers the tyrosinase activity. Post-peel penetration of ingredients is dramatically modified by the previous application of the peeling solution. The post-peel rate of penetration is much higher than the normal skin rate. The skin is much more permeable during the close post-peel period: sebum and corneocytes no longer act as a barrier and purely water soluble ingredients can rapidly pass through this modified epidermis.

\* Skinfold = more than 1 cm

† Glycolic acid to reduce stratum corneum thickness + tretinoine for stimulating basal layer turnover and regeneration and for reducing the stratum corneum permeability: this kind of pre-peel conditioning makes the skin much more permeable.

‡ Note that acetone not only degreases skin but also begins a protein denaturation that makes the skin more permeable.

§ Virtual mesotherapy: slight abrasion using sandpaper, application of specific vitamins and other elements on the skin, use of Excellderm (non-thermogenic radiofrequencies for inducing an intracellular penetration). Aestheticdermal.com

¶ see *Textbook of Chemical Peels* or [www.skintech.info](http://www.skintech.info)

\*\* I would use a pre-peel conditioning only in specific cases, as can be a phototype 4 or 5 patient with a long history of familial melasma.

So, high quantities of stimulating factors and antioxidants can reach the dermis. It is clinically evident that the residual inflammatory reaction is strong enough for stimulating the skin architectural rebuilding, but not strong and long enough for stimulating melanocytes or for slowing down the basal layer regenerative event sequences. Colleagues often ask to me how and why this cream can immediately stop the burning sensation, even if it is not a neutralizer (this cream is not a basic one) and if the frosting signs continue to appear normally, even after having applied the post-peel mask. I unfortunately cannot scientifically answer this question but have a theory: my understanding is that the post-peel mask antioxidant properties are such that they break the pro-inflammatory immediate post-peel reaction. It is well known that inflammatory reaction usually represents a big part of pain.

So to summarize: After having used many different peeling formulas and spending post-peel nights of insomnia, I decided that it should be possible to use TCA without the post-therapeutic nightmare. This is what brought me this Easy TCA formula: it is easy to do (10 minutes as a maximum for face and neck), not expensive, not painful (never needs any kind of anesthesia or painkiller), no skin conditioning (peeling solution and post-peel mask do this), patient's social life is possible (desquamation looks like a sunburn), no phototype limitation (phototype allowed: from 1 to 6), very little percentage of side effects (average 1.7% transitory side effects, duration less than 8 days\*). This peel, applied according to different protocols, allows for a wide spectrum of depths of action and therapeutic indications from active acne to pigmentation and acne scars, photoaging and deep, old stretch marks†. It can be applied to both face and body. In addition, it can be used during the same session with laser, IPL, mesotherapy, radiofrequencies, depilation, botulinic toxin‡, surgery, and telangiectasies treatment. Even hyaluronic acid injections for wrinkle treatment is allowed, immediately before Easy TCA Peel§.

In the same time, I decided to stop trying to guess what would be the best concentration of TCA for a patient, but only use the Easy TCA solution, which I progressively apply the necessary number of coats to get the desired frosting.

In the preceding paragraphs, I mentioned using only three different concentrations, and this will now be clarified. The main peel I use, on about 80%–85 % of my patients, is Easy TCA Solution, containing vitamins, antioxidants, AHAs and saponines, together with 15% w/w TCA. This peel benefits from the important post-peel mask protection against post-peel inflammatory reactions and immediately stopping post-peel burning. Depending on the number of coats I pass

on the skin, I drive my peel into epidermis, basal layer, Grenz zone, or even papillary dermis. Nevertheless, papillary dermis peel needs many coats of Easy TCA and this is uncomfortable for the patient. This is why I use a derivative of Easy TCA when I want to reach the papillary dermis: Unideep¶ has the same structure as Easy TCA. It is a peeling solution form which ingredients we can find 23% w/w TCA and an adapted post-peel mask with the same aim as Easy TCA. Sometimes, I need to go very deep into the skin for treating focal old lentigine or solar keratoses: naturally, we often can do it using successive focal coats of Easy TCA or Unideep, but the work is easier when using Only Touch Peel (OTP): it has same qualitative base solution than Unideep but contains 45% w/w TCA. OTP has no post-peel mask and has to be used in combination with Easy TCA for avoiding post-peel inflammation. Indeed, OTP without Easy TCA induces PIH in more than 60% of the cases. When done immediately before Easy TCA, the percentage of PIH is much less prevalent: a maximum of 10%, if the protocol is respected, even less if the patient does not scratch the scabs. OTP can be used on small surfaces only: it is always a focal peeling for treating lesions with diameter than 1 cm. OTP is applied, avoiding excess product, with a fine cotton bud or a wooden toothpick or skewer. Touch the lesion to be treated quickly, precisely, and once only with the chosen applicator and wait until the acid solution has dried completely. A significant frosting will appear quickly on the face, slowly on the body. If no such frosting occurs, however, repeat the application cautiously a few minutes after the solution has dried. Immediately after the frosting occurs, apply the first of the four basic protocols of Easy TCA on the entire treated area, including the one where OTP has just been applied, in order to obtain an even result and limit the risk of side effects.

## HOW PAINFUL IS TCA APPLICATION?

The burning sensation of TCA application is clearly linked to its concentration and to the skin permeability. One coat of 30% TCA will be very painful on thin skin, but very tolerable when applied on thick and oily skin. On the same patient, a 30% peel will be more painful compared to a 20 or 15%.

An elegant solution has been recently launched by Skin Tech Pharma Group: the medical device Easy TCA Peel Pain Control®. The peel is on average 25% more aggressive than the classical Easy TCA® 15%, but is 90% less painful. Frosting is achieved more easily and the patient only experiences a beginning burning sensation during the first 10 seconds, or after the third coat (when the peel is voluntarily done up to the papillary dermis). This lack of burning sensation provides total comfort to the patient.

## PEELS AND FIBROBLASTS

Dermal fibroblasts represent a major cell, not only in the understanding of peels: their correct stimulation by any means would lead to good skin rejuvenation. Fibroblasts have a dendritic appearance, like Langerhans cells, but have no immunological known function. Fibroblasts synthesise all the intercellular matrix components and play a vital role during the formation and the contraction of the granulation tissues that appear during the wound healing processes and post-peel regeneration events. Huge morphologic variations exist within

\* Unpublished statistic established on a total amount of 5000 peeling sessions in Clinica Hera, Empuriabrava, Spain: 28 transitory easy to treat side effects. No definitive side effect.

† See: [www.estetik.com](http://www.estetik.com), left column, treatment tips, peelings: you'll find there a complete description of active acne, stretch marks, melasma treatments, together with the protocol for local phenol peel around eyes and lips.

‡ Ten minutes after botulinic toxin injection, Easy TCA (as basal layer peel) can be performed. We never saw migration or shorter results after this association.

§ As everybody did, I also read the HA notice for injection, which says not to perform peelings after HA injections. Nevertheless, this recommendation is valid only because of the huge release of free radicals after usual peelings. Free radicals rapidly damage the HA polymer, breaking it and making his life shorter. Easy TCA post-peel mask scavenges free radicals at the same time they are produced, which can stop self-maintained free radical reactions, able to damage HA polymer.

¶ Easy TCA, Unideep and Only Touch are peelings developed by Skin Tech, based on my own formulas.

this cellular population: papillary dermis shows little horizontal fibroblasts (4–7 microns), their dendrites have contact with several collagen fibers. Stimulation of these fibroblasts is responsible for a dense deposit of horizontal neo collagen in the top of the papillary dermis.

Medium-depth dermis and deep dermis show larger fibroblasts, with a variable orientation. Dendrites have contact with one big bundle of collagen only. The very deep dermis, at the limit of hypodermis, contains very large fibroblasts (16 microns) with long dendrites (up to 180 microns), forming a continuous net.

Inflammatory reactions and healing processes induce the appearance of many hybrid cells, called myofibroblasts. These are phenotypically modified fibroblasts having some characteristics of muscle cells and which are responsible of the retraction processes during skin healing. Cytoplasm of myofibroblasts contain myofilaments connected with cell membranes, responsible for cell contraction and eventual scarring processes.

“Normal depth” peels will stimulate the full population of fibroblasts without overstimulating myofibroblasts. They give a better skin tension, without scarring (up to papillary dermis peels). Too-superficial peels (intraepidermal peels) are only able to slightly stimulate fibroblasts synthesis: the result on skin tension is weak. Deep peels will strongly stimulate fibroblasts and myofibroblasts, inducing a tridimensional tensing effect on facial tissues, giving a well-done phenol peel. Too-deep peeling, locally hypodermal peels, focal overpeels, leave the skin without any other regeneration possibility than the strong myofibroblastic contraction, bringing about unaesthetic scarring processes. We can therefore easily understand that scars only occur when something wrong has happened to the skin: it can be a problem induced by the physician having applied a too-concentrated solution or a too-strong pre-peel conditioning, or by a patient having scratched and infected newly peeled skin\*.

Nevertheless, it is largely admitted that the papillary dermis is the limit from which the scarring process can be switched on. This means that as long as a peeling does not enter into reticular dermis, there is no danger of scarring. The problem here is how to strictly limit the penetration of acids until the desired depth. One of the possible answers has been described in the TCA paragraph.

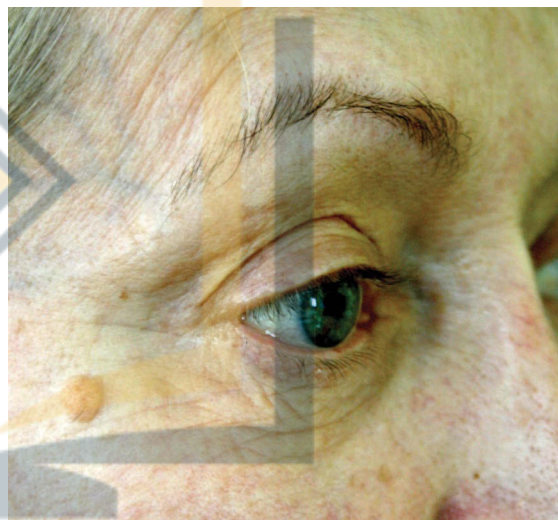
## ABOUT PHENOL

Many things have been said about this molecule, some true and others only urban legends. One example of truth: it is possible to iron completely the upper lip, using phenol peel. One example of legend: the patient can die right after the very first contact with the first drop of phenol. I will not say much about phenol peels, since a little part of a short chapter is not the right place for learning about it. Nevertheless, let's divide our phenol paragraph in two parts: local phenol and full-face phenol.

Local phenol is a very simple procedure, the fastest of all peels, and it gives amazing results. Chemical blepharoplasty and/or cheiloplasty (lip procedures) are easy to perform with phenol. The quantities necessary are largely under the threshold of toxicity and new formulas (see in the beginning of this chapter) are considered as self-blocking at the level of the upper part of reticular dermis. Moreover, new formulas no longer induce “porcelain skin,” and skin tanning can still be possible in the future. Local phenol peels are therefore one

of our frequent treatments for deep wrinkles of the upper lip, the eyelid area, and in some cases wrinkles between eyebrows, on the top of the nose pyramid. Lower lip and mental area are much more difficult to treat with local phenol. Often, at this level, a deep mechanical abrasion has to be used after phenol peel in order to achieve good results.

Special attention has to be paid to the patient selection: phototypes 2 and 3 are the best patients but patients showing yellowish sun-damaged skin should be avoided since post-phenol skin regeneration will completely renew the treated skin and it will appear like a baby skin surrounded by irregular-color skin (Figures 53.10–53.12). The case of freckles is also a trap. These clear phototypes are good indications, but freckles will completely disappear at the level of phenol peel and not on the rest of the skin. In these cases, a local phenol peel can be tried, together with a papillary dermis peel on the rest of the face, calling attention of the patient that, even in this



**Figure 53.10** Before local phenol (Lip and Eyelid Formula—Skin Tech).



**Figure 53.11** Eight days after local phenol lower eyelid—Unideep on the rest of the face for uniformization.

\* Some genetic disorders make the skin prone for post-peel scarring, like Ehler Danlos syndrome. Insulin-dependent diabetes is also a risk factor for deep peels. See Deprez 2016 (1) for more side effects.





**Figure 53.12** Eight months after local phenol lower eyelid and Unideep.

case, a demarcation line can be seen between the area treated by phenol and the one treated by TCA.

The post-peel period is dramatically important: see your patient nearly every day during the first week; this will allow you to detect infections, abnormal reactions, etc. For more practical information, please refer to the website [www.estetik.com](http://www.estetik.com), left column: "Peelings" and "Treatment Tips." There can be found clear tables about how to do the peel and the post-peel.

Full face peeling is another story, since the phenol toxicity has to be avoided or even treated in exceptional cases. Even if some physicians still feel comfortable in doing it as an office procedure, as in the past, I would strongly recommend performing this kind of peel in a secure surgical environment, with the help of a trained anaesthetist (nerve blocks associated to a deep sedation or neuroleptanalgesy). Nothing usually happens but all the lights are red and we have to be very careful, since an accident would never be forgiven. Strict application rules have to be followed. The post-peel period after phenol peel is quite uncomfortable for the patient, it looks like after a deep ablative CO<sub>2</sub> laser procedure. Procedure is specific, many details have to be respected if we want to do it very safely, and post-peel is difficult. I cannot describe here the full process of a full-face phenol peel and how to avoid all the traps. Nevertheless, Deprez 2016 (1) contains approximately 200 pages dedicated to all aspects of phenol and phenol derivatives-peel procedures.

Regardless, full face phenol peeling is one of the most satisfying treatments I perform: many patients look 15 or 20 years younger after a phenol peel. Skin aging goes many years back and goes on from there: the patient never again has as old a skin as they had before the peel. Full-face phenol peel can be combined with surgery, but not at the same time. When necessary, we perform a face lift as the first procedure and a phenol peel as second treatment, 6 months later, in order to achieve complete face rejuvenation. At the same time, we treat neck, décolleté, and hands by other procedures.

## CONCLUSIONS

The world of peeling continues to be discovered; its treasures are able to make a large majority of our patients happy. Nevertheless, we cannot begin our exploration of these treatments, groping our way along, following any light that can

appear in the sky as a shooting star: while we would look in this direction, we are at risk of falling into dangerous traps. Exploration of the world of peeling has to be done carefully, progressively, using good material, having at our disposal the necessary experience for passing from one step to the other. In short: deep full-face phenol or chemoabrasive techniques for stretch marks should not be the first peel we ever do. The problem will not be the procedure itself but what we will have to face and the problems we will have to solve later. The door of the world of peeling should open on VHS or HS peels only, and beginners should use depth 1 to 3 peels first, trying deeper treatments only when they are fully experienced.

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## Lasers and Light Sources for Vascular and Pigmented Components of Photoaging

Anne Marie Mahoney and Robert A. Weiss

### INTRODUCTION

Photoaging-related changes most commonly occur on the face, however, areas such as the chest and legs are also frequently involved. Two clinical manifestations of photoaging for which patients often present for treatment are vascular and pigmented lesions. Facial telangiectasias are the most common vascular lesion related to photoaging in Caucasians. In contrast, Asians with photodamage present more frequently with UV-induced pigmentation problems. The most common pigmentation concerns are lentigines, keratoses, and Hori's macules (1). This chapter reviews current treatment modalities of these common problems.

### VASCULAR LESIONS

The term *telangiectasia* refers to superficial cutaneous vessels visible to the human eye (2). These vessels measure 0.1 mm to 1.0 mm in diameter and represent a dilated venule, capillary, or arteriole (Figure 54.1). The vessel type determines the clinical presentation of telangiectasias. Arteriolar telangiectasias are small in diameter, bright red in color, and do not protrude above the skin surface. Venule-derived telangiectasias are wider, blue in color, and often protrude above the skin surface. Telangiectasias arising at the capillary loop initially present as fine, red lesions, but with time they often enlarge and become purple or blue in color (2).

There are four classifications of telangiectasias based upon clinical morphology: 1) simple or linear, 2) arborizing,

3) spider, and 4) papular. Linear and arborizing telangiectasias with red coloration are very common on the face, particularly on the nose and midface regions. These lesions are also seen relatively frequently on the legs. In addition, patients experience enlargement of slightly larger venulectases. These appear as purplish vessels on the cheeks, periorbital region, and vermillion. Papular telangiectasias are typically manifestations of genetic syndromes, such as Osler-Weber-Rendu syndrome, or occur in the setting of collagen vascular diseases. They are less frequently related to photoaging. Cherry hemangiomas are also seen, which are small round red-to-purple dome-shaped vascular ectasias scattered anywhere on the face or body. All forms of telangiectasias are thought to occur through the release or activation of vasoactive substances under the influence of a variety of factors, such as anoxia, hormones, chemicals, infection, and physical factors such as UV radiation, with resultant capillary or venular neogenesis (3).

Spider telangiectasias of the face are most commonly seen in patients with fair skin of Fitzpatrick Types I and II, indicative of this group's increased susceptibility to UV damage. Facial telangiectasias are especially common on the nasal ala, dorsal nose, and mid cheeks and are probably due to UV-induced vessel wall weakness that leads to persistent arteriolar vasodilation. Sun exposure damages and weakens collagen with cumulative exposures, resulting in ectasia. Additionally, there is a relatively high incidence of rosacea on the face, which may have a prominent telangiectatic component (Figure 54.2). Rosacea consists of



**Figure 54.1** (a) Telangiectasias on the cheek of a Caucasian female consistent with the early stages of photoaging. (b) The same patient following treatment with IPL has fewer visible vessels and smoother skin. IPL with double-pulse 570-nm filter, 2.4-msec + 6-msec pulse with 10-msec delay, and a fluence of 29J/cm<sup>2</sup> was used.





**Figure 54.2** Rosacea is thought to result in part from photoaging. IPL treatments reduce intensity and duration of flushing that worsens telangiectasias in rosacea. Before (a) and after (b) three treatments with VascuLight IPL (Lumenis, Santa Clara, CA) using a 550-nm filter, double pulse of 2.4- and 7-msec, delay of 10 msec, and a fluence of 27–29J/cm<sup>2</sup>.

frequent flushing associated with telangiectasias, papules, and pustules. It is the repeated flushing in rosacea, often caused by exercise, alcohol, and spicy food, which leads to the development of telangiectasias. Genetic factors also play a large role, as the rosy cheek appearance passes from one generation to the next in individuals susceptible to rosacea. Aging of the skin, particularly photoaging, causes more telangiectasias as collagen breakdown ensues (4). Repeated trauma to the face will also induce localized erythema and ultimately vascular dilatation.

Fortunately, the treatment of facial telangiectasias is relatively safe and more predictable than treatment of telangiectasias on other sites, particularly the legs. This is attributable to several factors. One is the ability of facial skin to heal quickly with fewer propensities toward scarring when treated with a similar depth of injury than other locations. Treatment results are often seen much more quickly as healing is much faster on the well-oxygenated skin of the face. Facial vessels also have the advantage of a more consistent depth than the legs. The vascular walls themselves are much thinner and uniform, and hydrostatic pressure plays no major role in pathogenesis. Occasionally, arterial pressure is a factor as seen in spider angiomas with a small central arteriole. This is important when deciding on a method of treatment, as sclerotherapy into a bright red arteriolar fed vessel on the cheek incurs more risks of necrosis than the use of laser or light to shut down the branches and shrink the arteriolar component.

Patients with telangiectasia of various types present for treatment primarily because of cosmetic concerns; therefore, it is important that the procedure be relatively risk-free without unsightly scarring. Various modalities can be used to treat telangiectasia on the face or other regions. Several of these modalities will be discussed in more detail in this chapter, including electrodesiccation, sclerotherapy, and a variety of lasers including the pulse-dye laser (PDL), long-pulse dye laser (LPDL), argon laser, frequency doubled neodymium:yttrium-aluminum-garnet laser (532Nd:YAG), intense pulsed light (IPL) in all its forms, and a variety of 1064nm long-pulsed Nd:YAG lasers (1064Nd:YAG).

## ELECTROSURGERY

Electrodesiccation is commonly used to treat facial telangiectasia because the device is readily available and relatively low cost, making it an accessible and affordable treatment worldwide. Electrodesiccation is a process in which heat is generated from resistance of tissues to the passage of a highly damped current from a single electrode. Dehydration occurs in the tissue immediately adjacent to the needle point, and as cellular fluids are evaporated, tissue destruction results. The vessel must be cauterized or electrocoagulated every 2 to 3 mm with very low amperage current (1–2 amps). Some degree of epidermal necrosis occurs due to the nonspecific nature of cauterization. Multiple treatments are typically necessary for successful treatment. Punctate white or pigmented scars may occur if excessive thermal damage occurs. Groove type scars along the nasal ala are the most common adverse effect of electrodesiccation.

Using the lowest effective fluence and the finest electrodes produces optimal results. Electrodes that are coated with Teflon so that only the tip of the electrode or one side of the electrode is exposed tend to provide the safest treatment. In addition, use of a bipolar current (with the patient grounded to a plate at a distance from the treated area) allows for effective treatment with a lower fluence. With bipolar treatment, the current passes through the cannulated vessel for several millimeters with relative selectivity. When performed with care, electrodesiccation is effective, but is best reserved for the smallest of telangiectasia. This technique has been popularized by Kobayashi who reports excellent results (5).

Serious adverse effects from electrosurgery such as disturbance of pacemakers and implantable cardio-defibrillators are extremely rare. To prevent disruption to electric currents in these devices, short bursts are recommended with minimal power settings. Rare instances of pacemaker interference with skipped beats and reprogramming have been reported in a survey of dermatologic surgeons performing electrocoagulation during cutaneous surgery. An incidence of 0.8 cases/100 years of surgical practice occurred, but may not be representative of all patients undergoing electrodesiccation of telangiectasia (6).



## LASERS

There are multiple lasers available for destroying facial telangiectasia. These lasers act by selectively heating the vessel to cause its destruction through the absorption of laser energy by oxygenated and deoxygenated hemoglobin. The advantages and disadvantages of presently available lasers are described below.

Several factors must be considered in selecting an appropriate laser for telangiectasia treatment. In general, the choice of wavelength(s) and pulse duration are related to the type and size of target vessel treated. Deeper vessels require a longer wavelength to allow penetration to their depth. Pulse duration must be matched to vessel size, as the larger the vessel diameter, the longer the pulse duration required to effectively thermally damage the vessel. The relative importance of hemoglobin absorption peaks in green (541 nm), yellow (585–595 nm) and red to infrared (800–1000 nm) shifts as the depth and size of blood vessel changes. Absorption by hemoglobin in the long visible to near infrared range appears to become more important for vessels over 0.5 mm and at least 0.5 mm below the skin surface (7).

It is important to note that while laser treatment of facial telangiectasias has yielded excellent results, the use of lasers to treat leg telangiectasias has been far less successful than sclerotherapy. This is likely related to insufficient vessel destruction by lasers, competition for the laser absorption from overlying melanin, and the failure of lasers to treat the increased hydrostatic pressure from the “feeding” venous system.

## Continuous Wave Lasers

### Carbon Dioxide Lasers

Carbon dioxide (CO<sub>2</sub>) lasers were used early on in an effort to obliterate telangiectatic vessels by means of precise vaporization without significant damage to adjacent tissue. Unfortunately, because the CO<sub>2</sub> laser is so well-absorbed by water in the epidermis and dermis overlying the blood vessel, non-specific thermal injury is guaranteed regardless of whether pulsed or continuous wave sources are used (8). All reported studies demonstrate unsatisfactory cosmetic results (9). Treated areas show multiple hypopigmented punctate scars with either minimal resolution of the treated vessel or neovascularization adjacent to the treatment site. Because of its non-selective action, the CO<sub>2</sub> laser has no advantage over the electrodesiccation needle and is associated with more adverse effects.

### Argon Laser

Argon (488 and 514 nm) and argon pumped continuous wave dye lasers (515–590 nm) are well-absorbed by hemoglobin and penetrate to the depth of mid-dermal vessels, over 1 mm into skin. Treatment parameters vary and laser powers of 0.8 w to 2.9 w, exposure times of 50 milliseconds (msec), 0.2 seconds (sec), 0.3 sec, and continuous and spot sizes of 0.1 mm and 1 mm have been used. Although the success rate in treating facial telangiectasia is acceptable (10,11), pitted and depressed scars, hypopigmentation, hyperpigmentation, and recurrence of veins have been noted. In addition, when compared to the PDL, the latter is more efficacious.

Adverse healing consequences occur with the argon laser due to competition for absorption of its wavelength (411 nm and 514 nm) from epidermal melanin as well as radial diffusion and dissipation of heat from the target blood vessels secondary to long pulse durations. Both of these factors result

in relatively nonspecific thermal destruction and thus this laser is not recommended.

### KTP 532nm Green Lasers

KTP crystals are highly reliable, convenient to work with, and easily available to laser manufacturers. While the mechanisms of these devices vary, each produce millisecond domain pulses at 532 nm. Pulsing in milliseconds allows vessel coagulation to occur without producing purpura. The various KTP lasers available differ in the spot size, which ranges from 0.5 to 4 mm in diameter.

Results of treatment of facial vessels have been excellent (12). KTP is typically used with a 2-mm spot, 10–20 msec pulse duration and 10–15 J/cm<sup>2</sup> of fluence. Cooling appears to be of significant benefit in protecting the epidermis, thus allowing use of higher, more effective fluencies. A randomized split face study using a larger spot size (5 mm), pulse duration of 18–20 msec and fluence of 8–11 J/cm<sup>2</sup> was compared to PDL and at 3 week follow-up there was a higher rate of clearance of facial telangiectasias in those treated with the KTP versus PDL (85% vs. 75%) (13). In addition, a recent study of 647 patients revealed that 78% of patients demonstrated clearance or marked improvement in vascular lesions after 6 weeks. In this same retrospective review, 6% of patients reported minor adverse effects and there was only a single report of bruising; there were no major adverse effects (14).

### Flashlamp Pumped-Pulsed Dye Laser

The traditional pulsed dye laser (PDL) (585 nm, 450 µsec pulse duration) is highly effective in treating a variety of cutaneous vascular lesions, including PWS and facial telangiectasia. The original PDL was developed for the treatment of port wine stains in children, where the average vessel is superficial and has a diameter of 100 µm and an average depth of 0.46 mm. Modern-day PDL is delivered entirely differently, using 595 nm, 1.5–20 msec pulse durations and synchronized skin cooling with a cooling spray or airstream.

In preliminary animal studies in the rabbit ear vein, approximately 50% of vessels treated with an effective concentration of sclerosant demonstrated extravasated RBCs, while after PDL treatment, extravasated RBCs were apparent in only 30% of vessels treated (15). Rabbit ear vein treatment with the PDL resulted in a relative decrease in perivascular inflammation compared to vessels treated with sclerotherapy alone.

The PDL treatment technique involves delivering a series of pulses overlapping 10%–50%, tracing the vessels to be treated with a 2, 3, 5, 7, 10 mm or elliptical delivery spot, and treating an area of interlacing telangiectasia with overlapping spots to cover the involved area. Delivery energies range from 5.0 to 14.0 J/cm<sup>2</sup> depending on the spot size used and are adjusted according to vessel response. The end-point is purpura or vessel spasm.

Purpura is a common occurrence after treatment with traditional PDL with short pulse durations, but is much less common with pulse durations longer than 6 msec. Initial studies using the 0.45-msec pulse duration flashlamp pumped-pulsed dye laser (FLPD) laser demonstrated high efficacy, but it was complicated by purpura that lasted for 1–2 weeks (16). In this study 182 patients treated with the 0.45-msec pulse laser at 6–7.75 J/cm<sup>2</sup> with a 5-mm diameter spot size were evaluated. Seventy six to 100% clearance was obtained in 83.5% of patients with the remainder having 51%–75% clearance.

A technique to increase efficacy and decrease purpura is to use double and triple pulses (pulse stacking) at sub-purpuric

fluences. Tanghetti used pulse stacking to increase efficacy on photoaging with reduced side effects, but found that multiple treatments with or without pulse stacking had excellent results on the signs and symptoms of photoaging including telangiectasias (17).

#### *Long-Pulse Dye Lasers*

Based on the theory of selective photothermolysis, the predicted pulse duration ideally suited for thermal destruction of vessels the size of leg telangiectasia (0.1–several mm in diameter) is in the 1–50 msec domain (18). Newer LPDLs with variable pulse durations as long as 40 msec (V-Beam Perfecta™, Syneron/Candela, Wayland, MA and Cynergy™, Cynosure, Chelmsford, MA) are now available. Each device uses a rhodamine dye to produce wavelengths of 585–595 nm. These longer pulse durations and longer wavelengths improve our ability to treat deeper, larger caliber vessels.

Newer FLPD lasers that extend the pulse duration to 1.5, 3, 6, 10, 20, and 40 msec and use dynamic or continuous air cooling have eliminated most of the pain and have minimized purpura associated with the first-generation FLPD lasers. Typical fluences of 10 J/cm<sup>2</sup> with a 10 msec spot size usually result in 90% resolution of facial telangiectasias in one treatment with minimal pain and purpura (Figure 54.3). Improvement in rough texture and pigmentation of photoaging is also seen.

Treatment of leg telangiectasias with this method has not produced satisfactory results, even with the use of longer pulsing. A study evaluating the LPDL of 595 nm using two different fluences of 20 J/cm<sup>2</sup> and 24 J/cm<sup>2</sup> demonstrated that after two treatments at 6 month follow-up, there was a 50% improvement in leg telangiectasias in 77% and 85%, respectively (19). In a single treatment of vessels less than 0.4 mm in diameter using the 595 nm, 1.5-msec PDL (Cynosure) and an experimental 595 nm, 4-msec PDL, clearing rates were not clinically significant with either device, and the rates of both hypopigmentation and hyperpigmentation were significant (20). Despite advances in laser therapy, sclerotherapy remains the gold standard for treatment of leg telangiectasias.

#### *The Copper-Vapor Laser*

The copper-vapor laser operates at two specific wavelengths, 578 nm (yellow) and 511 nm (green) and delivers a “quasi-continuous wave” composed of pulsed laser light energy

in 20-nanosecond pulses at a frequency of 15,000 pulses per second. This train of pulses interacts with tissue in the same manner as a continuous beam because of the accumulation of heat with the large number of pulses delivered. Due to the resulting thermal diffusion, it is necessary to electronically gate the pulse to a 20–50 msec duration.

These refinements should allow this laser to work within the thermal relaxation time of telangiectasia (21). When the laser is used with these refinements, it is somewhat safer and more effective than the argon laser for treatment of facial telangiectasia. It also has the advantage of leaving very minor superficial crusts overlying treated vessels in contrast to the very visible dark purpuric impact spots of the FLPD laser.

#### *Long Pulse Nd:YAG 1064nm*

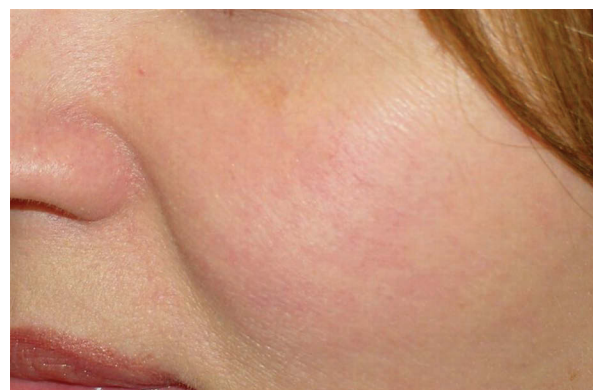
Long pulsed 1064-nm lasers have recently been developed in an effort to target deep, relatively large caliber cutaneous vessels. This wavelength achieves deep penetration and is not absorbed by melanin, thus allowing treatment of more darkly pigmented individuals. High energies must be utilized for adequate penetration. Only with sufficient fluence and facilitation of heat dissipation can the posterior wall of a larger diameter (1–2 mm) vessel filled with deoxygenated hemoglobin be reached and heated.

The newer pulsed 1064-nm lasers have pulse durations between 1–200 msec (Vasculight™, Lumenis, Santa Clara, CA; Cool Touch Varia™, CoolTouch Corp, Roseville, CA; Lyra™, Laserscope Lyra, San Jose, CA; Coolglide™, Altus, Burlingame, CA). Large-caliber vessels > 0.5 mm in diameter respond best to these lasers, however, recent data suggests that by using smaller spots and higher fluences even small vessels will respond. In initial studies with a first-generation 1064-nm fixed 6-mm spot delivery handpiece, optimal settings were fluences of 80–120 J/cm<sup>2</sup> and single-pulse durations of 10–30 msec (22). Experience indicated an approximately 75% resolution of leg telangiectasias at 3 months using 16-msec pulse durations with fluences of 130–140 J/cm<sup>2</sup> (22).

Larger violaceous vessels of the face may be treated with these devices (Figure 54.4). The fluence must be lowered by 30%–40% for facial vessels as compared to the legs. A recent study of facial telangiectasias on the nasal alae and tip demonstrated that vessels ranging in size from 0.2–0.3 mm decreased in size by 92% (23).



(a)



(b)

**Figure 54.3** Treatment with the long-pulsed dye laser (V-Star, Cynosure, Chelmsford, MA) improved this patient's telangiectasias, skin texture, and pigmentation. (2-msec duration, 2.5J, 10-mm spot, and three passes. (a) Pretreatment. (b) After treatment.





**Figure 54.4** Larger facial veins can be treated with the 1064-nm Nd:YAG laser (Vasculight, Lumenis, Santa Clara, CA). (a) Pretreatment. (b) 50% improvement 2 months after treatment.

It should be kept in mind that treatment with long pulse 1064-nm laser is relatively painful, and both cooling and topical anesthesia should be employed to minimize discomfort. Stacked pulsing CANNOT be performed with 1064 nm as there is high risk of heat buildup and skin breakdown, and 1064 nm should never be used faster than 1 Hz pulse rate and pulse positioning should never be closer than 1 mm apart. For patient comfort, epidermal cooling can be contact cooling or cryogen spray, which can be programmed both before and after the laser pulse. The concept behind applying the spray after the cooling pulse is for "thermal quenching" (U.S. Patent # 6451007, Koop, Baumgardener and Weiss) of the heat released from larger vessels following the laser heating. Topical lidocaine has also proven efficacious in reducing pain (24).

### INTENSE PULSED LIGHT SOURCE

The high-intensity pulsed light (IPL) source was developed as a device to treat ectatic blood vessels using non-coherent light emanating from a filtered flashlamp (Lumenis One™, Lumenis, Santa Clara, CA). Although Lumenis is the largest and most well known of the IPL device manufactures, other manufactures of pulsed light devices include Energis Technology, Swansea, UK, marketing an Energis Elite IPL system for hair removal only, and Danish Dermatologic Development, Hoersholm, Denmark which markets the Elipse system for hair and vascular indications. The Energis system is a low-output device, with 5–19 J/cm<sup>2</sup> output, spot size of 10 × 50 mm, pulse train length of 15–40 msec and pulses per train of 4 to 5. There is a fixed delay between pulses of 1.5 msec. By comparison, the Lumenis device is a high-output device with up to 90 J/cm<sup>2</sup> output, spot size of 8 × 35 mm, variable pulse lengths of 2–40 msec, and infinitely variable delay between pulses of 1 to 1000 msec.

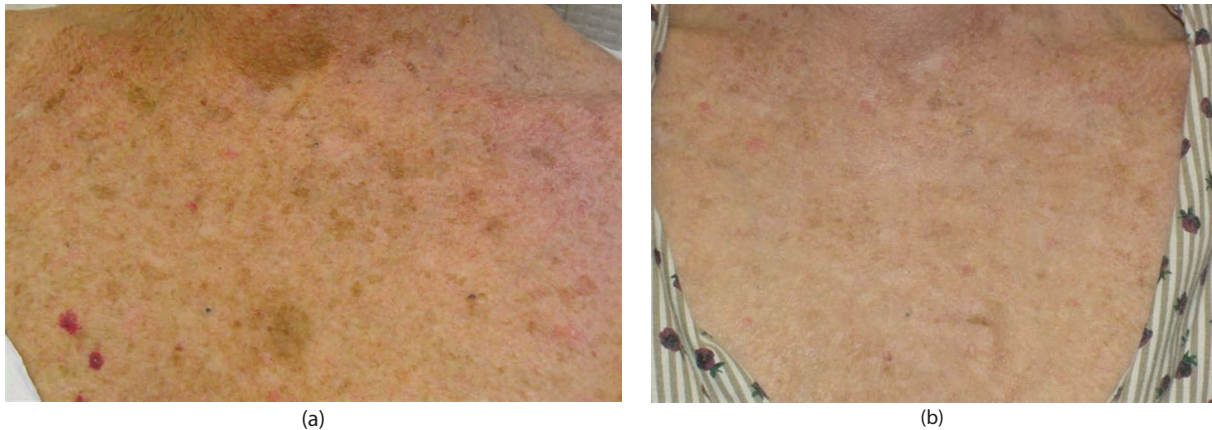
Selectivity for IPL is achieved primarily by manipulating pulse durations to match thermal relaxation times of vessels larger than 0.2 mm and by using filters to remove lower wavelengths of visible light. Fluence can be very high with the unit delivering up to 90 J/cm<sup>2</sup>. Sequential pulsing of 1–12 msec duration separated and synchronized with 1–100 msec rest intervals delivers wavelengths of 515–1000 nm. It is most commonly used with the 550 and 570 nm filters to deliver the yellow and red wavelengths and some infrared. The ability of IPL to produce a non-coherent light as a continuous spectrum lon-

ger than 550 nm was thought to have multiple advantages over a single-wavelength laser system. These advantages include absorption by both oxygenated and deoxygenated hemoglobin and absorption by larger blood vessels located deeper in the dermis. In reality, the primary advantage has been larger spot size and relatively low incidence of purpura on facial telangiectasia.

For facial telangiectasia, our experience has been that the 550 nm, 560 nm or 570 nm produce optimal results. When treating darker skin types and larger blood vessels, we choose a longer cut-off filter. For telangiectasia-predominant photoaging, the typical pulse durations are 2.4 msec + 6.0 msec with a 10-msec delay between pulses. The delay between pulses is increased to 20–30 msec in darker skin types as they are more prone to thermal injury. Typical pulse durations are 2.4 msec + 4.0 msec (double pulse) with a 10-msec delay between the pulses for pigmentation predominant photoaging. Typical fluences range from 24–38 J/cm<sup>2</sup> again related to the sensitivity of the skin and the degree of epidermal cooling. To minimize non-specific epidermal damage, the crystal is placed on a layer of ice-cold clear gel 2–3 mm in thickness for non-cooled crystals (floating technique). When using the Quantum IPL with a thermoelectrically cooled crystal, a thin layer of gel is used with the crystal resting directly on the skin, with the crystal at maximal cooling (direct contact technique).

Studies on the treatment of leg telangiectasia and poikiloderma of Civatte have proven the efficacy as well as limitations of IPL technology. Few studies have evaluated IPL efficacy purely on facial telangiectasia. Most studies with the IPL comment on its photorejuvenation effects that include elimination of lentigines, reduction of pore size, and minimization of fine wrinkles in addition to treatment of telangiectasia (Figure 54.5). Results from studies evaluating the IPL's efficacy for treating telangiectasias have been consistently promising. Bitter reported that the IPL used in "FotoFacial settings" produced > 75% improvement in telangiectasia in 38% of patients and > 50% reduction in telangiectasia in 70% of patients (25). Tanghetti performed a split-face randomized study comparing the efficacy of PDL versus IPL for the treatment of facial telangiectasias based on the Telangiectasia Grading Scale (TGS); she found that for both devices the mean TGS score was 3.3 at 3-month follow-up, demonstrating that IPL may be as effective as PDL (26).





**Figure 54.5** Poikilodermatous type of photoaging of the chest including telangiectasias and pigmentation. (a) Pretreatment of chest and neck areas. (b) Following one IPL treatment. Note the disappearance of cherry hemangiomas on the right side of the chest.

Another IPL device (Ellipse Flex, Danish Dermatologic Development, Hoersholm, Denmark) was used in 27 patients with facial telangiectasia. This IPL has a lower cut-off at 555 nm as well as an upper cut-off filter at 950 nm with a median wavelength at 705 nm delivered through a 10 x 48 mm crystal light guide. Fluences required to produce a slight bluing of the vessels ranged 13–22 J/cm<sup>2</sup>. Pulse durations were 10 msec for vessels < 0.4 mm in diameter, and pulse durations of 15 and 30 msec were used for larger vessels. Patients received from 1–4 treatments with an average of 2.54 treatments. Seventy nine percent of patients had greater than 50% clearing with 38% having 75 to 100% clearance (27).

The development of the short pulse-long pulse protocol utilizing 2.4–3 msec and 7 msec pulses separated by a 10–20 msec delay employing the 560-nm or 570-nm filter has yielded the best results for leg veins using the IPL device (28). By combining a shorter pulse (2.4–3 msec) with a longer pulse (7–10 msec) it is theoretically possible to ablate smaller and larger vessels overlying one another in the dermis. Smaller, more superficial vessels absorb the shorter pulses more selectively, while the longer pulses are absorbed by the larger diameter, deeper vessels. New contact epidermal cooling devices improve treatment results by allowing larger fluences with less risk to the epidermis.

### THE ROLE OF COOLING

The concept of cooling the skin in an effort to protect the epidermis during laser treatment of dermal targets was first studied by Gilchrist, who incorporated the use of ice prior to argon laser treatment of port wine stains (29). Skin cooling during skin laser therapy offers multiple benefits, including cooling and protecting the epidermis, preventing other collateral dermal damage, and also reducing the discomfort associated with treatment. Cooling is especially critical in the treatment of larger telangiectasia due to the high fluencies required for efficacy. By cooling the skin, collateral injury is limited.

Several cooling modalities have been used including water-cooled chambers applied directly to the skin through which the laser beam is directed, cooling coupling gels, and refrigerated spray cooling devices. Preliminary results suggest

that cooling helps to spare epidermal damage, hence allowing use of higher fluencies and yielding more damage of the targeted vessels and achieving a greater degree of clearing per treatment (30,31). Cooling has a minor role in the treatment of pigmented lesions.

### PIGMENTED LESIONS

Pigmented lesions are another common manifestation of photodamage for which patients often present to dermatologists for treatment. Sun damage induces pigmentation changes in several manners. In the development of ephelides (freckles), the pattern of melanin deposition is altered. Ephelides classically occur in a photodistributed pattern, favoring the face, shoulders, and extensor arms, and darken with increased sun exposure. Lentigos are the most common photoaging-associated pigmented lesions and occur due to UV-induced melanocyte proliferation. The treatment of ephelides and lentigos will be the focus of this section.

### Q-Switched Lasers

Several laser systems have been shown to be effective in the treatment of lentigines. These systems include the 510-nm PDL, the frequency doubled Q-switched (QS) Neodymium:Yttrium-Aluminum-Garnet 532 nm (QS 532 nm Nd:YAG) laser, the QS Ruby laser, and the QS Alexandrite laser (32) (Figure 54.6). As in most aesthetic procedures, the risk of adverse effects is an important consideration. Dark-skinned patients have a higher epidermal melanin content and are more likely to develop complications such as hyperpigmentation. Studies of the use of Q-switched lasers in dark-skinned patients have indicated that the risk of post-inflammatory hyperpigmentation (PIH) is approximately 10% to 40% (33).

QS ruby and QS Alexandrite wavelengths are well absorbed by melanin. The greater depth of penetration can be a disadvantage because there is a potential for permanent follicular melanocytic damage causing leukotrichia when a high fluence is used. When QS lasers have been compared to long pulsed lasers, interesting findings regarding optimal efficacy and adverse effects have been shown. An in vivo study



**Figure 54.6** Solar lentigo. (a) Before treatment. (b) Following one treatment with Q-switched Ruby laser at 4 J/cm<sup>2</sup>.

of 34 patients compared a QS 532-nm Nd:YAG laser to a long pulse 532-nm Nd:YAG laser (34). Results showed that the long pulse 532-nm laser (6.5 J/cm<sup>2</sup>, 2-mm spot size, 2-msec pulse duration) resulted in a lower risk of PIH when used to treat lentigines in Asians.

### Q-Switching versus Millisecond Pulse Durations

QS lasers generate high-energy radiation with very short pulse duration. This produces intense energy that leads to a rapid rise in temperature (one thousand degrees Celsius) within the target subcellular chromophore. As the laser pulse duration is shorter than the thermal relaxation time of the target, a temperature gradient develops between the target and its surrounding tissue (35). When the temperature gradient collapses, it generates localized shockwaves causing the fragmentation of its targets. This photomechanical reaction leads to melanosome disruption that occurs after nanosecond pulse durations of Q-switching (36).

### Picosecond Lasers

Very recently it has been recognized that picosecond lasers, which were developed for tattoo removal, can be quite effective for the treatment of photo-induced hyperpigmentation (37). The pulse duration of a picosecond is at a minimum ten times shorter or quicker than that of the QS lasers. This faster pulse duration results in enhanced melanosome destruction. The wavelengths of light that are available in picosecond include 532 nm, 755 nm, and 1064 nm; however, it is the 755 nm that has been used for treatment of hyperpigmentation. Weiss et al have reported that adding a focused lens array which divides the picosecond 755 nm into 140 microbeams shows significant improvement for photoaging and fine wrinkles, based on physician and patient assessment (Weiss et al, LSM 2015, in press). This study resulted in FDA clearance for wrinkles, and results showed that a 755-nm picosecond laser can be used to treat solitary, discrete solar lentigines to diffuse dyspigmentation. Picosecond 755-nm laser coupled with a focused lens array achieves significant improvement for photoaging with less downtime and less pain than other modalities. Figure 54.7 shows one of the patient results from the study (Weiss et al, LSM 2015, in press).

### IPL

Photothermal effects, such as those produced by the IPL, were initially considered more efficacious when treating lentigines. Some promoted the concept that the photomechanical effect of QS lasers was undesirable for lentigines and this has been confirmed by a number of investigators (38). Use of IPL for photoaging in Asians has been shown to increase collagen and decrease melanin without significant adverse effects in the treatment areas and relatively high patient satisfaction rates (39). There have been few reports of IPL-induced long term hyperpigmentation (40,41), although temporary hypopigmentation is a well-recognized side effect. To minimize risks of scarring, it is important to select a pulse duration that is shorter than the thermal relaxation time of the epidermis. The epidermal relaxation time is estimated to be approximately 10 msec if the epidermal thickness is 100 µm, so that pulse durations of less than 10 msec with IPL are preferred for pigmented lesions (42).

### SELECTING THE LASER

When treating epidermal pigmented lesions such as lentigines, patients should be informed about advantages and disadvantages of each mode of treatment. IPL typically results in little downtime and may also improve rhytids. Lasers, however, may be the preferred choice for patients in terms of fewer treatments and cost effectiveness. Test areas with two different devices may be performed. If a test spot clearance is satisfactory, without development of PIH, then it is best to use the device that performed best. Patients are advised that clearance may take 1 to 4 sessions. If PIH develops at any time during the treatment phase, treatment is stopped, hydroquinone or an equivalent is applied daily, and the patient is followed until resolution of PIH occurs. Typically we use IPL if multiple pigmented lesions are scattered around the face, but QS Ruby if a few isolated lentigines are targeted.

### PRE-TREATMENT AND POST-TREATMENT CONSIDERATIONS

One cannot overemphasize the importance of pre-treatment skin preparation to patients planning to undergo laser or light





**Figure 54.7** 56 year old patient with significant photoaging, (a) Before, (b) Results after four treatments with 755 nm using FOCUS handpiece, 5000 pulses, fluence of 0.57 J/cm<sup>2</sup> using a 6-mm handpiece.

treatment for pigmented components and vascular components of photoaging. The use of topical bleaching agents such as hydroquinone, vitamins C and E, and UVA/UVB sunscreens of SPF 30 or higher should be initiated 2 weeks prior to the laser/IPL surgery and then resumed 5 to 7 days post-treatment or when epidermal crusting resolves. Treatment should continue for at least 6 months after a course of treatment. Use of sunscreens should become a lifelong habit.

## SUMMARY

Treatment of vascular and pigmented lesions in adults can be very successful. Practically all laser devices available in the visible light range, except for red, produce good results on facial vascular lesions. Q-switching is an important tool for treatment of pigmentation. Knowledge of the lesion type and differences in response by size and location can assist in selecting the procedure most likely to achieve successful results. Wavelengths of lasers may be fine-tuned for size or color of individual telangiectasias. Although electrocautery is used frequently, a more selective method such as laser or IPL is usually a better choice, with far less risk of scarring. Larger cavernous lesions may require deeper penetration of 1064 nm (infrared) wavelengths used cautiously. The benefit of IPL in photoaging is the smoothing of skin and reduction of irregular pigmentation in addition to treatment of vascular lesions. Considering the skin type of the patient, the nature of the lesion, and performing test areas when indicated can ultimately lead to an effective and safe treatment of pigmented and vascular lesions due to photoaging.

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## Nonablative Laser Rejuvenation

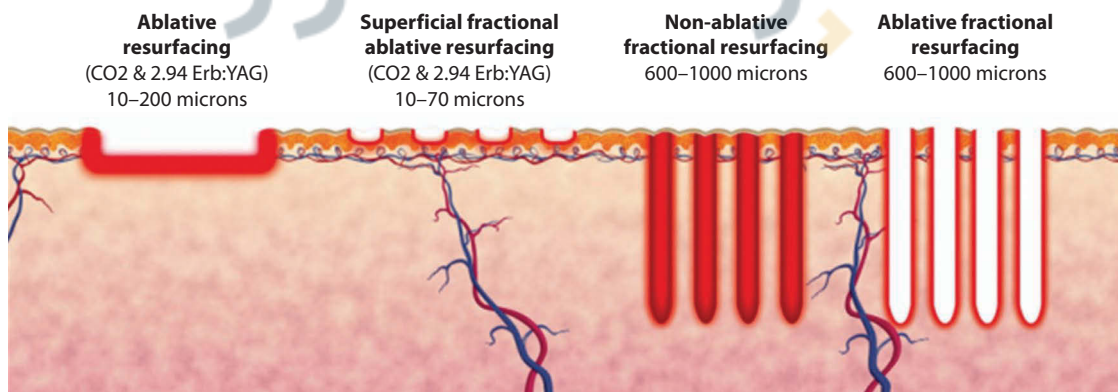
Christian R. Halvorson, Karen L. Beasley, and Robert A. Weiss

### BACKGROUND

The development of fractional photothermolysis is one of the most important discoveries in the field of laser medicine and surgery. This concept has revolutionized laser skin resurfacing. Previously patients could only significantly enhance their skin through fully ablative lasers, like the 10,600-nm carbon dioxide (CO<sub>2</sub>) or the 2940-nm erbium-doped yttrium aluminum garnet (Er:YAG) lasers. These laser treatments required at least 1 to 2 weeks of recovery depending on the depth of resurfacing and the type of laser utilized. With deeper resurfacing procedures, patients could experience considerable discomfort and side effects. Results could be exceptional but patients soon became aware of the potential disadvantages of aggressive procedures. Besides the potential side effects of infection or permanent scarring, many patients who were treated with deep CO<sub>2</sub> laser resurfacing experienced prolonged erythema of the skin for 6 months to a year. Many also developed unexpected permanent hypopigmentation of their treated skin (1,2). In addition, patients also became aware of the stark contrast between their beautiful resurfaced facial skin, which was now adjacent to their severely sun-damaged neck and chest. Fully ablative laser resurfacing was fraught with severe complications when used off the face. Darker skin types were not candidates for the procedure. With these limitations, traditional deep ablative resurfacing began to decrease in popularity.

Nonablative infrared lasers were developed in hopes of remodeling and rejuvenating the skin with fewer side effects. Infrared lasers, like the 1320-nm neodymium:yttrium aluminum garnet (Nd:YAG), 1450-nm diode and 1540-nm erbium:glass lasers were developed. These lasers demonstrated modest improvements in fine rhytides and acne scars (3–5). Additionally, these lasers were able to produce results in patients with darker skin types (6). But the results from these nonablative lasers often paled in comparison to traditional ablative procedures, leading patients and physicians to seek more efficacious treatments and resulting in the development of newer, more effective lasers and delivery systems. A comparison of types of ablative and nonablative lasers is shown diagrammatically in Figure 55.1.

Despite the more limited efficacy of nonablative treatments, it is important to consider the risk-benefit ratio of a laser procedure and the potential for post-procedure down time. In our current culture, many patients do not want to take the risk of a serious side effect in exchange for a cosmetic improvement. Furthermore, many patients have busy work or social schedules that do not allow for extended recovery times. Nonablative fractional laser (NAFL) resurfacing allows for real results with less side effects and downtime. This technology also allows for the treatment of darker skin types and can successfully treat a multitude of skin conditions and body areas. The disadvantages, compared with ablative resurfacing, are the need to return for multiple treatments and the decrease in efficacy.



**Figure 55.1** Schematic representation of laser impact from several lasers: (a) Ablative resurfacing (CO<sub>2</sub> and 2.94 Erb:YAG) 10–200 microns; (b) superficial ablative resurfacing (CO<sub>2</sub> and 2.94 Erb:YAG) 10–200 microns; (c) non-ablative fractional resurfacing (thermal only without vaporization) 600–1000 microns; (d) ablative fractional resurfacing, 600–1000 microns. (From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.)

## FRACTIONAL PHOTOTHERMOLYSIS

During fractional photothermolysis, the laser creates microscopic noncontiguous columns of thermal injury in the dermis, referred to as microthermal zones (MTZ) (7) (see Figure 55.2). Each MTZ is surrounded by a limited zone of heat-shocked tissue as well as a larger zone of healthy unaffected tissue. The MTZ allows for transport and extrusion of necrotic dermal content through the compromised dermal epidermal junction (8). The precise nature of the coagulated tissue allows for quicker healing and recovery (7–9). Immunohistochemical studies have shown increased collagen III production around treated MTZs by 7 days and replacement of damaged collagen in the MTZs by 3 months post treatment. In addition, histology also reveals that there is a localized, well-controlled melanin release and transport mechanism using microscopic exudative necrotic debris (MEND) as the vehicle for pigmentary redistribution (9). In other words, NAFL resurfacing improves pigmentation by shuttling the melanin through the MENDs where it is exfoliated off the skin. The initial paper by Manstein et al. also reported that there was little to no pigmentary alteration in dark skinned patients when utilizing laser treatments with low to medium MTZ densities per treatment (7). This essential combination of creating a precise injury that has an enhanced healing rate coupled with the ability to build collagen and redistribute pigment is the hallmark of fractional photothermolysis.

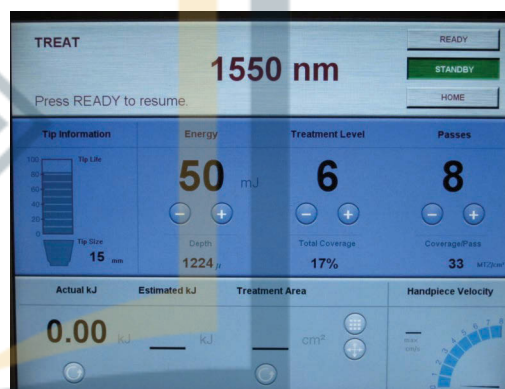
## NONABLATIVE FRACTIONAL LASERS (NAFL)

Two main NAFL families currently dominate the world laser market: the Solta family of nonablative fractional lasers (Solta Medical, Inc., Hayward, CA) and the Palomar family of nonablative fractional lasers, which were acquired by Cynosure in 2013 (Cynosure, Inc., Westford, MA). Both families contain a variety of effective nonablative resurfacing lasers. The laser wavelengths used in both families are in the infrared region of the light spectrum and utilize water as their tissue target or chromophore. The families differ by using slightly different wavelengths in their respective lasers. But the most striking difference between the two families is the manner in which the laser energy is delivered to the skin. Besides these two main families, there is a multi-wavelength nonablative fractional laser also by Cynosure,

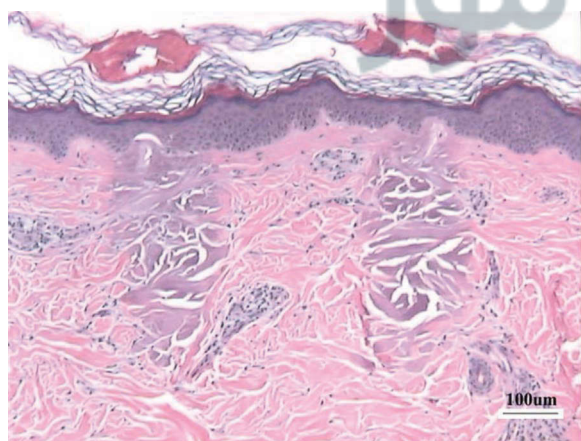
a fractional 910-nm laser by Syneron Medical Ltd. (Irvine, CA), a fractional Q-switched 1064-nm by Alma Lasers (Buffalo Grove, IL), and a new 1440-nm home-use device by Tria Beauty, Inc. (Dublin, CA). There are also other NAFLs that are marketed outside the United States or that are currently in development.

## THE SOLTA FAMILY OF NAFL

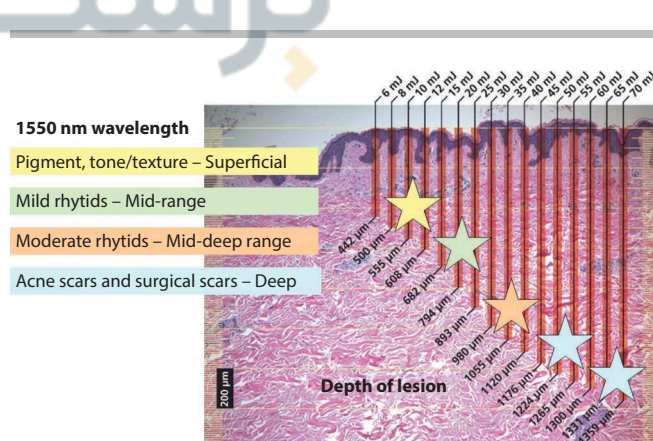
The Solta family consists of the Fraxel division of laser devices and the Clear + Brilliant™ laser system. The Fraxel division includes the Fraxel 1550-nm laser, the Fraxel DUAL 1550/1927-nm laser, a stand-alone Fraxel 1927-nm laser, and the Fraxel refine 1410-nm laser, which is mostly marketed outside of the United States. All of the Fraxel laser systems have an interface that allows for a customized treatment by controlling the pulse energy, treatment level, and number of passes (see Figure 55.3). The pulse energy is delivered in millijoules (mJ) and it controls the depth of penetration or the depth of the MTZ. Different depths of the skin can be treated for different indications (see Figure 55.4). The treatment level (TL) is the percentage of skin treated or



**Figure 55.3** Treatment screen of a 1550-nm nonablative fractional laser. (From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.)



**Figure 55.2** Microthermal zones of damage from a 1550-nm nonablative fractional laser. (From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.)



**Figure 55.4** Approximate depths of thermal impact by fluence. (From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.)



covered by MTZs during one treatment session and it controls the aggressiveness of treatment. The number of passes is also set during the procedure. A pass is defined as a single unidirectional motion over the skin that lays down one row of MTZs. On average, 6–8 passes are performed. Additional passes can be applied to individual areas of scarring or deep lines. The total energy delivered per treatment session is constant regardless of the number of passes. For example, the quantity of energy per pass decreases as the number of passes increase. Alternately, the quantity of energy per pass increases as the number of passes decrease. To increase patient comfort, a higher number of passes with less density of MTZ per each pass can be performed. Typically, passes are delivered by alternating horizontal and perpendicular passes within one cosmetic unit at a time. Before a laser treatment series begins, the skin surface can be measured and an estimated energy total kilojoule (kJ) of the treatment can be computed by the laser treatment screen according to the set treatment parameters.

All of the Solta lasers have a continuous motion handpiece, which is equipped with rollers. The Fraxel laser treatment tip comes in two different sizes, 15 mm and 7 mm. Smaller cosmetic units like the eyelid, nose, or lip can be treated with the smaller tip (see Figure 55.5). The Clear + Brilliant system™ is a nonablative fractional 1440-nm laser. It is based on the technology of the Fraxel lasers and has a similar, yet more simplified interface. In all the NAFL devices by Solta Medical, the handpieces should be positioned perpendicular to the skin and should not be lifted while the footswitch is depressed. Handpiece velocity is also measured on the laser during treatment. Passes should be smooth and controlled and stay within the recommended velocity levels. If the treatment passes are delivered too fast, the laser microdots become oval rather than round and therefore the energy delivered may be reduced by up to 50%, which leads to undertreatment. As with the stamping technique of delivering fractional laser, the pulses delivered by the rolling technique may not be uniformly distributed. Some pulses overlap while others are adjacent and some are farther apart.

### FRACTIONAL 1550-NM LASER

The Fraxel 1550-nm laser, which is the most extensively studied nonablative resurfacing laser, was introduced in 2006. It is indicated for moderate skin damage resurfacing for periorbital

wrinkles, pigmented lesions, dyschromia, scars, melasma, and actinic keratoses. It can deliver laser energy ranging from 4 to 70 mJ. Its treatment levels range from 5% to 48% coverage. Maximum absorption depth into the skin is estimated to be 1400–1500 µm deep. As discussed previously, different depths of the skin can be treated for different indications and treatment levels can be adjusted to determine the aggressiveness of treatment. For example, to target a deep scar with the Fraxel 1550-nm laser, a higher energy like 50–70 mJ would be selected. An example of an aggressive treatment level would be 12 and would cover 35% of the facial skin in one treatment. In patients with darker skin types (Fitzpatrick types IV–VI), the density must be delivered at a lower treatment level, such as 4%–6% or 11%–17% coverage, to reduce the chances of postinflammatory hyperpigmentation (PIH). See recommended treatment settings in Table 55.1.



**Figure 55.5** Tip for rolling nonablative fractional device. This must be replaced every 5–10 treatments. (From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.)

**Table 55.1** Fraxel 1550-nm Laser Treatment Settings

Indication	Mild-Moderate Parameters	
	Pulse Energy (Depth)	Treatment Level (Coverage)
<b>Fitzpatrick Skin Phototype I–III</b>		
General Resurfacing (pigmented lesions, textural irregularities, fine lines)		
Face	10–25 mJ	4–8 (11%–23%)
Eyelids (within orbital rim)	10–20 mJ	4–7 (11%–20%)
Off-face	10–25 mJ	4–7 (11%–20%)
<b>Deep Wrinkles, Acne Scars</b>		
Face	25–70 mJ	5–9 (14%–26%)
Off-face	25–40 mJ	4–8 (11%–23%)
<b>Melasma (evaluate patient after each treatment, 2–3 treatments)</b>		
Face	6–15 mJ	5–8 (14%–23%)
Surgical scars	40–70 mJ	5–8 (14%–23%)
<b>Fitzpatrick Skin Phototype IV–VI</b>		
General Resurfacing (pigmented lesions, textural irregularities, fine lines)	10–25 mJ	3–7 (9%–20%)

(Continued)

**Table 55.1** Fraxel 1550-nm Laser Treatment Settings (*Continued*)

Indication	Mild-Moderate Parameters	
	Pulse Energy (Depth)	Treatment Level (Coverage)
Eyelids (within orbital rim)	10–20 mJ	3–6 (9%–17%)
Off-face	10–25 mJ	3–6 (9%–17%)
<b>Deep Wrinkles, Acne Scars</b>		
Face	25–70 mJ	4–7 (11%–20%)
Off-face	25–40 mJ	4–6 (11%–17%)
<b>Melasma (evaluate patient after each treatment, 2–3 treatments)</b>		
Face	6–15 mJ	3–7 (9%–20%)
Surgical scars	40–70 mJ	4–7 (11%–20%)

Source: From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.

### FRACTIONAL 1550-NM AND 1927-NM LASERS

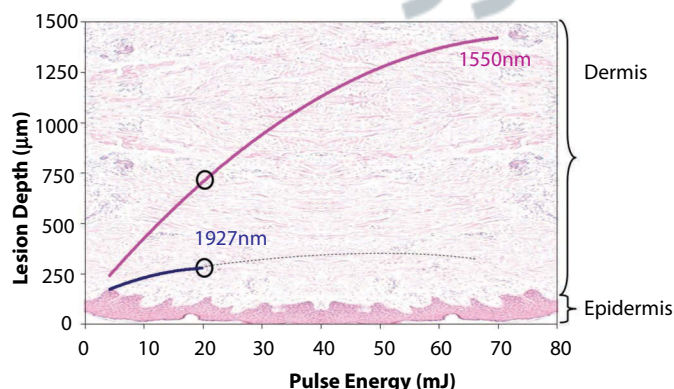
Fraxel DUAL 1550/1927 was introduced in October 2009. Both lasers are housed in the same platform and function independently of each other so that a fully 1550-nm or 1927-nm treatment may be performed. Additionally, both lasers can be layered on top of each other for a combination treatment. The thulium 1927-nm wavelength has a higher absorption coefficient for water than the 1550-nm wavelength so it has a greater ability to target the epidermis. Figure 55.6 illustrates the absorption of the two wavelengths. Energy fluences for the 1927-nm laser range from 5 to 20 mJ and the TL ranges from 20 to 70% coverage. It has a maximum depth of penetration of 200  $\mu$ m into the skin. Therefore, this wavelength is more effective in treating epidermal processes, like actinic keratoses. An average energy and treatment level for a patient with a Fitzpatrick skin type I–III would be 10–20 mJ and a TL of 3–6 that corresponds to 30%–45% coverage. See recommended treatment settings in Table 55.2. In 2011, the Fraxel 1927-nm laser became available in a stand-alone laser platform. The 1927-nm wavelength is currently FDA-approved in the treatment of actinic keratoses.

The 1550-nm and the 1927-nm lasers can be combined for a “DUAL” resurfacing treatment. When combining the wavelengths, 2–4 passes of 1550-nm are performed over the treatment

area to target the dermis and then 2–4 passes of 1927-nm are performed to provide an epidermal treatment. Laser energies and treatment levels are set lower than if the lasers are used separately. Our experience has taught us to use caution when treating with both wavelengths on the same day. Recovery times and discomfort may be greater than if the lasers were used individually. See recommended treatment settings in Table 55.3.

### FRACTIONAL 1440-NM LASER

The Clear + Brilliant system™ is a fractional diode 1440-nm laser that was launched in May 2011. It is indicated for general skin resurfacing. The laser has a fixed spot size of 140  $\mu$ m. Energy can be delivered on a low (4 mJ), medium (7 mJ), or high (9 mJ) level. The depth of penetration ranges from 280 to 390  $\mu$ m depending on the energy level used. The low level setting corresponds to 4% coverage, the medium level corresponds to 7% coverage, and the high level corresponds to 9% coverage. It utilizes a disposable treatment tip per treatment. It can be used in all skin types, although we have experience with other 1440-nm devices and find that the risk of postinflammatory hyperpigmentation is higher with 1440-nm than with 1540–1500-nm devices. It is marketed as a preventative laser treatment and it provides a superficial treatment with minimal down time. It is marketed to medical spas primarily.



**Figure 55.6** Comparison of thermal impact depths for 1550-nm vs. 1927-nm lasers. (From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.)

### THE CYNOSURE ICON (FORMERLY PALOMAR) FAMILY OF NAFL

With the acquisition of Palomar, the Cynosure family (Cynosure, Inc., Westford, MA) of nonablative fractional lasers (NAFL) now includes primarily a 1540-nm erbium:glass laser, with different sets of delivery lenses. These devices are based on the stamping mode of delivering fractional energy with the microdots of energy coming through microlens arrays. The latest generation of Cynosure's NAFL is available through the Palomar Icon™ Aesthetic System platform, which has four unique 1540-nm handpiece microlenses: the original 10 mm and 15 mm microlenses, the 15 mm XF Microlens, and the XD Microlens. Figure 55.7 illustrates the Icon 1540-nm interface. Furthermore, the new XD Microlens can be used on any of the previous generation 1440-nm and 1540-nm fractional handpieces with the appropriate software and factory calibration.

The original fractional microlenses are composed of a microlens array that delivers a lattice of optical microbeams

**Table 55.2** Fraxel 1927 nm-Laser Treatment Settings

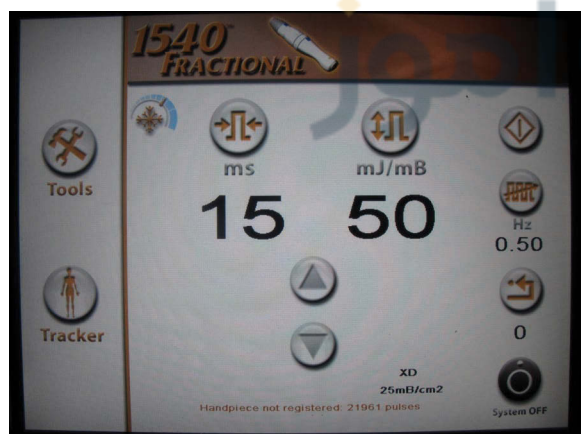
Indication	MODERATE PARAMETERS	
	Pulse Energy (Depth)	Treatment Level (Coverage)
<b>Fitzpatrick Skin Phototype I–IV</b>		
<b>General Resurfacing</b>		
Face	5–20 mJ	1–5 (20%–40%)
Eyelids (within orbital rim)	5–20 mJ	1–4 (20%–35%)
Off-Face	5–15 mJ	1–4 (20%–35%)
<b>Fitzpatrick Skin Phototype I–III</b>		
<b>Actinic Keratosis</b>		
Face	10–20 mJ	3–5 (30%–40%)
Off-face	10–20 mJ	2–4 (25%–35%)

Source: From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.

**Table 55.3** Fraxel 1550-nm /1927-nm DUAL Laser Treatment Settings

1550 nm and 1927 nm treatment parameters					
Indication	Moderate Parameters: 1550 Nm		Moderate Parameters: 1927 Nm		Total Coverage
	Pulse Energy (Depth)	Treatment Level (Coverage)	Pulse Energy (Depth)	Treatment Level (Coverage)	
<b>Fitzpatrick Skin Phototype I–III</b>					
<b>General Resurfacing</b>					
Face	10–25 mJ	3–5 (9%–14%)	5–20 mJ	1–3 (20%–30%)	(30%–40%)
Eyelids (within orbital rim)	10–20 mJ	2–4 (7%–11%)	5–20 mJ	1–3 (20%–30%)	(30%–35%)
Off-face	10–25 mJ	1–3 (5%–9%)	5–20 mJ	1–2 (20%–25%)	(25%–35%)
<b>Fitzpatrick Skin Phototype IV–VI</b>					
<b>General Resurfacing</b>					
Face	10–25 mJ	1–4 (5%–11%)	5–20 mJ	1–3 (20%–30%)	(25%–35%)
Eyelids (within orbital rim)	10–20 mJ	1–3 (5%–9%)	5–20 mJ	1–3 (20%–30%)	(25%–35%)
Off-face	10–25 mJ	1–3 (5%–9%)	5–20 mJ	1–2 (20%–25%)	(20%–30%)

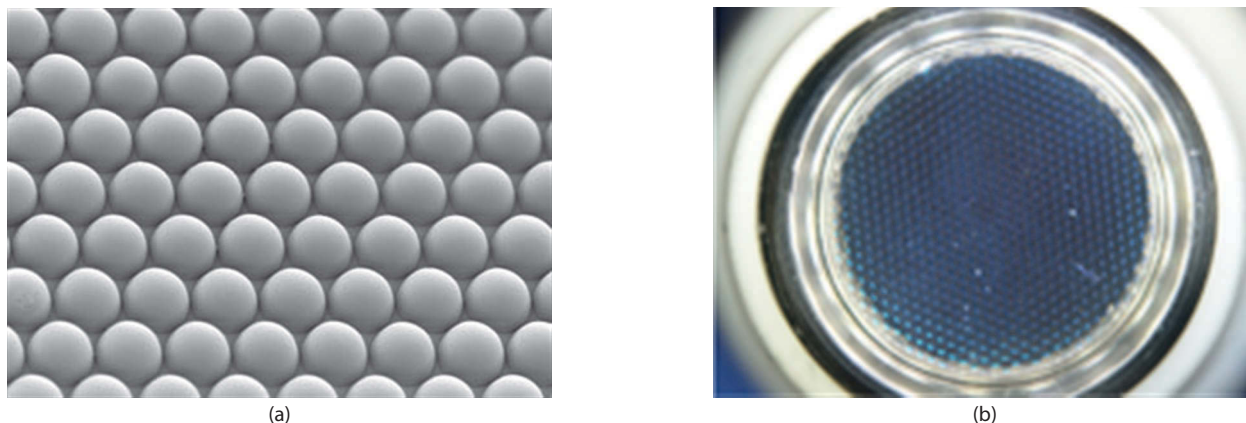
Source: From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.



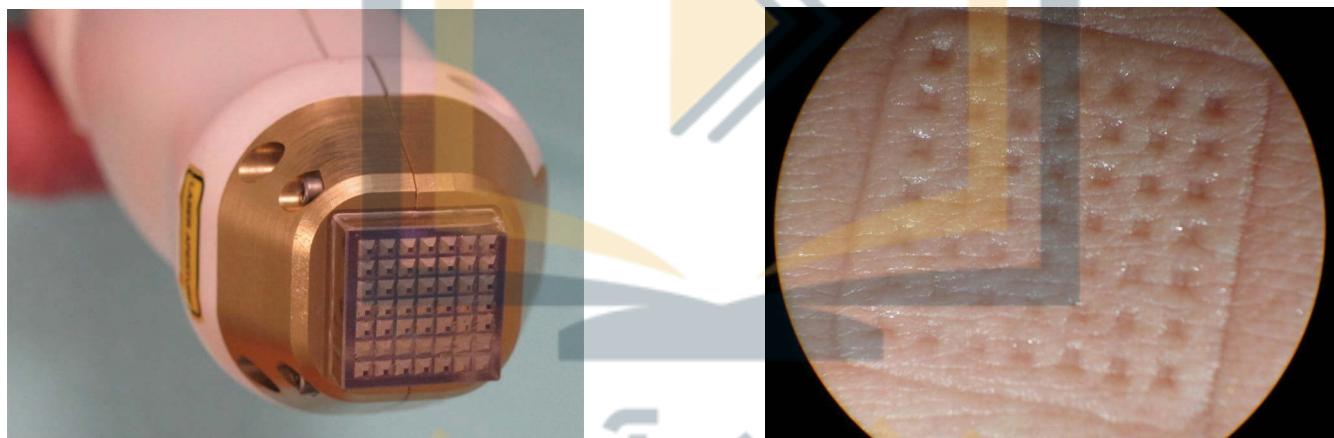
**Figure 55.7** Treatment screen for a 1540-nm fractional laser. Here each pulse is 15 ms and each thermal zone is 50 mJ/cm<sup>2</sup>. Coverage is determined by the percent overlap of each stamp. (From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.)

that create microdenatured columns in the skin. The microlens is responsible for taking a single beam of laser energy and separating it into smaller laser beams at a predetermined pitch. See Figure 55.8a/b for photos of the original microlens. The XD Microlens is a chilled 12 × 12 mm square sapphire contact window that is composed of 49 micro-compression pins each co-aligned with a microbeam. Figure 55.9 depicts the XD Microlens tip, which allows for much deeper (XD = extra deep) penetration of energy by compression of water from the upper layers of dermis. This new microlens is used with manual compression into the skin to achieve deeper penetration of laser energy into the dermis. Figure 55.10 illustrates the effect on skin after compression with the XD tip. The act of compression displaces water into interstitial spaces of the skin. With less water to absorb, the scattering of laser light is reduced which enables increased absorption of light by deeper targets. The compression into the skin not only enhances beam penetration, but it also enhances skin cooling due to less heating in the epidermis. Better cooling means decreased epidermal temperature and injury. Clinically, this translates into fewer side effects, less downtime, and a more comfortable treatment. The XF optic provides higher coverage per pulse compared to the original optics, allowing faster treatment times, similar to 1440-nm but with the added benefit of increased depth due to the increased penetration of the 1540-nm wavelength.





**Figure 55.8** (a) Micro lens array for a stamped 1540-nm nonablative fractional laser; (b) 15 mm handpiece lens array. (From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.)



**Figure 55.9** XD optic showing optical prongs to displace water out of tissue. (From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.)

**Figure 55.10** XD optical prong pattern after 30 seconds of compression on the skin. (From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.)

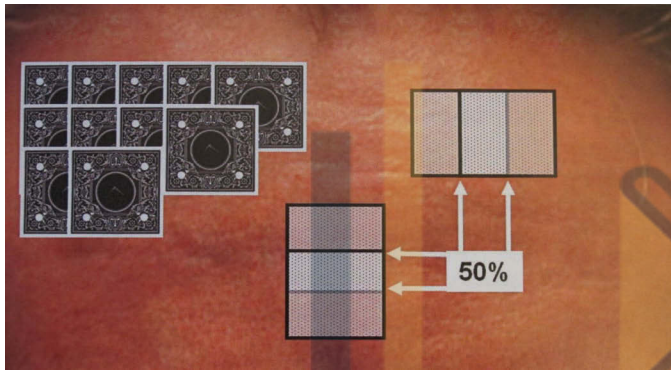
These laser treatments are customized by controlling the microbeam energy, the amount of overlap between pulses, and the number of passes. The pulse energy is delivered in millijoules per microbeam (mJ/mb) and it controls the depth of penetration or the depth of the MTZ. Energies should be adjusted for the skin condition being treated. Lower energies are utilized for more superficial conditions and higher energies, which penetrate deeper, are utilized for deeper indications. The percentage of skin treated or covered by MTZs during one treatment session is determined by the amount of overlapping passes performed with the stamping technique of the microlens. More overlapping and passes increase the aggressiveness of treatment (total surface coverage). Additionally, use of manual compression with the standard and XD Microlens is suspected to increase penetration of laser energy into the skin.

Regardless of tip or wavelength utilized, the laser procedure is delivered in a stamped method. Multiple stamps are delivered onto the skin with varying amounts of overlap. Rows of stamps are performed in a single cosmetic unit at a time. The rows may be overlapped from 0% to 50% depending on the wavelength, the microlens used, the desired total coverage, and the rate at which the coverage is administered. One pass is completed when the entire cosmetic unit is covered by the stamps. Passes are then alternated between perpendicular and horizontal directions. This is also known as the tile cascade method (see Figure 55.11). One to five passes are typically performed, depending on the total desired coverage. It is important to keep the treatment tip in full continuous contact with the skin during each laser pulse. As with the roller technique for delivering fractionated laser pulses, when overlapping

stamped pulses you also produce laser impact sites, which are not evenly distributed on the skin surface.

### FRACTIONAL 1540-NM LASER

The 1540-nm original microlenses utilized in the Palomar StarLux™ and Artisan™ and now the Icon laser platforms are available in two sizes: a flat, circular 10 mm tip with a microbeam density of 100 microbeams/cm<sup>2</sup> and a flat, circular 15 mm tip with a microbeam density of 320 microbeams/cm<sup>2</sup>. The pulse durations are adjustable to 10 or 15 milliseconds (ms). The 10 mm tip can deliver energies between 14–70 mJ/mb, with a smaller number of higher energy laser beams that penetrate deeper with a wider coagulation column diameter



**Figure 55.11** Tile cascade pattern for a stamping nonablative fractional laser showing 50% overlap pulse to pulse. (From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.)

compared to the 15 mm tip. The 15 mm tip can deliver energies between 3–15 mJ/mb. The standard 10 mm tip averages a 725  $\mu$ m depth of penetration while the 15 mm tip averages a 600  $\mu$ m depth of penetration. See Table 55.4 for recommended treatment settings.

The Palomar Icon Aesthetic System can use a standard 10 mm or 15 mm handpiece, but additionally has the 12  $\times$  12 mm XD Microlens (25 microbeams/cm<sup>2</sup>) and the 15 mm XF Microlens (115 microbeams/cm<sup>2</sup>). The XD Microlens delivers laser energies from 20–70 mJ/mb. The XF Microlens emits energies from 6–60 mJ/mb. The XF Microlens provides higher coverage per pulse compared to the original optics allowing fast treatment coverage comparable to the 1440-nm with the added benefit of increased depth. Average depth of penetration of the microbeam is approximately 750  $\mu$ m for the XF and 1060  $\mu$ m for the XD Microlens. Pulse stacking has been studied with the XD Microlens. For example, three stacked pulses with the Lux 1540 XD tip have reached depths of 1900  $\mu$ m into the dermis. Settings for the XD Microlens are typically from 40–70 mJ/mb. Three to six passes with overlap ranging from 10%–50% depending on the desired total coverage and clinical condition treated can be utilized. See Table 55.5 for recommended treatment settings.

### THE MULTI-WAVELENGTH FRACTIONAL 1440/1320-NM LASER

The Affirm™ Multiplex laser (Cynosure, Inc.) is a fractional laser that emits a combination of 1440-nm and 1320-nm Nd:YAG wavelengths. The Multiplex technology is defined as the sequential emission of two laser wavelengths using one laser fiber. It has a diffractive lens array that consists of 1000 diffractive elements per cm<sup>2</sup> to affect more surface area per single pulse. The laser uses combined apex pulse (CAP) technology. CAP technology reportedly delivers apex pulses of high-fluence

**Table 55.4** StarLux 1540-nm Laser Treatment Settings

	Superficial/Moderate Corrections	Deep Corrections
Handpiece (mm)	15	10
Millijoules per microbeam	6–15	25–50
Pulse width (ms)	5–10	5–10
Passes (20%–50% overlap)	2–5	2–4
Number of treatments	3–5	3–5
Treatment intervals (weeks)	3–4	3–4

Source: From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.

**Table 55.5** Icon 1540-nm Laser Treatment Settings

	15-mm tip	10-mm tip	XD Microlens	XF Microlens
Millijoules per microbeam	10–15	40–70	40–70	30–50
Pulse width (ms)	10–15	10–15	15	10–15
Number of passes (20%–50% overlap)	3–4	3–6	3–6	1–2
Number of treatments	2–5	2–5	2–5	1–2
Treatment intervals	3–4 weeks	3–4 weeks	3–4 weeks	1–3 months

Source: From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.

regions for collagen remodeling and low-fluence regions to stimulate collagen growth. The 1440-nm wavelength heats a column of tissue to a depth of 300  $\mu\text{m}$  and the 1320-nm wavelength penetrates deeper. Cynosure's Smart Cool™ air cooling system attaches to the handpiece to protect and cool the skin. Usually, topical anesthesia is not necessary. A 14 mm spot size is used; usually 2 passes are suggested with 15%–20% overlap. Four to six treatments spaced 3–6 weeks apart are necessary for improvement in superficial scars and photodamage. The 1440-nm laser has a maximum energy of 8 J and the 1320-nm laser has a maximum energy of 14 J. Typical treatment parameters for skin rejuvenation would be 2–3 J/cm<sup>2</sup> for the 1440-nm component and 6–10 J/cm<sup>2</sup> for the 1320-nm component.

## OTHER FRACTIONAL LASERS

A fractional Q-switched ruby laser (TattooStar FRx, Asclepion Laser Technologies, Jena, Germany) has been reported to improve melasma in Korean patients (12). A 1540-nm fractional erbium:glass laser (Mosaic, Lutronic Co., Ltd., Seoul, South Korea) has been studied in the treatment of male and female pattern hair loss and in skin rejuvenation (13–15). The 1540-nm and 1340-nm fractional lasers are available through the DEKA Dot platform (DEKA laser, Firenze, Italy). In the United States, there is also a fractional 910-nm laser by Syneron Medical Ltd. and a fractional Q-Switched 1064-nm by Alma Lasers, which has been shown to improve superficial rhytides (16). A home-use device (Tria Age-Defying Eye Wrinkle Correcting Laser, Tria Beauty, Inc., Dublin, CA) has also been recently FDA-cleared and is marketed for the treatment of multiple signs of facial aging. Prototypes of a fractional 1940-nm and a 1208-nm laser have also been studied.

## CLINICAL CONSIDERATIONS

Before initiating a NAFL treatment series, several clinical considerations need to be carefully examined. Specifically, the treating physician and support staff should carefully consult with each patient about the indications for treatment, expected results of treatment, and review the treatment process in detail.

Also, a medical history and physical examination must be performed to identify contraindications or impediments to treatment. Patient selection for NAFL resurfacing is very important. The ideal candidate has mild to moderate skin damage or discoloration. Those with severely damaged skin, deep acne scars/wrinkles, or extreme laxity will benefit more from an ablative fractional/fully ablative or surgical procedure. Patients must also be counseled to have realistic expectations of the treatment results. NAFL resurfacing yields slow gradual improvement over a series of treatments. Reduction of fine lines and scars occur but they are not completely removed. Definitive improvement in brown pigmentation and actinic keratoses can be seen but patients must be counseled that they may recur with continued sun exposure and time. Daily sun protection is necessary for all patients post-treatment. Also, the final results from treatment may take 3–6 months after the last treatment. This time is necessary to achieve the full effect of collagen remodeling from the NAFL. During the consultation, the treatment experience is also discussed in detail. Patients are instructed that a series of treatments will be necessary. Usually, the treatment can be accomplished over two to five sessions spaced 4–6 weeks apart, depending on the clinical indication. Topical anesthetic will be applied to the treatment area for approximately 60 minutes before the laser treatment and the treatment itself will take

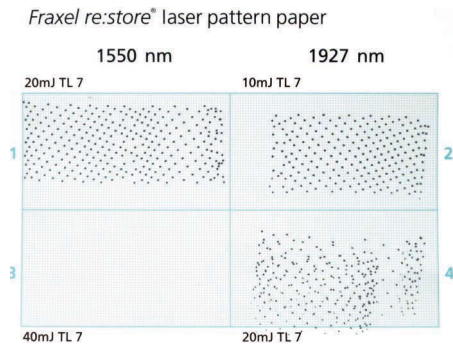
20–40 minutes depending on surface area treated. Cool air and cold ice rollers can be used to increase comfort during the treatment. A prickly heat sensation or a zap of heat can be felt on the skin depending on which NAFL system is used during the treatment. Pain levels appear to be roughly equivalent in treatment-naïve patients and those with a history of past laser treatments (17). Post-treatment, patients may feel like they have a sunburn. Redness and swelling should be expected initially. Skin dryness with a flaky dot appearance can last a few days up to 2 weeks depending on treatment intensity and also body area treated. During the consultation, side effects are discussed, a medical history is reviewed, and physical examination of the treatment area is performed. Contraindications include oral retinoid use within the last 6 months, predisposition to keloid formation or excessive scarring, and lesions that appear to be suspicious for malignancy in the treatment area. I would also include recent sun exposure or tanned skin, pregnancy, an active infection in the treatment area, or a medical condition that compromises healing time or predisposes to infection. Determining any history of herpes simplex virus (HSV) infection is a mandatory portion of the medical history as reactivation of the virus can occur with NAFL treatment. Pretreatment with an oral antiviral is recommended. For darker skin types or patients prone to hyperpigmentation, pre- and post-treatment with a hydroquinone bleaching cream is recommended and strict sun protection is discussed with all patients.

## TREATMENT PREPARATION

To prepare for NAFL treatment, patients are asked to remove all jewelry and makeup and to cleanse their skin with a mild cleanser. Consents are reviewed and signed. Premedication with an oral antiviral for patients with a history of HSV is documented. Pretreatment standardized photographs are taken. My practice primarily uses the Visia™ and IntelliStudio™ (Canfield Scientific, Inc., Fairfield, NJ) systems. The treatment area is wiped with 70% isopropyl alcohol and allowed to dry. A moderately thick (¼ inch) amount of a topical anesthetic is applied. We use a compounded topical anesthetic with 15% lidocaine and 5% prilocaine in an ointment base for the treatment of the face or localized body area. A two treatment area restriction should be considered when using such stronger, compounded topical anesthetic agents. Lidocaine overdose is possible and lidocaine toxicity has been reported during NAFL treatment (18). The fractional laser consensus panel recommends to limit the area to 300–400 cm<sup>2</sup> to minimize potential lidocaine toxicity (19). If larger skin surfaces are to be treated, a standard topical anesthetic cream can be utilized like lidocaine 5% cream (L.M.X.5, Ferndale Healthcare Inc., Ferndale, MI). The topical anesthetic should be blended into the perimeter of the anticipated treatment area to ensure comfort. After 60 minutes, the topical anesthetic is removed with a dry gauze pad. Use of eye protection for the patient, practitioner, and support staff is mandatory during the NAFL treatment. If the eyelid skin inside the orbital rim is to be treated, intraocular metal eye shields should be inserted. Handpieces are sanitized with Sani-Cloth® (Professional Disposables International, Inc., Orangeburg, NY) before each treatment. Laser system tests are performed to ensure a properly performing laser. A paper test strip is available with use with the Fraxel laser systems (see Figure 55.12).

When treating the patient with any NAFL, divide the treatment area into smaller cosmetic units. For example, the cosmetic units of the forehead, cheeks, nose, lip, and chin are treated as separate areas. These units can be customized to





**Figure 55.12** Test strip utilized with Fraxel laser systems.

the preferences of the individual practitioner. Completely treat one cosmetic unit at a time. Be careful not to overlap treatment between the individual cosmetic units that could cause over treatment. In my practice, we use forced cold air during the treatment, which has been shown to reduce pain but can also affect the thermal laser injury (20).

### POST-CARE ROUTINE

After the NAFL treatment is concluded, patients receive a light-emitting diode treatment (Gentlewaves, LLC, Virginia Beach, VA) to reduce the intensity and duration of post-treatment erythema (21). They are also treated with a thermal water spray (Avene Thermal Water, Pierre Fabre Dermo Cosmétique USA, Parsippany, NJ) to alleviate discomfort, and a physical sunblock is applied before they leave the office. The next treatment is scheduled in 3–6 weeks.

Gentle post-care at home is paramount. In my practice, we supply our patients with a skin care kit to be used during their laser treatment series. It consists of a gentle cleanser, non-occlusive moisturizer, thermal water spray, and a physical sunblock. Post-care instructions are reviewed verbally with the patient and they are also sent home with written instructions. The patient is instructed to cleanse the treated area twice a day with the gentle cleanser and immediately apply the gentle moisturizer. The broad-spectrum sun protection must be applied before exposure to sunlight and reapplied as necessary. Patients can also apply cool compresses or cold packs and spray their face with the thermal water spray as needed to help reduce swelling and discomfort. Narcotic pain medication is rarely necessary, but over-the-counter acetaminophen can be taken if needed. Patients should sleep with their head elevated for the first few nights to help diminish swelling. They should avoid vigorous exercise or activity while swollen or red. They should avoid smoking and excessive alcohol consumption. Strict sun protection must be observed using a broad-spectrum SPF of 30 or greater. Direct sun exposure should be avoided for 3 months post-treatment. Topical products and medications can usually be restarted 3–7 days post-treatment depending on the level of treatment and sensitivity of the patient. Those patients prone to PIH or who are being treated for melasma will restart a topical hydroquinone cream 1 to 4 days post-procedure.

Ablative and nonablative fractional lasers are increasingly being utilized for enhanced topical drug delivery. This concept can be utilized in post-treatment care, and in our practice we frequently apply a topical serum containing L-ascorbic acid, alpha tocopherol, and ferulic acid, as well as topical

corticosteroids and bleaching agents to take advantage of this opportunity for enhanced epidermal absorption.

### COMPLICATIONS

Complications are a possibility with any nonablative fractional laser treatment, even though the potential for and the incidence of serious side effects are exponentially less than with traditional ablative resurfacing. Nonablative skin resurfacing has a low complication rate. Most side effects are transient in nature. Possible side effects include erythema, swelling, blistering, scarring, infection, pigmentary changes, herpes reactivation, and acne flare-up. Transient erythema and swelling is normal and the patient should be instructed to expect this. In a review of short-term adverse effects from 1550-nm NAFL treatment by Fisher et al., all patients displayed post-treatment erythema, and edema was present in 82% of patients (22). Prolonged erythema, massive swelling, blistering and subsequent scarring can occur if aggressive treatment parameters are utilized and overheating of the skin occurs. These side effects can be easily avoided with prudent selection of treatment parameters. Infection is rarely seen with nonablative fractional resurfacing. Hyperpigmentation definitely occurs and is more prevalent with darker skin types (IV–V). In a retrospective review of Fitzpatrick skin types IV–VI treated with a 1550-nm erbium-doped fractional nonablative laser (Fraxel Re:Store SR 1550; Solta Medical, Inc., Hayward, CA), PIH occurred in 5 out of 115 total laser sessions (4%), with only one case lasting longer than a month (2 months total) (23). This can be reduced with pre- and post-treatment with hydroquinone bleaching agents and strict sun protection. Also, treatment densities and fluencies must be lowered to reduce this side effect. Reactivation of herpes simplex is another real side effect. Patients who are prone to herpes simplex should be pretreated with oral antivirals. There have also been three cases of herpes zoster along the trigeminal nerve which have been reported after NAFL. Acneiform eruptions can also occur. If patients are prone to acne, they can be treated with oral antibiotics, like minocycline or doxycycline and/or a topical antibiotics, such as clindamycin. The nonablative resurfacing complication rate has been studied over several years (25–27). A retrospective study of 961 nonablative 1550-nm laser treatments recorded a 7.6% complication rate (27). The two most common complications encountered were acneiform eruptions and herpes simplex reactivation. However, these were only encountered at a 1.87% and 1.77% rate respectively. Rarely, ulceration can occur, as reported recently in two cases of stable surgical scars that ulcerated following nonablative fractional resurfacing with a 1550-nm device (Fraxel Re:Store) (28). Both of these cases involved the use of intralesional lidocaine for pre-treatment anesthesia. Overall, NAFL is a very safe treatment and has minimal side effects at the recommended settings.

### CLINICAL APPLICATIONS

Nonablative lasers have been used to treat an expansive range of conditions. Numerous clinical studies have shown its effectiveness in a variety of treatments (19,29,30). The main areas of treatment include photodamage and wrinkling, scars, stretch marks, pigmentary disorders, and actinic keratoses. Evolving applications include the enhancement of drug and topical product delivery, which takes advantage of increased permeability of the epidermis and dermis after treatment. Case reports have also shown NAFL treatment to be useful in miscellaneous other applications that include improving residual

hemangiomas, minocycline induced pigmentation, nevus of Ota, granuloma annulare, and male and female pattern hair loss, among other applications described below (13,14,31–34).

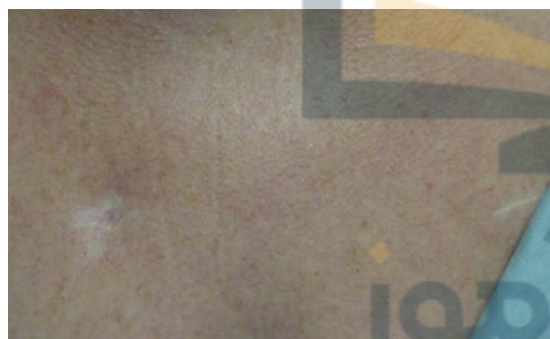
## PHOTODAMAGE

Many studies have examined nonablative fractional resurfacing of the face and non-facial skin for improvement in sun-induced discoloration and wrinkles. In 2007, Wanner et al. examined 50 patients with mild to moderate photodamage, rhytides, and dyspigmentation who received three successive treatments with a 1550-nm erbium-doped laser (Fraxel SR750, Reliant Technologies, Inc.,<sup>\*</sup> Mountain View, CA) (35). At least 51%–75% improvement in photodamage was observed in 73% and 55% of facial and non-facial (chest and neck) treated skin at the 9-month follow-up. Fractional laser photothermolysis for photoaging of the hands has also been examined in a small cohort of patients by Jih et al. (36). In this study, ten patients were randomized to receive five NAFL treatments with a 1550-nm laser (Fraxel SR) to either their left or right hand. Statistically significant improvements in skin pigmentation and skin texture were noted at the 1- and 3-month follow-up visits. Skin biopsies were also taken at baseline and at 1- and 3-month follow-up visits. The post-treatment biopsies showed thickening of the epidermis and notably increased collagen density in the papillary and upper reticular dermis. Moreover, the study was safe and showed limited side effects. In 2010, a 1550-nm laser consensus panel published their recommended

treatment settings for photoaging (19). The panel recommended settings of 10–20 mJ of energy with a treatment level of 7–11 to improve dyschromia in patients with Fitzpatrick skin types I–III. For rhytides, specifically in the periorbital region, the consensus recommended settings of 30–70 mJ of energy using a TL of 7–11 with eight passes. For dyschromia of the neck and chest the panel recommended 10–40 mJ of energy using a TL of 7–11 and eight passes for Fitzpatrick skin types I–III. Newer versions of the 1550-nm laser, as well as other NAFLs of other wavelengths, have been developed since the consensus meeting. Hopefully another consensus meeting will be planned that will include recommended treatment guidelines for all NAFL systems. Results using a 1550-nm laser for the treatment of photodamage on the chest are shown in Figure 55.13.

In 2014, Brauer et al. examined a 1927-nm nonablative thulium laser for the treatment of photo-induced pigmentation (Fraxel DUAL, 1550/1927 Laser System, Solta Medical, Inc., Hayward, CA) (37). Forty patients with photo-induced facial pigmentation received two treatments at an energy level of 10 mJ, coverage of 40%, and 4–6 passes. At 1 and 3 months after the second treatment, overall improvement was graded as moderate to very significant in 82% and 69% of subjects, respectively. Lentigines and ephelides also showed moderate to very significant improvement in 68% and 51% of subjects at 1 and 3 months follow-up, respectively. Treatments were well-tolerated with a mean pain sensation of 4.3 on a 10-point scale, with no serious adverse events. A clinical example of results with this device is shown in Figure 55.14.

<sup>\*</sup> Reliant Technologies, Inc. was acquired by Solta Medical, Inc. in 2008



(a)

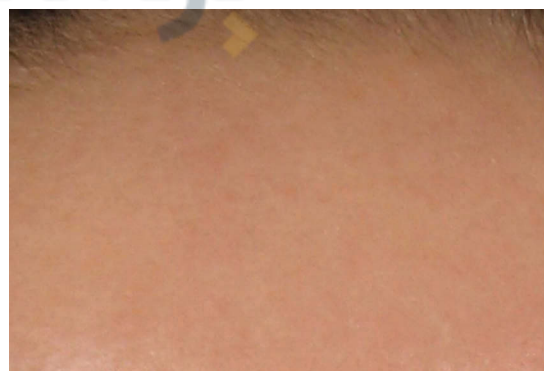


(b)

**Figure 55.13** Photodamage of the chest (a) before and (b) after two treatments with a 1550-nm device. (From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.)



(a)



(b)

**Figure 55.14** Improvement in pigmentation (a) before and (b) after one treatment with a 1927-nm device. (From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.)

In 2014, Marmon et al. reported mild improvement in skin texture, pigmentation, and wrinkling when treating Asian subjects with a 1440-nm diode-based nonablative fractional laser (Clear + Brilliant™ Laser System, Solta Medical, Inc., Hayward, CA) (38). In this study, ten Chinese subjects with Fitzpatrick skin types III–V received four treatments spaced 2 weeks apart. Side effects most often included erythema and edema, which were transient in all, with one patient developing a discrete area of postinflammatory hyperpigmentation that resolved by the end of the study. The authors suggest that this low energy, less intense treatment may be a good option for skin of color patients who may have only a mild degree of photodamage. This same laser has also been evaluated as a treatment for large pores. In a 2013 study, Saedi et al. reported 20 patients who received six treatments spaced 2 weeks apart at the highest tolerable energy (39). Utilizing pore scores calculated with use of the Visia-CR imaging system (Canfield Scientific, Inc., Fairfield, NJ), the study found a 17% average reduction in pore score, which was statistically significant. Subjects and investigators also scored their improvement, with both groups rating the appearance of pores, skin texture, and overall appearance as moderate to markedly improved.

Photodamage can also be addressed with a new, FDA-cleared 1440-nm home-use device (Tria Age-Defying Eye Wrinkle Correcting Laser, Tria Beauty, Inc., Dublin, CA). Rahman et al. recently reported significant improvement utilizing this device for periorbital wrinkles in 45 subjects who treated themselves daily for 8 weeks (40). The subjects were followed for 12 weeks after the final treatment, with improvement being sustained in 81% of subjects. Treatments were generally about 1 minute per eye and were well tolerated with no serious adverse events.

NAFL treatments can also be combined with other modalities on the same day for the treatment of photoaging. In 2012, Kearney et al. reported synergistic results when IPL was combined with a 1550-nm fractional device (Fraxel Re:Store) (41). In our practice, we commonly combine IPL with the 1927-nm thulium laser in a single treatment session.

## SCARS

Improvement in scars may significantly improve a patient's self-esteem and quality of life. Common causes of scars include surgery, trauma, and acne. Two main categories of scars include atrophic and hypertrophic scars. They can vary in color from white to red to brown. In 2007, an initial pilot study by Vasily studied 31 subjects with 13 surgical scars and 18 traumatic scars utilizing the Lux 1540-nm 10 mm handpiece (42). Treatments utilized laser energies in the range of 30–60 mJ, a 10 ms pulse duration, and three to five passes, with a series of one to eight treatments being performed. A blinded observer noted 51%–75% improvement in scars at 1-month post-treatment in 59% of patients. The most improvement was seen after the first three treatments. These results have further been confirmed in a more recent study utilizing a NAFL for the treatment of surgical scars and skin grafts following Mohs surgery (43). NAFL treatment of surgical scars has also been compared to and outperformed the standard scar treatment of pulsed dye laser (PDL) (44). This was shown in a randomized, blinded split-scar study performed in 12 patients comparing a 1550-nm erbium-doped fiber laser (Fraxel SR) against the 595-nm V-Beam PDL (Candela Corporation, Inc., Wayland, MA). Fluences of 70 mJ, TL 8, and 16 passes were performed on the NAFL side and 7.5 J/cm<sup>2</sup>, pulse duration of 0.45 ms, and a spot size of 10×3 mm were

performed on the PDL side. After a series of four treatments, greater overall mean improvement was seen in the NAFL side (75.6%) compared to the PDL side (53.9%).

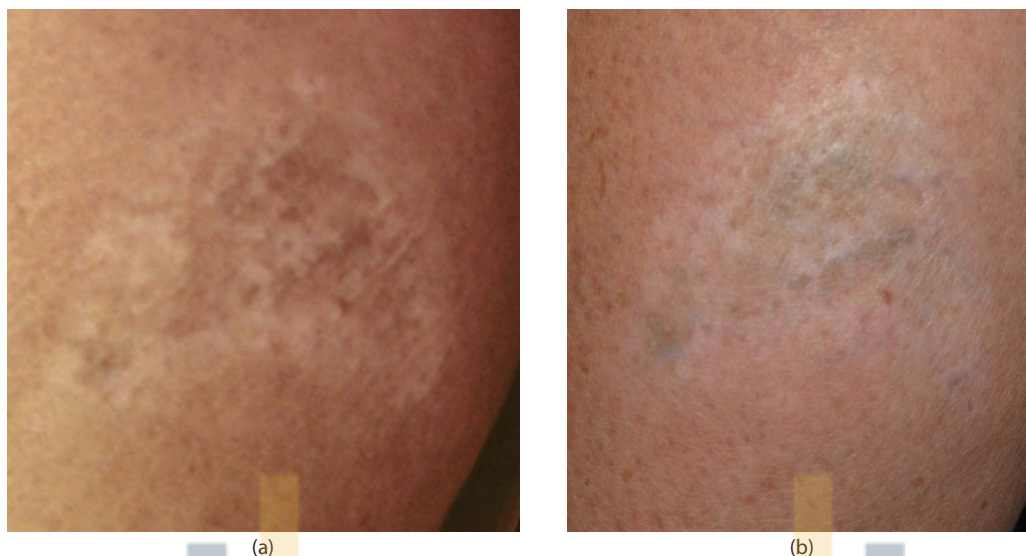
Hypopigmented scars, hypertrophic scars, and traumatic thermal burn scars have also been studied in small cohorts and have demonstrated improvement with NAFL (45–49). In a study by Massaki et al., 14 patients with hypopigmented scars were treated with an erbium-doped 1550-nm fractionated laser in combination with either topical bimatoprost twice daily plus topical tretinoin 0.05% or pimecrolimus 1% cream daily for those who could not tolerate tretinoin. After a mean of 4.5 treatment sessions, follow-up results 4 weeks after the last treatment revealed significant improvement in scar pigmentation (greater than > 50%) in 12 of 14 patients with improved clinical scores that persisted for a mean follow-up of 20.1 months. The 1550-nm NAFL consensus panel recommended settings of 50–70 mJ of energy with a TL of 7–11 for skin types I–III in the treatment of surgical scars (19). Flattening and improvement in hypopigmentation of a scar is shown in Figure 55.15.

Nonablative fractional resurfacing has also been reported to be effective in the treatment of contracted scars. In a 2015 case report, Finney et al. reported improvement in a contracted right lower extremity scar following treatment with the Fraxel DUAL (50). The patient underwent six treatments spaced 4–8 weeks apart, receiving three treatments with the 1927-nm thulium laser (10 mJ, 30% density, eight passes) and three with the 1550-nm laser (40 mJ, 17%–26% density, eight passes). Following the treatments, the patient had subjective and objective improvement in their range of motion, as well as 50%–75% improvement in scar texture and discoloration.

Like surgical scars, acne scars can be cosmetically and psychologically disturbing to patients. NAFL therapy has been proven successful in the treatment of acne scars. In 2008, Weiss et al. presented a retrospective analysis of over 500 acne scar treatments with the Lux 1540-nm laser (51). The fluences used were 50–70 mJ with a minimum of three passes. Results assessed by blinded photographic evaluation showed a median of 50%–75% improvement in acne scars with 85% of patients rating their skin as improved. In 2012, Bencini et al. evaluated 87 acne scar patients with moderate to severe acne scarring who were treated with the same device (52). Four passes with 50% overlap at 15 ms pulse duration with energies ranging from 50–60 mJ were utilized. At 6 months follow-up, 80/87 (92%) of patients had marked improvement and 7/87 (8%) showed a moderate improvement as graded by two blinded independent physicians. In a more recent study by Sardana et al., use of the Lux 1540-nm laser with a 10-mm handpiece was found to markedly improve atrophic acne scars, particularly boxcar scars (53). In this study, 35 patients received six treatments with four passes per treatment and energies ranging from 70–100 mJ. At 6 months follow-up, there was a reduction in mean counts of ice-pick, rolling, and boxcar scars, although only boxcar scars showed a statistically significant reduction (52.9%). Additionally, 46.7% of patients reported moderate improvement of 25%–50%, 26.7% reported marked improvement of 51%–75%, and 3.3% reported near total improvement. Acne scars have also been studied with the Fraxel 1550-nm SR750 by Alster et al. (54). Fifty-three patients with acne scarring received three laser treatments. Ninety percent of patients were found to have 51%–75% improvement by an independent observer.

Nonablative fractional laser treatments can also be utilized for the treatment of postinflammatory erythema secondary to acne. In a 2014 study by Park et al., 12 Korean patients





**Figure 55.15** Flattening and improvement in hypopigmentation of a scar (a) before and (b) after several 1550-nm treatments. (From Beasley KL, Weiss RA, In: Goldman MP, Fitzpatrick RE, Ross VE, Kilmer SL, Weiss RA, eds. *Laser and Energy Devices for the Skin*. Boca Raton, FL: CRC Press, 2013; pp. 178–91. With permission.)

with acne erythema received a total of three split-face treatments with a 1550-nm erbium:glass nonablative fractional laser and a PDL (55). Both treated sides had statistically significant improvement in erythema, with no significant difference between them. Patients rated their improvement as either good or excellent in 91.7% of the fractional treated side, and 75% of the PDL-treated side.

The 1550-nm laser consensus panel meeting recommended treatment settings for acne scars that depended on skin type (19). They recommended settings of 30–70 mJ at TL 7–11 for 8–12 passes for skin types I–III and 30–70 mJ, TL 4–5 and 8 passes for skin types IV–V. We presently employ the Icon system with the 1540-nm XD handpiece for scars and anecdotally have seen responses for scars resistant to all other NAFL devices.

## STRIAE

Striae are disrupted areas of collagen in the skin that can occur during growth spurts, topical and oral steroid use, and conditions of hormonal change like pregnancy. It is estimated that 75%–90% of women develop some degree of stretch marks during pregnancy. Previous treatments have been ineffective or inconsistent and not suitable for all skin types (56). The Palomar<sup>†</sup> 1540-nm NAFL is currently the only laser to gain FDA clearance for the treatment of striae. One of the first studies with the Lux 1540-nm laser evaluated 51 subjects with striae of the abdomen, legs, buttocks, arms, and flanks (57). Fluences of 35–55 mJ with a 10 mm tip or 12–14 mJ with a 15 mm tip were utilized. Two to four treatments were performed. Patient and independent observer evaluations noted improvements of 50% or greater in all patients at 6 months past the last treatment. More recently, Wang et al. compared the clinical efficacy of the Lux 1540-nm with a low energy 1410-nm nonablative fractional laser (Emerge<sup>™</sup>, Cynosure, Inc., Westford, MA)(58). Ten patients with abdominal striae were treated, with half of their abdomens

being treated with the 1540-nm device (XD: 50 mJ, 15 ms pulse duration, 2 passes; XF: 50 mJ, 15 ms pulse duration, two passes, 25% total density) and the other half being treated with the 1410-nm device (30 J, 5 passes, 16% density). Six treatments were performed 2–6 weeks apart, with two blinded dermatologists scoring the photographs. All patients demonstrated clinical improvement, with “good” or “excellent” improvement seen in 33% of those treated with the 1540-nm device and 28% of those treated with the 1410-nm device. “Mild” or “fair” improvement was seen in 66% of those treated with 1540-nm and 72% of those treated with 1410-nm. These differences were not statistically significant. All patients were either “very satisfied” or “moderately satisfied.” Of note, transient hyperpigmentation appeared to last longer in those treated with the 1410-nm device.

Fraxel 1550-nm NAFL has also been studied in the treatment of striae even though it is not FDA-approved for this indication. Twenty patients with striae were studied over six laser treatments. Randomly selected photos of eight patients were chosen from the study and an independent observer rated a 26%–50% level of overall improvement in five out of eight patients (59). In a separate study, Guimarães et al. treated 10 cases of striae on the breasts following breast augmentation with a 1550-nm erbium:glass laser (Sellas, Dinona Inc., Seoul, South Korea)(60). Each patient underwent four to eight treatments at 4-week intervals, with fluences of 80–100 mJ, a density of 100 points per area, and one treatment pass. Responses were graded by subjects and two plastic surgeons 4 weeks following the last treatment session, with 50% of graded scores being between 9 and 10 on a 0 (no improvement) to 10 (total improvement) scale. Nonablative 1550-nm treatments have also been compared to ablative CO<sub>2</sub> fractional laser resurfacing in Asian patients with striae on the abdomen, both with significant clinical and histologic improvement. There was no significant difference in clinical efficacy between the modalities based on blinded physician evaluations (61). The 1550-nm NAFL consensus panel recommended settings of 40 mJ, TL 10 for eight passes in skin types I–III and reducing the TL to 4–7 in darker skin types (19). When treating striae, a

<sup>†</sup> Reliant Technologies, Inc. was acquired by Solta Medical, Inc. in 2008

laser test area is always suggested. Pre- and post-treatment with a hydroquinone bleaching cream is suggested in darker skin types and areas prone to prolonged PIH, like the legs.

## PIGMENTARY DISORDERS

Increased pigmentation of the skin, as seen in photodamage and melasma, has shown improvement through NAFL treatment. Melasma can be quite distressing to patients and is notoriously difficult to treat. It is a chronic skin condition seen primarily in female patients. It presents as a brown patchy discoloration over sun-exposed areas like the cheeks, forehead, upper lip and sometimes arms. Contributing factors include sun exposure, hormones, pregnancy, and genetics (62). Many therapeutic modalities have been investigated over the years but none have been shown to be uniformly successful (63,64). When the first 1550-nm laser was released, several melasma studies were initiated. The first pilot study was by Rokhsar et al. using a 1550-nm laser on 10 patients (65). It showed some success with 60% of patients demonstrating excellent clearing and 30% of patients demonstrating mild improvement. Another study compared 1550-nm laser resurfacing with a topical bleaching cream through a split-face study (66). The patients in the study showed preference to the topical bleaching cream side over the 1550-nm NAFL side. The 1927-nm laser is not approved for melasma but initial reports seem promising. An initial pilot study by Polder et al. used the Fraxel 1927-nm thulium laser in the treatment of 18 patients with melasma (67). They performed three to four treatments using 10–20 mJ of energy, a TL corresponding to 20%–45% coverage, and eight passes. Using the standard Melasma Area and Severity Index (MASI) and blinded independent photographic review of standardized photos, they reported a 51% reduction in the MASI scores at 1-month follow-up. There was still a 33% and a 34% reduction of MASI score at 3- and 6-months follow-up, respectively. More recently, a retrospective analysis of 20 patients treated with the same device found that 60% of subjects had more than 50% clearance of their melasma at 4 weeks after a single laser treatment (68). At 4 weeks after the treatment, mean MASI scores improved by 35%, and by 53.8% at the 6- to 12-month follow-up visit. Treatment settings included fluences between 10–20 mJ, with 60%–70% surface area coverage, and total energies ranging between 1.72 to 4.42 kJ. Notably, 7 of 15 patients who successfully followed up had recurrence of their melasma with time. Although NAFL treatment has shown success in the treatment of melasma, its long-term efficacy is still limited by the inherent recurrence rate of melasma. In some patients with melasma, we have also noted increased hyperpigmentation and prolonged erythema after NAFL treatment. Most NAFL and other laser treatments for melasma work best when combined with topical depigmenting skin care and aggressive sun protection.

## ACTINIC KERATOSES

An actinic keratosis (AK) is a precancerous, epidermal, scaly macule or papule that occurs on sun-damaged skin. Treatment of these lesions is indicated, as there is potential for them to progress into invasive squamous cell carcinoma. The 1550-nm and the 1927-nm wavelengths have been studied and have shown success in the treatment of these precancerous growths. Initially, the 1550-nm fractional laser (Fraxel Re:Store) was investigated in a small group of men with AKs (69). These men underwent five nonablative resurfacing laser treatments every 2 to 4 weeks. Energies ranged from 20–70 mJ during the treatment but the

majority of treatments were performed at 70 mJ. Treatment level 11 was utilized and 8–10 passes were performed. A baseline biopsy was performed and then repeated at the 3-month follow-up visit, immediately adjacent to the initial biopsy site. AKs were counted at 1-, 3-, and 6-month follow-up visits. There was clinical improvement after each session, although histologically, precancerous changes were still evident. At the 1-, 3-, and 6-month visits, actinic keratosis percent reduction from baseline was 73.1%, 66.2%, and 55.6%, respectively. With the introduction of the 1927-nm thulium fractional laser, more AK treatment studies began. This superficial wavelength selectively targets the epidermis and upper dermis and was postulated to have greater efficacy in the treatment of the mostly epidermal AKs. Weiss et al. studied 24 subjects with facial AKs receiving up to four treatments with a 1927-nm fractional laser (Fraxel DUAL)(70). One month after the final treatment, average AK clearance was 91.3% by an independent physician assessment. At 6 months after the treatment, average AK clearance was 86.6%. Histologic clearance of AKs was also documented in 87.5% of those subjects who were biopsied. They found this treatment to be well tolerated by patients, without incidents of infection, scarring, or pigmentary changes. Friedman et al. performed split-face laser treatment of AKs using a 1550-nm laser in combination with the 1927-nm laser versus the 1927-nm laser alone (71). They found that the side treated with 1927-nm alone had greater clinical reduction in AKs as well as improvement in sun-induced pigmentation. The 1927-nm laser has also been applied to the treatment of AKs of the lip, otherwise known as actinic cheilitis (72). This condition is notoriously hard to treat, prone to recurrence, and can be quite painful for patients. Anolik et al. performed a retrospective chart analysis of a small group of actinic cheilitis patients and found that after 1–2 treatments patients were improved by 50%–75% or 75%–100% as rated by blinded, non-treating staff dermatologists (73). The procedure was well tolerated by patients and had much less side effects when compared to ablation, surgery, or treatment with topical chemotherapeutic agents or immune modulators.

Nonablative fractional resurfacing has also been reported to be safely and effectively combined with fractional ablative therapy for the treatment of actinic keratoses. In 2015, Ortiz et al. reported a 91% clearance rate in AKs treated with a hybrid 1470/2940-nm fractional laser when utilized on 115 AKs located on the scalp, chest, forearms, and dorsal hands (74).

## ENHANCEMENT IN DELIVERY OF TOPICAL DRUGS AND PRODUCTS

Research in this application of fractional lasers is still in its infancy but there is expanding evidence supporting its role in a variety of applications, particularly with ablative fractional devices. One area of particular promise is the use of ablative and nonablative fractional resurfacing for enhancement of photodynamic therapy (PDT), as laser pretreatment offers the potential of increased ALA uptake with shortened incubation and potentially improved clinical outcomes. Enhanced absorption of ALA has been demonstrated in an in vivo study that showed skin pretreated with a 1550-nm fractional erbium:glass laser (Mosaic) followed by topical 5-aminolevulinic acid had a statistically significant increase in porphyrin fluorescence compared to areas not exposed to the laser (75). In my practice, we often pre-treat our PDT patients with two to four passes of NAFL, which allows for faster penetration of topical levulinic acid. This is especially helpful in areas like the arms and legs in which incubation time with levulinic acid would otherwise be hours. Enhancement of platelet-rich plasma (PRP) treatment of

the skin has also been studied in NAFL (15). The study showed that when PRP was combined with NAFL with a 1550-nm laser (Mosaic), patient satisfaction and skin elasticity increased while erythema index of the skin decreased. Histologically, those patients who were treated with PRP plus NAFL demonstrated increased length of the dermal epidermal junction, increased amounts of collagen, and increased numbers of fibroblasts compared to patients treated with fractional laser alone.

## OTHER CLINICAL INDICATIONS

Nonablative lasers have also been reported to be effective in the treatment of a variety of other conditions. In 2014, Hsu et al. reported favorable outcomes using a 1450-nm diode laser for the treatment of xanthoma disseminatum (76). This same laser has also been reported to effectively treat xanthelasma palpebrarum (77). Beleznyay et al. reported a case of lupus miliaris disseminatus faciei treated with nonablative fractionated resurfacing utilizing a novel 1565-nm device (78). Nonablative fractional resurfacing utilizing the Lux 1540-nm laser with the XD handpiece has also been shown to be effective in the treatment of iatrogenic hypopigmentation secondary to intralesional steroid injections (79). In 2014, Emer et al. reported improvement in a case of lupus pernio using combined pulsed dye laser and nonablative fractional resurfacing (Fraxel DUAL) (80). In 2013, Narinesingh et al. reported improvement in the waffle pattern of a meshed skin graft following treatment with a 1540-nm fractionated erbium: glass laser (81). Macular seborrheic keratoses have been shown to be safely and effectively treated with a 1927 thulium fiber laser (82). Isolated steatocystoma multiplex has also been reported to be substantially improved with two treatments of a 1450-nm diode laser and a 1550-nm fractionated erbium-doped fiber laser (83).

## CONCLUSIONS

Fractional photothermolysis, and its application of NAFL skin resurfacing, is still considered a leading groundbreaking technology. Both the stamping modes and the continuous motion handpiece with rollers are effective methods to deliver the energy. New understanding and engineering has allowed the development of optics that drive energy more deeply and more safely to obtain better results on scars and other clinical problems. NAFL's proven efficacy in a multitude of skin conditions leads us to utilize NAFL as an integral part of our daily practice of dermatology and medicine. Clinical applications include among others, photodamage, wrinkling, scars, stretch marks, pigmentary disorders, and actinic keratosis. Study after study demonstrates that fractional resurfacing is effective, safe, and has minimal side effects. NAFL will continued to be studied and is still in its infancy when related to enhancement of topical therapies

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# Cryolipolysis for Non-Surgical Fat Reduction

Christine C. Dierickx

## INTRODUCTION

Liposuction is the second most popular aesthetic surgery procedure with 1.4 million procedures worldwide in 2015 (1). As patients seek to reduce subcutaneous fat resistant to diet and exercise, there has been increased interest in non-surgical alternatives because of risks from invasive surgical procedures, anesthesia, and downtime for recovery. According to the American Society of Aesthetic Plastic Surgeons, there were 135,448 non-surgical fat reduction procedures in 2014, including cryolipolysis, high intensity focused ultrasound, and diathermy heating. In 2015, there were 160,763 such procedures, reflecting an 18.7% increase in non-surgical body contouring procedures (2).

Numerous non-invasive body reshaping technologies have been studied, including low level laser therapy, ultrasound, radiofrequency, diathermy heating, and infrared light. This chapter focuses on cryolipolysis, the most widely used (3) and well-established non-surgical body contouring procedure (Figure 56.1).

## BACKGROUND History

There have been numerous published clinical observations describing adipose tissue sensitivity to cold, inducing panniculitis, a localized inflammation in subcutaneous fat. In 1902, there were reports of firm nodules noted under the chins of children in response to acute cold injury (4). There were also case reports of cold-induced panniculitis in children, teenagers, and adults (5–10). The unique case report of “popsicle panniculitis” was reported in 1970 for an infant that developed a red, indurated nodule and subsequent fat loss in the cheek after sucking on a popsicle (11). Researchers Dieter Manstein, MD, PhD and R. Rox Anderson, MD of the Wellman Center for Photomedicine at Massachusetts General Hospital, a teaching affiliate of Harvard Medical School, drew upon these case reports and recognized the potential for controlled cooling to selectively target undesirable adipose tissue.

## Pre-Clinical and Clinical Studies

In 2008, Manstein et al. published a seminal cryolipolysis proof-of-concept porcine study (12) and results were confirmed in a subsequent porcine study by Zelickson et al (13). The pre-clinical studies investigated the effect of controlled surface cooling and the resulting damage to subcutaneous fat. Porcine pre-clinical studies were conducted for initial exploration, dosimetry evaluation, and safety assessment measuring lipid levels. The investigators found that selective reduction in superficial fat was achieved by the cryolipolysis procedure without causing injury

## BODY SCULPTING

**175,000** total procedures

**24,000** liposuction

**89,000** cryolipolysis  
“fat-freezing”

**24,000** laser lipolysis

**38,000** non-invasive  
treatment of fat and cellulite

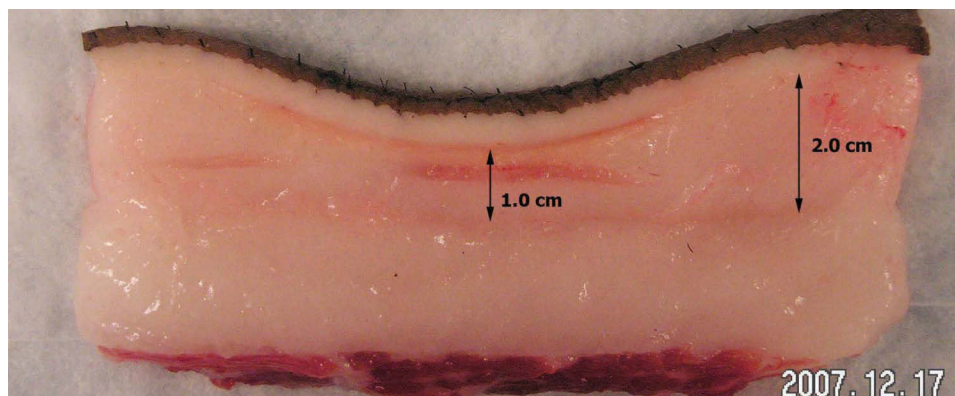


**Figure 56.1** Cryolipolysis accounted for 51% of all body contouring procedures. (Courtesy of the ASDS 2013 Survey on Dermatologic Procedures.)

to the epidermis or dermis (12–13). The investigators found that 80% of the superficial fat layer was removed at 3.5 months post-treatment for a total fat loss of 40% (12). Figure 56.2 shows gross pathology with significant fat reduction visible in the cryolipolysis-treated porcine adipose tissue.

Histological analysis from the porcine models found that controlled, selective cooling could be utilized to induce an inflammatory response in the subcutaneous fat approximately 24 hours following the cold treatment. Time course evaluation found that the inflammatory response intensified as adipocytes were surrounded by histiocytes, neutrophils, lymphocytes, and other mononuclear cells, leading to subsequent digestion of the fat cells. The inflammatory process was found to decline 90 days post-treatment, as shown in Figure 56.3. It appeared that the lipids remained trapped





**Figure 56.2** Gross pathology sections of cryolipolysis treated porcine tissue showing reductions in superficial fat layer 90 days after treatment. (From Zelickson B et al., *Dermatol Surg*; 35(10):1462–70, 2009. With permission.)

within the subcutaneous tissue until they were digested and cleared by a natural inflammatory process. This resorption took place over more than 90 days, resulting in a very gradual displacement of the lipids. The pre-clinical safety studies established no effect on serum lipid levels in the animal models, while attaining significant reduction in subcutaneous fat without damaging the skin (12–13).

Thereafter, clinical studies demonstrated safety and efficacy in human subjects. Clinical studies were carried out to investigate the efficacy of cryolipolysis to the flanks and back in 32 subjects with efficacy assessed by ultrasound measurement of the fat layer, pre- and post-treatment clinical photograph comparison, and physician assessment. The study found an average fat reduction of 22.4% in subjects assessed 4 months post-treatment (14). A separate flank cryolipolysis study of 10 subjects assessed efficacy and effect on nerve fibers following cryolipolysis. The investigators found an average fat layer reduction of 25.5% at 6 months post-treatment. While approximately one-third of the subjects experienced transient reduction in sensation in the treated site, all subjects experienced restoration of sensation within 7 weeks (mean 3.6 weeks) (15). Numerous clinical study publications on over 4000 study subjects have reported on the safety, efficacy, and tolerability of cryolipolysis for fat reduction in a variety of areas including the abdomen, flanks, inner thighs, outer thighs, back, arms, pseudogynecomastia, banana rolls, and knees (16–27).

Cryolipolysis is approved for fat reduction in over 70 countries worldwide including China, Canada, Europe, Brazil, and Australia. Cryolipolysis received FDA clearance for fat reduction for the flanks in 2010, for the abdomen in 2012, for the thighs in 2014, the submental area in 2015, and the back, bra area, and underneath the buttocks in 2016. It is cleared in Taiwan for the flanks and abdomen.

### Mechanism of Action

Controlled cooling for selective damage of subcutaneous fat is based upon the premise that lipid-rich adipocytes are more susceptible to cold injury than water-based cells; this is related to the higher phase transition temperature of lipids compared to water. Intracellular “lipid ice” is believed to form approximately 10°C degrees warmer than water ice (12, 28). Figure 56.4 shows the crystallization of porcine fat at various temperatures

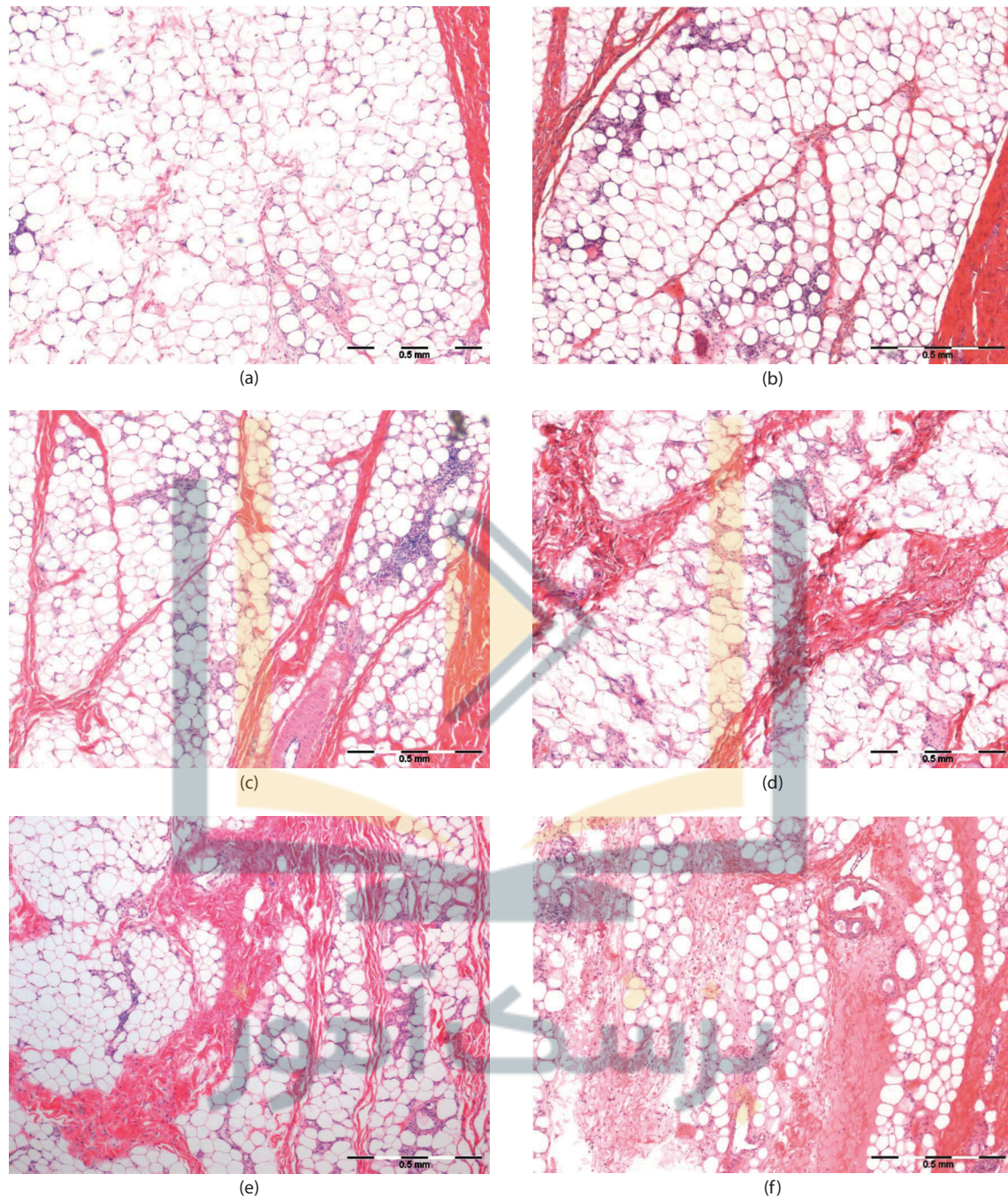
(12). The images were obtained by cross-polarized microscopy of a 1 mm quartz cuvette filled with heated pig lard. Needle-like crystals are visible at room temperature storage and cloudy crystallization of the porcine fat is shown when the temperature is lowered to 10.3°C. The lipid crystallization shown in these images demonstrates the higher phase transition temperature for adipocytes which leads to their greater susceptibility to cold injury.

Cryolipolysis induces apoptosis, a natural form of cell death, causing a wound healing cascade which results in the eventual clearing of apoptotic bodies through the lymphatic system (12). As opposed to necrosis caused by acute injury, apoptosis is programmed cell death which is a normal part of cellular homeostasis. During apoptosis, a defined sequence of morphological and biochemical events occur, such as membrane blebbing, shrinkage of the cell, aggregation of chromatin, and the appearance of apoptotic bodies (29–31).

Experimental studies have confirmed that cryolipolysis induces apoptosis, a natural form of cell death, which causes a wound healing cascade and the eventual clearing of the apoptotic bodies through the lymphatic system. By immunohistochemical stain for Caspase-3 activity and H&E stain to examine cellular morphology, histological analysis provides evidence of apoptosis following cryolipolysis treatment. As shown in Figure 56.5, human fat treated by cryolipolysis was harvested post-treatment, fixed, sectioned, and stained with cleaved Caspase-3 antibody, an immunohistochemical assay specific for an apoptosis enzyme. Sequential activation of caspases plays a central role in the execution phase of cell apoptosis. Human adipose tissue harvested following cryolipolysis shows evidence of cells undergoing apoptosis compared to the untreated control tissue.

Histology analysis using H&E stain further illuminates the cryolipolysis mechanism of action. Human abdomen tissue treated 2 weeks prior to harvest, Figure 56.6, shows adipocyte death by apoptosis and initiation of the phagocytosis process. The apoptosis is evidenced in the “blebbing” of cellular membranes, resulting in misshapen fat cells. Phagocytosis is shown by the presence of multinucleated macrophages which will engulf and digest dead adipocytes.

In summary, histology studies provide experimental evidence, both by Caspase-3 and H&E stains, that cryolipolysis



**Figure 56.3** Progression of inflammatory response in cryolipolysis treated porcine tissue: (a) 3 days, (b) 7 days, (c) 14 days, (d) 30 days, (e) 60 days, and (f) 90 days. (From Zelikson B et al., *Dermatol Surg*; 35(10):1462–70, 2009. With permission.)

induces adipocyte death by apoptosis. Histology demonstrates the Caspase-3 activity indicative of apoptosis and the resultant apoptotic cellular morphology, phagocytosis process, and eventual clearing of the affected adipocytes.

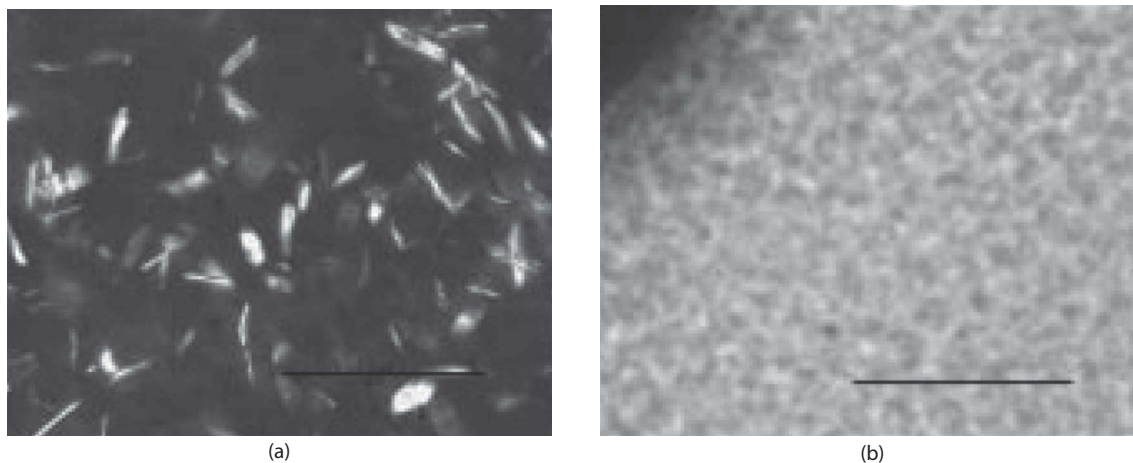
### CLINICAL TECHNIQUE

The cryolipolysis system (CoolSculpting, ZELTIQ Aesthetics) consists of a control unit and portfolio of applicators for various treatment areas (Figure 56.7). There are several vacuum

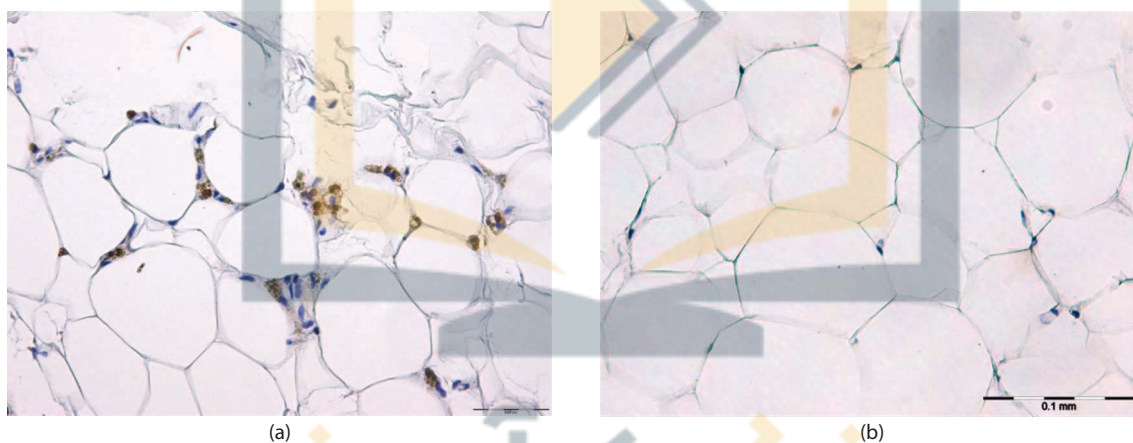
applicators which pull the targeted tissue into a cup and apply surface cooling from parallel panels. The vacuum applicator sizes and cup curvature accommodate a range of patient sizes and treatment areas, such as abdomens and inner thighs. A non-vacuum conformable surface cryolipolysis applicator allows treatment of fibrous, non-pinchable fat in areas such as the outer thigh “saddlebag” bulges.

The most popular treatment areas are the flanks and abdomen, followed by the back, thighs, arms, and chest. Potential treatment areas are shown in Figure 56.8.

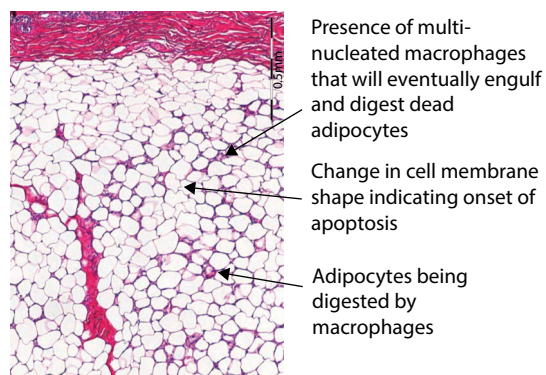




**Figure 56.4** Microscopic images of frozen porcine fat. (a) Needle-like crystals at 21°C after storage overnight at room temperature. (b) Cloudy crystallization at 10.3°C with cooling rate of approximately 10 °C/minute. Bar represents 1 mm. (From Manstein D et al., *Lasers Surg Med*; 40:595–604, 2008. With permission.)

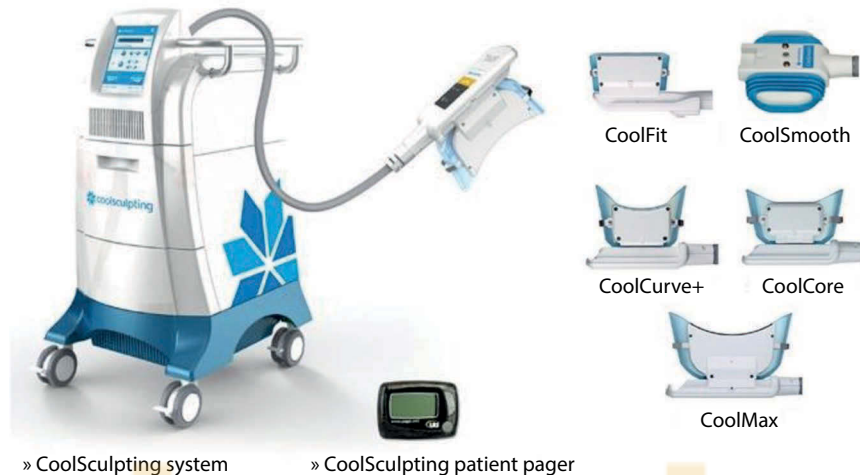


**Figure 56.5** (a) Human abdominal tissue stained with Caspase-3 assay showing evidence of apoptosis 2 days following cryolipolysis treatment. (b) Untreated control tissue.

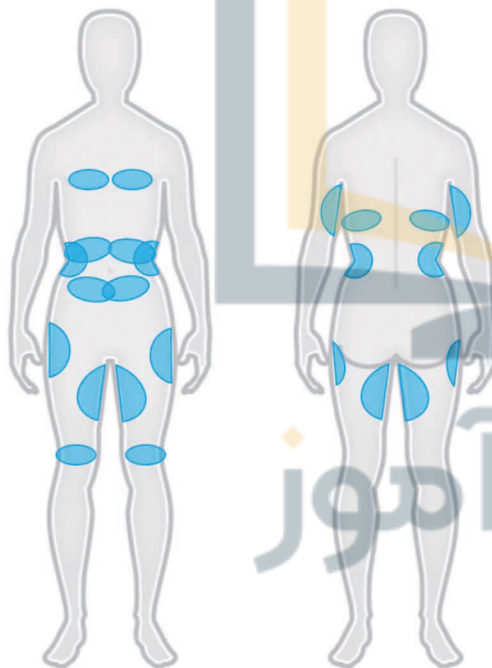


**Figure 56.6** Human abdominal tissue harvested 14 days post-treatment showing evidence of adipocyte death by apoptosis and initiation of the phagocytosis process.





**Figure 56.7** Cryolipolysis system consists of a control unit and applicators for various treatment areas. (Courtesy of CoolSculpting by ZELTIQ Aesthetics.)



**Figure 56.8** Cryolipolysis has been used to non-invasively reduce fat in the abdomen, flanks, thighs, back, arms, knees, submental area, and chest.

### Patient Selection

While it is not an appropriate treatment for weight loss and obesity, cryolipolysis is a safe and effective fat reduction method for appropriate patients. The key patient selection criterion is whether the tissue can be drawn into a vacuum cup or whether the treatment area can be covered by the non-vacuum surface applicator. For areas such as the abdomen and flanks, the curved cup vacuum applicators are appropriate. For areas such

as the upper arms and inner thighs, the flat cup vacuum applicator is appropriate. To reduce a double chin, the small cup vacuum applicator is appropriate. The subcutaneous fat should be pinchable and readily drawn into the vacuum applicator cup. For fibrous, non-pinchable areas, such as the outer thighs, the non-vacuum conformable surface cryolipolysis applicator can be applied. For larger bulges, multiple applicator placements may be needed to adequately cover the target area.

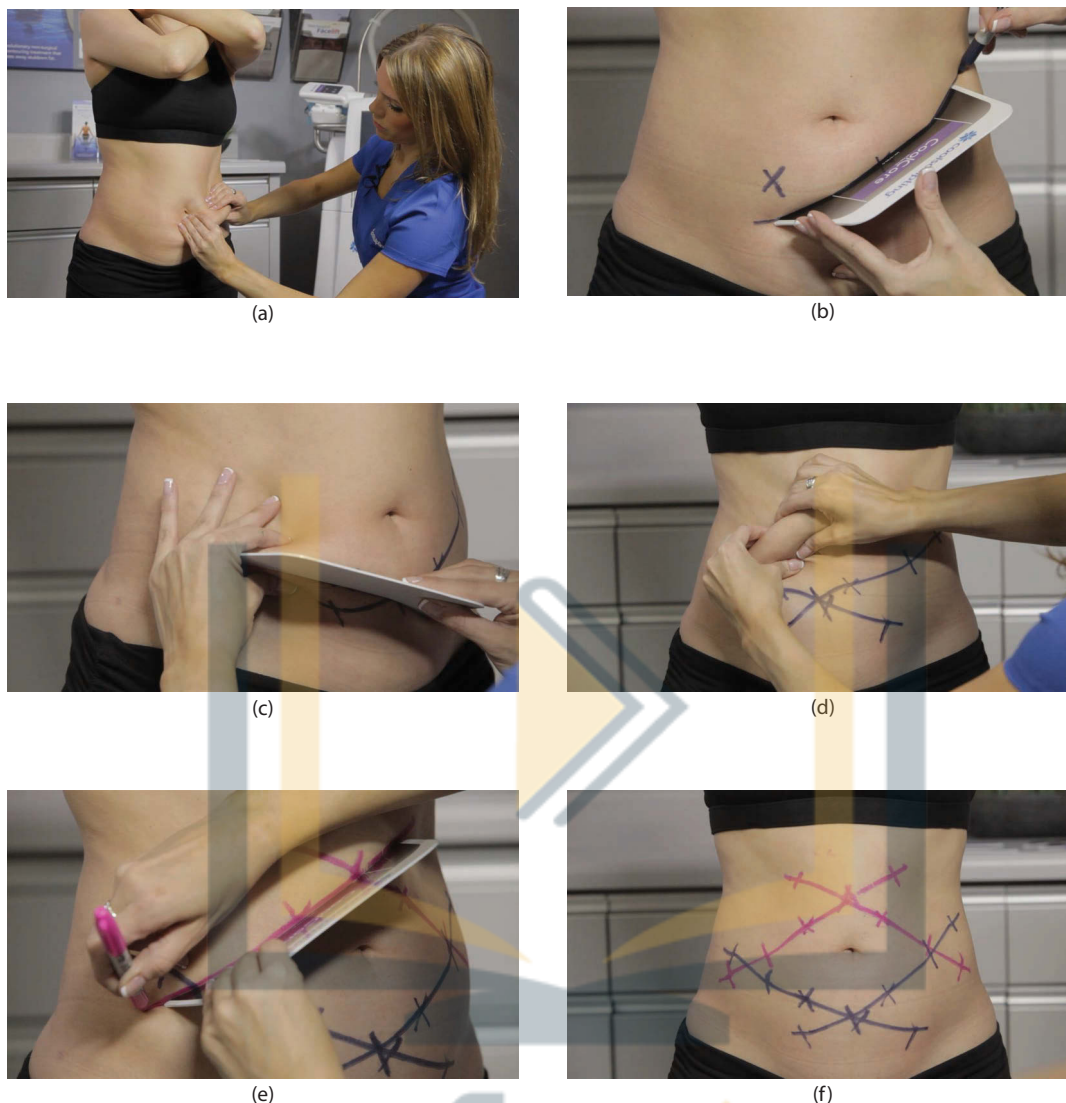
### Assessment Technique

For the assessment, patients should be undressed to allow the clinician to fully view the potential treatment areas relative to the whole body. The patient should be asked to indicate his/her areas of concern in order to develop a treatment plan. The areas of concern should be firmly pinched to assess whether the subcutaneous fat can be treated using a vacuum cup applicator. The clinician should notice the orientation with which the fat presents on the patient's body; templates can be used to plan applicator placements and mark the body. Figure 56.9 demonstrates the assessment and marking techniques and resultant marked treatment plan on a patient's abdomen.

### TREATMENT METHOD

Once the assessment and planning is complete, the treatment procedure can begin (Figure 56.10). To ensure safety, a protective gelpad is first draped over the treatment area. The clinician should ensure that the entire treatment area is covered by the gelpad to prevent freeze injury to the skin. The gelpad should be carefully smoothed to remove trapped air bubbles.

Next, the applicator should be positioned over the marked treatment area and vacuum suction should be initiated to engage the targeted subcutaneous fat. The clinician should visually observe the tissue draw into the vacuum cup to ensure that sufficient draw is achieved. In general, the tissue should fill at least half of the vacuum cup, reaching the horizontal retaining bar between the parallel cooling panels. If insufficient tissue fills the cup, the vacuum suction can be turned off, the applicator and gelpad removed, a new gelpad



**Figure 56.9** Assessment and treatment planning technique for a patient's abdomen: (a) assess lower abdomen; (b) mark lower right; (c) mark lower left; (d) assess upper abdomen; (e) mark upper abdomen; (f) marked abdomen.

placed over the treatment area, the applicator should be repositioned, and vacuum suction should be reinitiated. In the case of the non-vacuum conformable surface applicator, the applicator is secured with straps rather than vacuum suction. Once the applicator is secured to the patient, the cooling cycle can be initiated.

The patient should be comfortably positioned on the treatment bed. Generally, the patients find the treatment very tolerable and are able to engage in activities such as reading or sleeping during the treatment. Current vacuum applicator treatments are 35–60 minutes and the non-vacuum treatment is 75 minutes duration. When the vacuum applicator is removed, patients will typically see a very stiff, red tissue mass which gradually thaws as shown in Figure 56.11. It is recommended that the cold, stiff tissue is vigorously massaged for 2 minutes immediately post-treatment to enhance treatment efficacy (32).

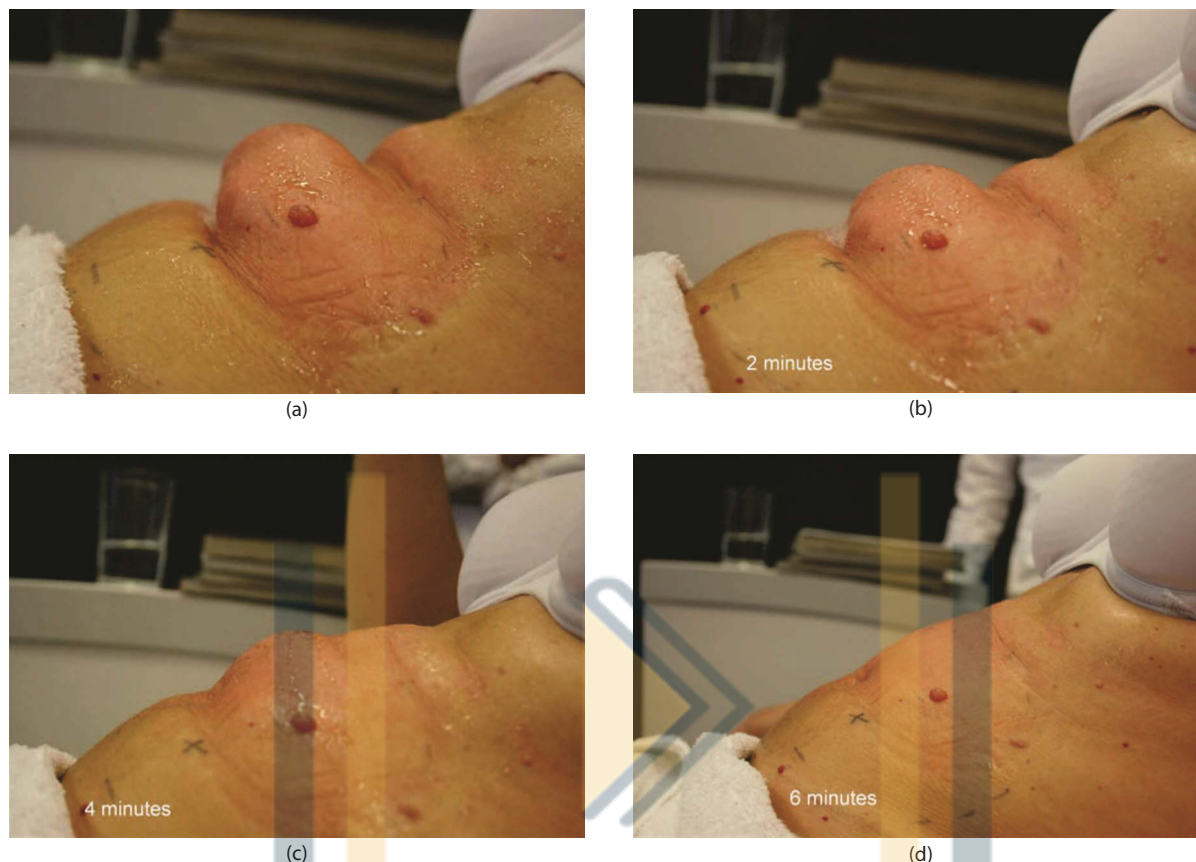
## SIDE EFFECTS

Cryolipolysis treatment side effects are typically mild and self-resolving. During treatment, the patients will experience sensations of pulling and pinching, when using a vacuum applicator. As the cooling plates lower tissue temperature, there will be cold, tingling, stinging sensations, sometimes described as aching and cramping. These sensations will usually subside as the treatment area becomes cool and numb. Immediately after treatment, the treatment area will be cold, red, and firm. There may be some transient blanching and mild bruising. As blood perfuses the treatment site, there will be tingling and stinging sensations. Finally, it is important to prepare the patient for common side effects in the days and weeks after treatment. There may be continued redness, bruising, and swelling. The treatment area will be ended with ongoing sensations of itching, sensitivity, and tingling. Numbness in the treatment site may last several weeks but will self-resolve.



**Figure 56.10** Treatment method for inner thigh cryolipolysis. (a) Treatment area is assessed with the patient standing and the untreated leg slightly raised. (b) The peak of the treatment site is marked. (c) The gelpad is placed over the treatment area, ensuring the peak of the bulge is covered. (d) The CoolFit applicator is placed over the treatment area. (e) During the treatment, the patient is seated with the leg slightly bent. (f) After treatment, the treatment area is massaged for 2 minutes.





**Figure 56.11** Post-treatment photographs of an abdomen treatment demonstrate the stiff “butter stick” nature of the tissue (a) immediately following cryolipolysis, followed by resolution at (b) 2, (c) 4, and (d) 6 minutes. (From Dierickx CC et al., *Dermatol Surg* 2013; 39(8):1209–16, 2013. With permission.)

The aforementioned side effects are common and should be discussed with patients to help them understand what they are likely to experience during and after cryolipolysis treatment. There are also very rare side effects associated with cryolipolysis. Vasovagal symptoms, such as dizziness, nausea, and fainting, may occur during or immediately after treatment. Late-onset pain may begin several days post-treatment and last several weeks, causing intense pain in the treatment site. Subcutaneous indurations, hardness and nodules in the treatment site, may occur but will resolve spontaneously, typically in 3–6 months. Hyperpigmentation may occur after treatment but will self-resolve. And the most serious rare side effect is paradoxical adipose hyperplasia, a visible enlargement in the treatment area which may develop 4–5 months post-treatment and require surgical intervention.

## EFFICACY

Cryolipolysis efficacy has been measured by several techniques including ultrasound and caliper measurements, circumferential measurements, 3D quantification of volume reduction, and blinded, independent review of clinical photographs. From published clinical studies compiled in Table 56.1, the mean ultrasound measurements of fat thickness reduction ranged from 1.9 to 8.3 mm and 10.3% to 25.5%. The mean caliper measurements of fat thickness reduction ranged

from 14.7% to 23.0% reduction. Single studies showed mean 0.9 cm circumferential reduction in the inner thigh, 2.4 cm circumferential reduction in the flanks, and 39.6 cm<sup>3</sup> volumetric reduction in treated flanks. Blinded, independent photo review was conducted in several studies with correct identification of baseline photographs ranging from 79.0% to 94.4%.

Based on the compilation of these various studies, the overall mean ultrasound fat layer reduction thickness was 20.5% and 4.6 mm. Compiled mean caliper fat layer reduction was 22.5%. The independent photo review was 88.4% correct, on average. Representative efficacy results are shown in Figures 56.12 through 56.17.

## CLINICAL PEARLS

The key steps for cryolipolysis success are patient counseling and realistically setting expectations. In the patient consultations, it is important to inform patients of the sensations they will experience during and after cryolipolysis, so they will be well-prepared and not fearful of the unfamiliar sensations. Also, it's important to set realistic expectations for cryolipolysis results. In general, patient selection is important since there will be modest reductions in fat and cryolipolysis should not be regarded as a method for weight loss. When putting together a treatment plan, some patients may need multiple treatments over the same area or treatments over several areas

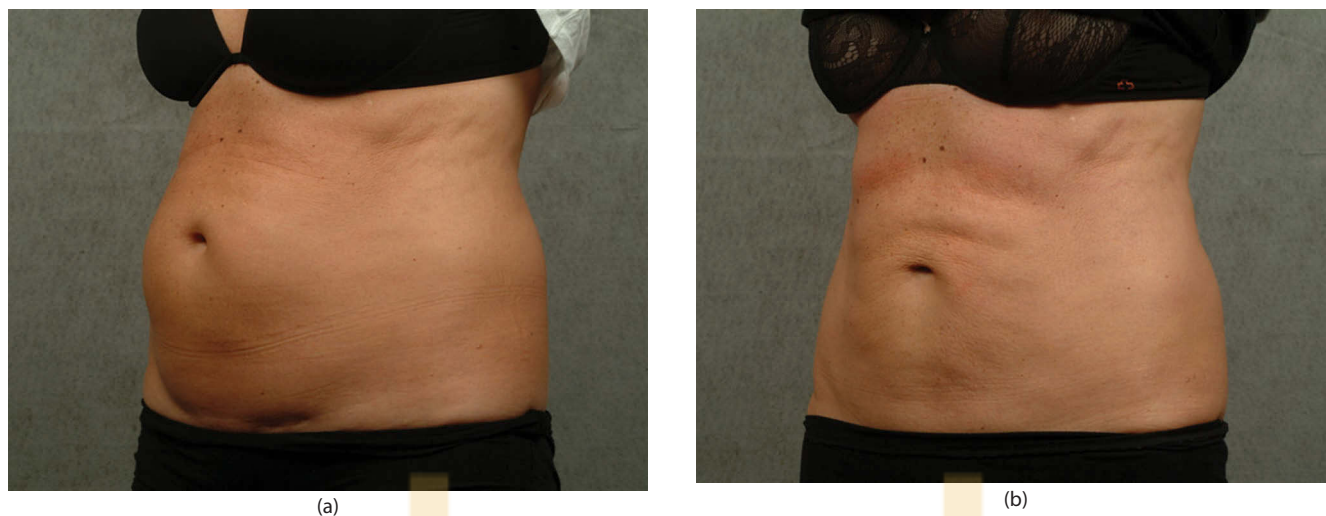
**Table 56.1** Efficacy Measurements Summarized

Efficacy Measurement Method	Treatment Sites	Reference	n	Mean Value	Compilation Mean	Range
Ultrasound fat layer reduction (%)	Inner thighs	Boey et al., 2014	11	20%	20.5%	10.3%–25.5%
	Abdomen	Sasaki et al., 2014	6	19.6%		
	Abdomen	Boey et al., 2014	10	14.9% (massage)		
	Abdomen	Boey and Wasilenchuk, 2014	10	10.3% (non-massage)		
	Various	Kotlus and Mok, 2013	59	25%		
	Pseudogynecomastia	Munavalli, 2013	18	18.3%		
	Flanks and back	Dover et al., 2009	10	22.4%		
	Flanks	Burns et al., 2010	41	17.6%		
Ultrasound fat layer reduction (mm)	Flanks	Coleman et al., 2009	9	25.5%	4.6 mm	1.9–8.3 mm
	Inner thighs	Boey et al., 2014	11	3.3 mm		
	Outer thighs	Stevens et al., 2014	40	2.6 mm		
	Various	Kotlus and Mok, 2013	59	8.3 mm		
	Inner thighs	Zelickson et al., 2015	43	2.6 mm		
	Abdomen	Boey et al., 2014	10	2.7 mm (massage)		
	Abdomen	Boey and Wasilenchuk, 2014	10	1.9 mm (non-massage)		
Caliper fat layer reduction (%)	Various	Sasaki et al., 2014	85	21.5%	22.5%	14.7%–23.0%
	Various	Dierickx et al., 2013	518	23%		
	Abdomen and flanks	Shek et al., 2012	21	14.7% (one tx)		
	Abdomen	Shek et al., 2012	10	21.2% (two tx)		
	Flanks	Shek et al., 2012	6	17.7% (two tx)		
	Flanks	Garibyan et al., 2014	11	79%		
Independent photo review (%)	Outer thighs	Stevens et al., 2014	40	87%	88.4%	79.0%–94.4%
	Inner thighs	Zelickson et al., 2015	43	91.0%		
	Flanks	Kaminer et al., 2009	50	92%		
	Flanks	Bernstein et al., 2014	10	94.4%		
	Pseudogynecomastia	Munavalli, 2013	18	80%		
	Abdomen	Mayoral et al., 2012	20	86%		
Circumferential reduction (cm)	Inner thighs	Zelickson et al., 2015	43	0.9 cm	0.9 cm	n/a
3D circumferential measurement (cm)	Flanks	Brightman and Geronemus, 2011	1	2.4 cm	2.4 cm	n/a
3D volumetric measurement (cm <sup>3</sup> )	Flanks	Garibyan et al., 2014	11	39.6 cm <sup>3</sup>	39.6 cm <sup>3</sup>	n/a

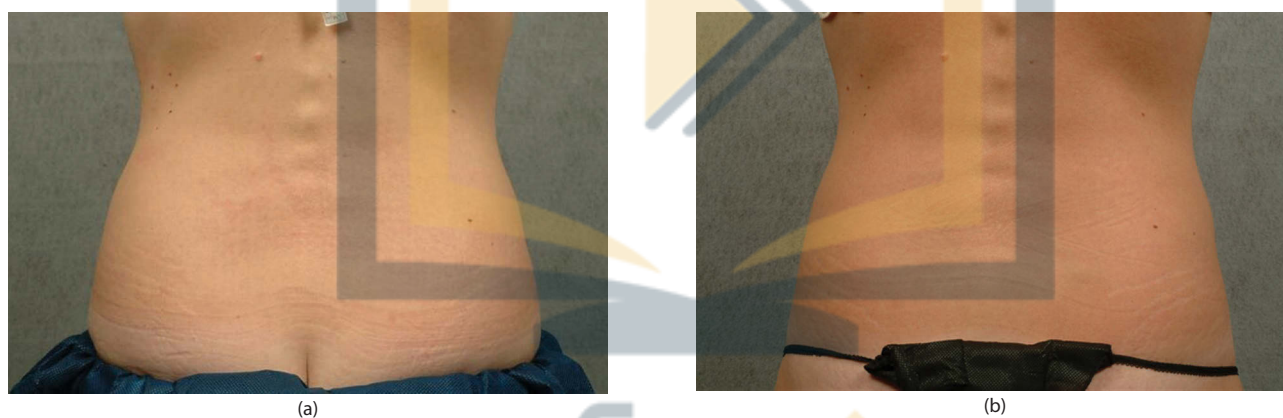
in order to achieve their body contouring goals. These plans should be discussed with the patient ahead of time to ensure realistic expectations and patient satisfaction.

One additional clinical tip is the management of late-onset pain, one of the rare side effects mentioned previously.

Late-onset pain has been described as deep pain, stabbing, shooting, and burning sensations, hypersensitivity, and severe pins and needles. Many practitioners have reported successful mitigation of these intense pain sensations by having patients wear compression garments, icing the treatment area, applying



**Figure 56.12** Abdominal treatment (a) before and (b) 3 months after treatment showing visible reduction in adipose tissue. Caliper measurements showed 2.1 cm reduction.



**Figure 56.13** Flank treatment (a) before and (b) 2.5 months after treatment, showing visible reduction in excess fat. Caliper measurements showed 1.1 cm reduction.



**Figure 56.14** Unilateral treatment of right outer thigh. (a) Pre-treatment and (b) 4 months post-treatment, demonstrating reduction of "saddlebag" bulge. (From Stevens WG, Bachelor EP, *Aesthet Surg J*; 35(1):66–71, 2015. With permission.)





**Figure 56.15** Inner thigh treatment (a,b) before and (c,d) 4 months after treatment, showing reduced inner thigh contour. (From Zelickson BD et al., *Lasers Surg Med* Jan 13, 2015. With permission.)



**Figure 56.16** Male chest (a) before and (b) 2 months after single cycle cryolipolysis treatment. (From Stevens WG, Pietrzak LK, Spring MA, *Aesthet Surg J*; 33(6):835–46, 2013. With permission.)



**Figure 56.17** Upper arm treatment (a) before and (b) 4 months after treatment, showing aesthetically pleasing reduction in subcutaneous fat. (From Stevens WG, Pietrzak LK, Spring MA, *Aesthet Surg J*; 33(6):835–46, 2013. With permission.)

heating pads, stretching, using sleep aids at night, and taking prescription analgesics.

## CONCLUSION

Cryolipolysis has proven to be a popular non-invasive body contouring method with reports of over 3,000,000 treatment cycles worldwide. In addition to being a popular treatment, cryolipolysis has clinically proven safety, efficacy, and patient tolerability. Based upon scientific evidence of adipocyte susceptibility to cold injury, this novel method of controlled cooling can reduce subcutaneous fat without surgical intervention. With proper patient assessment, treatment planning, and applicator placement, high patient satisfaction and aesthetically pleasing body contouring results can be achieved.

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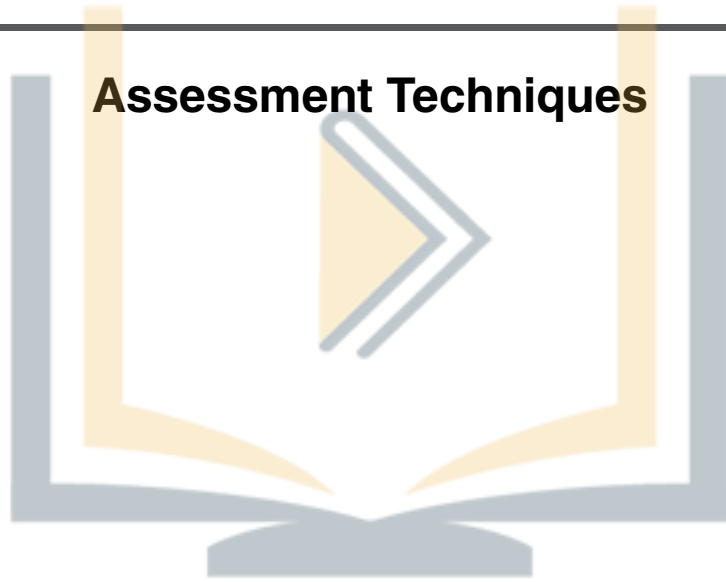


پزسک آموز

# Section VII

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**Assessment Techniques**



بزنسک آموز



پزسک آموز



# Using the Behind-the-Knee Test to Evaluate Lotion Transfer from Products to Skin

Miranda A. Farage

## INTRODUCTION

Manufacturers of consumer products consistently look for new ways in which their products may provide benefits to the consumer. A growing trend for absorbent consumer products is to incorporate lotions and emollients on the surface to improve overall consumer comfort during product use. There are a number of examples of such products currently on the market. Facial tissues impregnated with lotion have been shown to facilitate the healing of redness and dryness that may occur to the area around the nose as a result of repeated wiping when suffering from a cold or allergy (1). Benefits of topical administration of skin protectants from baby diaper topsheets clinically reduced skin erythema and skin barrier damage in infants (2). Petrolatum emollient impregnated in diapers reduced skin surface roughness, skin erythema, and diaper dermatitis compared to identical diapers without emollient (3,4). A combined zinc oxide/petrolatum (ZnO/Pet) formulation was demonstrated to actually protect skin from damage when subsequently challenged with a model irritant (0.5% sodium lauryl sulfate) under 24-h occlusive patch (5). The protective effects of this technology also provide advantages in feminine protection products (6). More recently, a cumulative irritation study was conducted on lotioned feminine pads (7). Volunteer subjects were patched on the upper arm for 21-days. Cumulative irritation scores for the sites patched with the sample feminine pads with lotion were significantly lower than the sites patched with feminine pads without lotion.

Lotion transfer is primarily a surface phenomenon. Molecules of lotion on the surface of the product transfer to the surface of the skin. Once on the skin surface, the lotion acts as a barrier to moisture loss and provides lubrication (8). It follows that, in order for lotion technologies to provide benefits for absorbent products, the lotion must first be able to transfer to the skin surface. While this point may seem obvious, it provides a unique challenge when dealing with absorbent products. After all, the main function of the product is to absorb material, not release it to the skin. A reliable means to measure the relative quantities of various lotions which transfer from absorbent products, and the potential effects of other product characteristics, is key in the development of lotion-containing products that will provide skin benefits. Measuring lotion transfer from absorbent consumer products in a traditional in-use study has proven to be challenging. Differing use patterns and habits among panelists often leads to a high degree of variability in the results.

A modification of the behind-the-knee (BTK) protocol has been utilized to evaluate lotion transfer from emollient-coated menstrual pads and pantliners. The BTK protocol

using the popliteal fossa as a test site was originally developed to evaluate both the inherent chemical irritation, and the potential for mechanical irritation of substrates and products that come into prolonged or repeated contact with the skin (9). However, we have found that this method effectively measures the amount of lotion formulations that transfer to the skin with greater consistency and reliability than measurements conducted in the course of clinical in-use studies (10). When coupled with evaluations for skin effects, the visual benefits from lotioned absorbent products can be demonstrated.

## METHODS

Both the BTK and clinical in-use protocols were conducted in accordance with the Declaration of Helsinki, and were reviewed and accepted by the Institutional Review Board of the research facility (11). All subjects reviewed and signed an informed consent prior to the study. Subjects were excluded from participation in the study if they had a history of sensitivity to skin tapes, bandages or adhesives, if they had any condition in the test area which would prevent the collection tapes from adhering to the skin (i.e. piercings, wounds, etc.), or if they were currently menstruating.

## Test Products

Menstrual pads and pantliners are constructed of a surface layer (topsheet), an absorbent core, and a moisture-impermeable backing (12). The topsheets are made from non-woven polyethylene-based or polypropylene-based copolymeric fabrics, perforated polyethylene films, or some combination of these. The absorbent core may consist of cellulose fluff pulp, or superabsorbent gel materials (AGM) designed to hold larger volumes of fluid. In addition, some menstrual products contain emollients on the surface to reduce skin irritation during use. These are applied in stripes on the topsheet that may vary in number, position and size. Test products used in specific studies are detailed in the appropriate figure or table captions.

## Pressure Measurement

Pressure sensors (Tekscan® Prosthetic Model 9801, Tekscan, Inc., South Boston, MA) were applied to the test areas. Currently marketed feminine hygiene pads were worn by the panelists in the traditional way, i.e., as in an in-use clinical study, and in a BTK application (13). Pressure was measured as participants engaged in three normal activities for 2-minute time periods: walking, sitting, and standing. The average pressure data collected for all three pads in both the genital and BTK locations was determined.

## Lotion Measurement

Lotion was quantified by gas chromatography with flame ionization detection. The thin film dressing tapes containing lotion from either the BTK or clinical in-use wear protocols were extracted for 30 min on a wrist action shaker using 10 mL of diluent. The extracts were passed through a 0.45 µm filter (13 mm Acrodisc® Syringe Filters with GHP Membrane, Pall Corporation, East Hills, NY). Samples and standards were then passed through a gas chromatographic column (a constant flow of 1.5 mL/min, initial oven at an initial temperature of 100°C, with an increase in temperature of 10°C/min up to 200°C, then 15°C/min up to the detector temperature of 325°C). Behenyl alcohol, a component of all the lotion formulations, served as a marker for lotion transfer. A calibration curve was generated based on the three standard concentrations of behenyl alcohol. (0.1, 0.05, and 0.004 mg/mL behenyl alcohol in 1L of hexane containing 50 mg tricosanol). The mass of lotion transferred was determined from the mass of behenyl alcohol and its concentration in the of lotion formulation. Recovery was 90%–110%, based on spiking of the collection tape.

## BTK Lotion Transfer Protocol

The BTK lotion transfer protocol has been described previously (8). Panels of healthy adults were enrolled for participation. Subjects were instructed to refrain from using lotions, creams, or any other skin preparation on the area of the skin to which product was to be applied. Subjects with preexisting irritation or discomfort in the area behind the knee or leg varicosities were excluded. To collect transferred lotion, sections of waterproof, thin film dressing tape (Tegaderm™ tape, 3M™, St. Paul, MN) were applied on the popliteal fossa of each leg. Test pad samples were placed on top of the collection tapes and held in place on the popliteal fossa by an elastic knee band of the appropriate size. Samples were placed randomly on the right or left test sites. Following application of the pads, subjects left the test facility and participated in normal daily activities. Specific protocol modifications for individual studies are detailed in the appropriate figure or table captions. One-way analysis of variance (ANOVAs) were conducted for the 3- and 6-hour data. A two-way ANOVA was used to compare time (two measurements), treatment (six pads), and their interaction. All computations were carried out using PC SAS®, Version 9.2.

## Clinical In-Use Protocol

The clinical in-use protocol has also been described previously (10). Healthy, adult, non-menstruating female subjects, ages 18–55, were enrolled. The subjects were randomized into three groups of 12 each, and each group was assigned to wear two of the six test products (i.e., feminine protection pads with lotion) in random order. A crossover design with a 24-hour washout period was used for the pairwise comparisons, such that each subject served as her own control. Consequently, the study had two phases, each involving 24 hours of pad wear time with one of the two comparison products to which the subject was assigned.

Immediately prior to the start of phase one, pubic hair present on the site of application was clipped by the study technician. Four sections (22 mm × 44 mm) of the thin film dressing tape were applied to each side of the labia majora (two per side) oriented parallel to the vaginal opening. Panties and test pads were dispensed, and the subjects wore the pads for 3 hours. During this time, subjects participated in normal activities of daily living. After 3 hours of wear, two of the four tape sections (in random order) were removed for determining lotion transfer. An activity log for the 3-hour wear time was

completed. After removal of two sections of collection tape, the same fresh test product was reapplied as needed by the test subject for the remainder of the total 24-hour test period (approximately 21 additional hours of wear time). Subjects were given enough test products so that they could change pads at approximately 3- to 5-hour intervals and once after overnight wear during the test period. The subjects kept a test product use log to record the times when each test product was removed, and the total number of pads used. After the pads had been worn for a total of 24 hours, subjects returned to the test facility, and the two remaining sections of collection tape were removed, to complete phase one. Following the 24-hour “washout” period, the entire process was repeated for phase two using a different product. The 3- and 24-hour data were analyzed using two-way ANOVA, involving period (i.e., pre- and post-crossover), treatment, and their interaction. For the combined data, a two-way ANOVA was used involving hour (i.e., 3- and 24-hour measurements) and treatment.

It should be noted that the clinical in-use study was a crossover design. Subjects used one test product in phase 1, and another product in phase two after a 24-hr washout period. To determine whether the washout period was effective, results were evaluated separately for the same products used during the first test period (phase 1) versus the second test period (phase 2). Significant differences between the amount of lotion transferred when the products were used first (phase 1) compared to the same products used second (phase 2), indicated the washout period was not effective. In these instances the 3-hour data were treated separately. For the 24-hour data, there were no significant differences for products tested during phase 1 vs. phase 2. Therefore, the 24-hour data for both phases were combined.

## Arm Patch Protocol

To examine the potential effects of lotions as a barrier to prevent irritation, testing was carried out on the upper arm in a previously described protocol (14). For each subject, test sites equally spaced along the upper arms (from shoulder to elbow) were identified. Each site consisted of a measured area circumscribed using indelible markers. On each arm, sample placement for test and control samples was random. Designated test sites were pretreated with weighed amounts of lotion formulations applied to the center of the test sites. Care was taken to be consistent in the amount of pressure used and the duration of the application. The lotion was allowed to penetrate the test sites for 10 minutes prior to further treatment. Lotion was not applied to the control sites. After lotion pretreatment, all test sites received a 0.3-mL patch of 0.5% sodium lauryl sulfate (SLS) using a commercially available patch (Webril patch, Professional Medical Products Company). Patches were in place for 24 hours. Evaluations of reactions on the test and control sites were conducted after a 30-minute acclimation period in a temperature- and humidity-controlled room. Sites were evaluated for transepidermal water loss (TEWL) using an evaporimeter (Dermalab, Cortex Technology, Denmark), and for erythema by visual assessment using the BTK grading scale of 0–4, where “0” is no apparent cutaneous involvement and “4” is moderate-to-severe spreading erythema (15). Test sites were evaluated prior to any treatment (baseline values) 30 minutes after removal of the 24-hour patch with SLS or saline, and daily thereafter for a total of 4 days. The average of all scores post baseline (PBA, or post-baseline average) was determined and differences between treatments were compared by ANOVA on ranks for erythema and nonparametric analysis of covariance

(ANCOVA) with baseline values as a variable for TEWL. Results were unadjusted for multiple comparisons.

### Modified Forearm Controlled Application Test Protocol

The modified forearm controlled application test (mFCAT) protocol has been described previously (1,16,17). Two test sites were identified and demarcated on the volar surface of each forearm. Treatment and sample placement was randomized among the four total test sites. In two of the four demarcated test sites, the skin of the forearm was pretreated with 24-hour patch of 0.1% SLS on study day 1. On days 2 and 3, the forearms were wiped in a controlled manner with lotioned feminine pads. Treatment with the samples on day 2 consisted of a total of 120 swipes/test site. One swipe was equal to a back-and-forth motion with sufficient pressure to insure direct contact with the skin. Five fresh pads were used to administer the 120 swipes (24 per pad). On day 3 a total of 60 swipes/test site were done. Once again, five fresh pads were used to administer the 60 swipes (12 per pad). Evaluations of reactions on the test and control sites were conducted after a 20-minute acclimation period in a temperature- and humidity-controlled room for each subject. TEWL measurement and visual assessment for erythema was conducted as described above. Dryness was evaluated on a scale of 0–6, with “0” indicating perfect skin and “6” indicating severe scaling with bleeding cracks (1). Reactions at test sites were evaluated prior to any treatment (baseline value), after patching with SLS (post-irritation value), and before and after swiping with product. Differences between the two lotion treatments were compared using stratified Cochran-Mantel-Haenszel (CMH) for erythema and dryness, and Wilcoxon signed rank for TEWL.

## RESULTS

### Comparing Relative Pressure on Test Samples

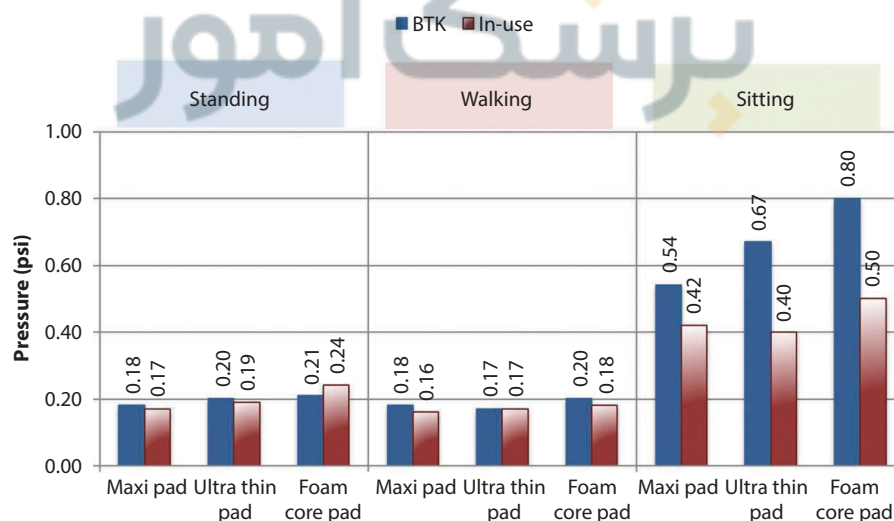
As mentioned above, lotion transfer is primarily a surface phenomenon where molecules of the lotion on the surface of

the product transfer to the surface of the skin (8). It follows that the amount of pressure exerted on the test sample could significantly alter the amount of lotion that is transferred. In order to validate the use of the BTK in lotion transfer we compared the pressure on test samples using the two application methods—in-use and BTK (13). Currently marketed feminine hygiene pads were worn by the panelists in the traditional way, i.e., as in an in-use clinical study, and in a BTK application. Pressure was measured as participants engaged in three normal activities for 2-minute time periods: walking, sitting, and standing. The average pressure data collected for all three pads in both the genital and BTK locations are shown in Figure 57.1. In both protocols, the pressure was relatively small (i.e., under 1 psi). For all three sample products, pressure measured at about 0.2 psi in both the BTK and the in-use protocol when subjects were standing or walking. When sitting, pressures were higher in both protocols: 0.5–0.8 psi in the BTK and 0.4–0.5 psi in the in-use clinical. The results obtained in this study confirm that pressure exerted from feminine hygiene pads in the BTK clinical test model is comparable to real product wear conditions; pressure was virtually identical for standing and walking positions, and somewhat higher for the sitting position.

### Transfer as a Function of the Amount of Lotion on the Pad

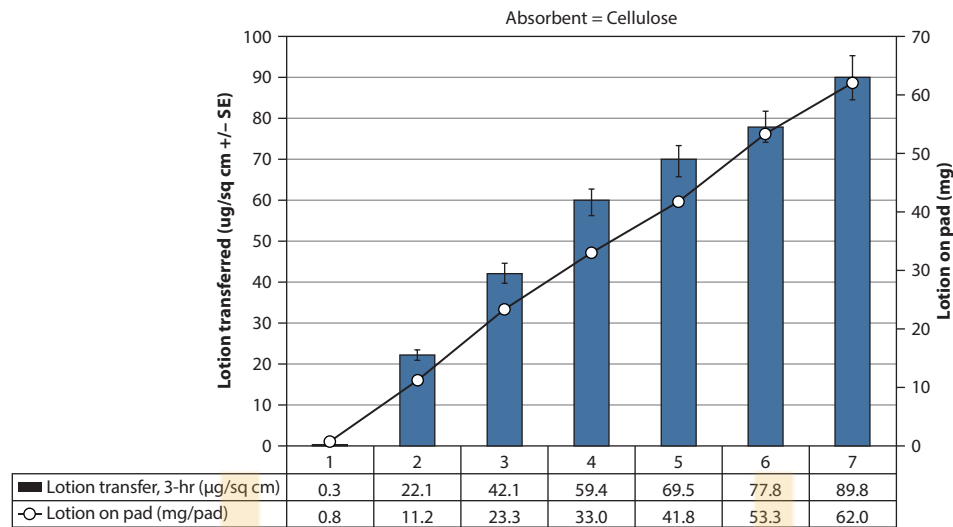
Since lotion transfer is a surface phenomenon, there will be a range of concentrations of lotion on the pad where the amount of transferred lotion will increase proportionally. There will also be a range of concentrations where no additional lotion will transfer, i.e., where the surface of the collection tape is full. In this range, additional lotion, either from an increase in concentration on the pad, extended wear time, or from multiple applications of fresh pads, will not result in additional lotion transferred to the collection tape. Studies were conducted to define the range of lotion concentrations and exposure durations that could be utilized in the BTK.

A test product series was constructed to evaluate the effective concentration range for lotion transfer (18). These



**Figure 57.1** Comparison of pressure exerted by the sample in the BTK and in-use clinical studies. Participants engaged in three normal activities for 2-minute time periods: standing ( $n=3$ ), walking ( $n=4$ ), and sitting ( $n=4$ ). Multiple pressure readings were taken for each participant during the 2-minute activity interval and averaged for the individual. Individual averages were used to calculate an overall average pressure in psi. This study has been detailed in an earlier publication (13).





**Figure 57.2** Lotion transfer as a function of starting amount on pad. Lotion transfer from Test Product Series I was evaluated using the BTK. These test products were identical in construction, with a cellulose absorbent core and a pad thickness of 7 mm. Lotion was applied to the pads in a regular configuration of 5 stripes of 5 mm width each. The amount of lotion on the pads ranged from 0.8 to 62 mg/pad, as shown in the table at the bottom of the graph. Two sections of collection tape were affixed to the skin at each test site, followed by application of the test product samples. After 3 hours of exposure in the BTK, the test sample was removed, and one piece of collection tape was removed from each test site for analysis. A fresh test product was applied for 3 more hours (i.e., a total of 6 hours of application), following which the second collection tape was removed and analyzed. The amount of lotion transferred (in µg/cm<sup>2</sup>) is compared to the starting amount of lotion on the pad (in mg/pad) for each of the test products. This study has been detailed in an earlier publication (18).

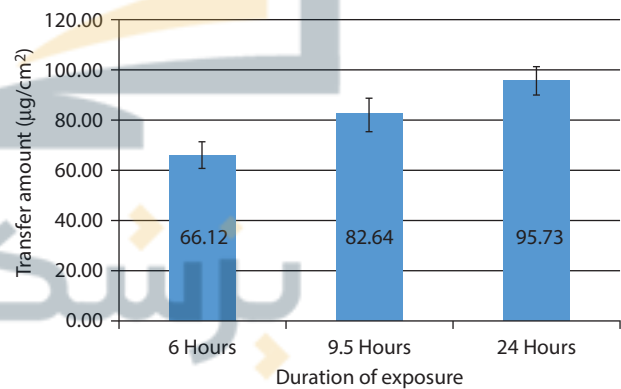
menstrual pad samples (Test Product Series I) were identical in every respect except the amount or lotion applied to the surface. As shown in Figure 57.2, for this range of lotion concentrations (0.8–62 mg/pad, as shown in the table at the bottom of the graph), the amount of lotion which transferred to the collection tape in the BTK after a 3-hour application was directly proportional to the amount which was applied to the pad. The 6-hour sample application showed similar results (data not shown). The data confirm that the range of lotion quantities tested (0.8–62 mg/pad) resulted in effective transfer of the lotion to the surface in a linear fashion.

**Transfer as a Function of Duration of Exposure**

This test was conducting using identical pantiliner test product samples with different durations of exposure in the BTK. The pantiliner test product contained 22.7 mg/pad on the surface, and samples were evaluated after 6, 9.5, and 24 hours. As shown in Figure 57.3, the product transferred increasing amounts of lotion with longer durations of exposure: 66.12 ± 5.23 µg/cm<sup>2</sup> of lotion with 6-hour exposure, 82.64 ± 6.54 µg/cm<sup>2</sup> at 9.5 hours, and 95.73 ± 5.23 µg/cm<sup>2</sup> at 24 hours.

**Influence of the Absorbent Core on Lotion Transfer**

The two basic options for absorbent cores have very different chemical and absorbent characteristics. Cellulose material absorbs by capillary action whereas AGM absorbs by ionic action. If the lotion is composed of nonpolar components, AGM will have a lower affinity for the lotion and therefore release it more easily. Thus, the type of absorbent core would be expected to influence the amount of lotion that will transfer from the product. Further, because of the superior absorbent qualities of AGM,



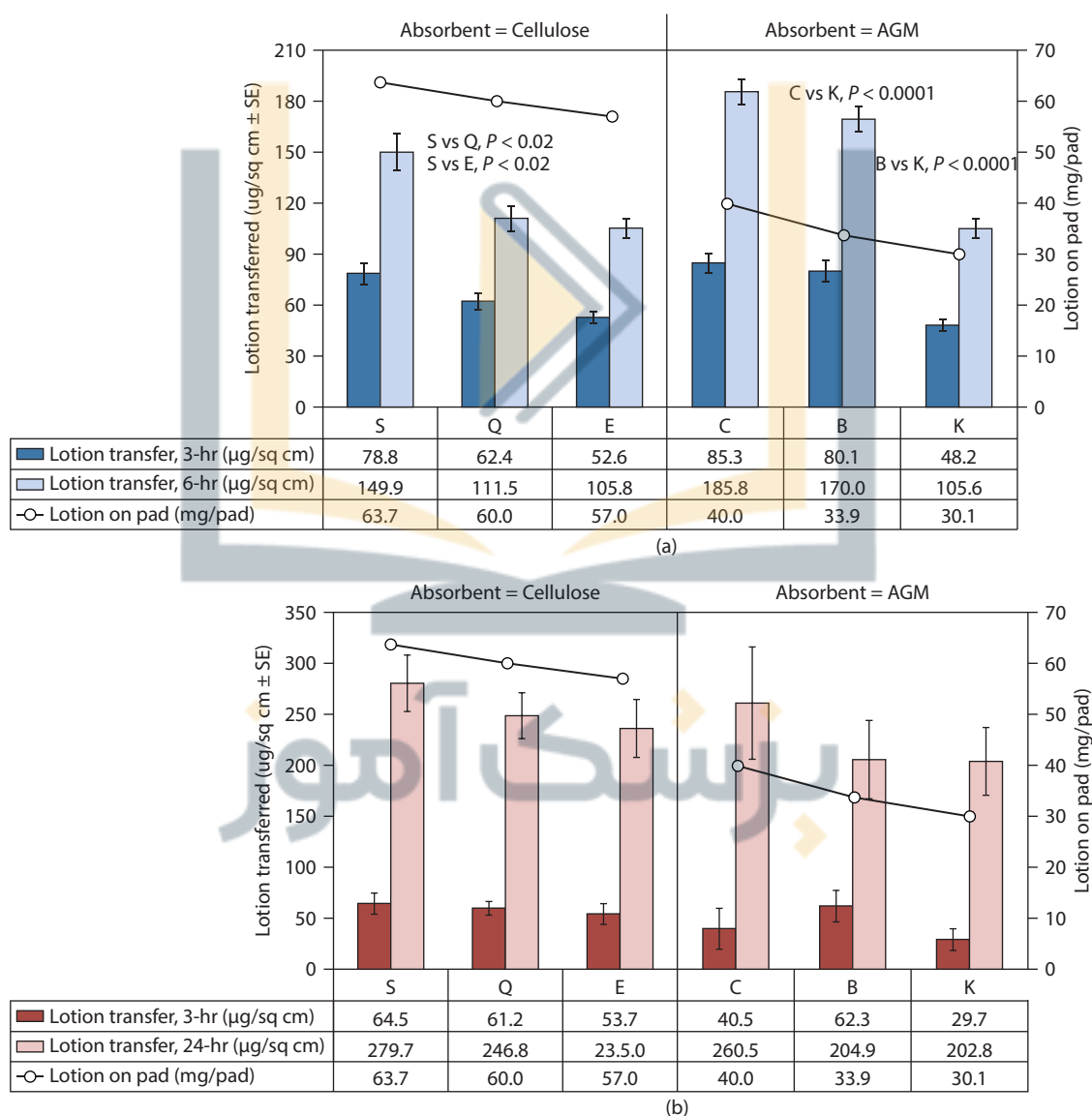
**Figure 57.3** Lotion transfer as a function of duration of exposure. Lotion transfer was evaluated in the BTK from a pantiliner test product with an AGM absorbent core and a pad thickness of 1.8 mm. Lotion was applied in 10 stripes of 4 mm width each in the crosswise direction on the product. The total lotion concentration on the pantliners was 22.7 mg/pad. Two sections of collection tape were affixed to the skin at each test site, followed by application of the test product samples, followed by application of the pantiliner test product samples. After 6 hours, one sample was removed and one of the two sections of tape on that side was carefully collected for analysis. The same pantiliner sample was placed back on the same site for a further 3.5 hours. At this time (i.e., after 9.5 hours of exposure on that site), this same pantiliner sample was removed and the other tape section on that side collected for analysis. After 24 hours, the pantiliner on the other leg was removed and both collection tape sections were removed.

pads constructed with this material can be much thinner than those constructed with cellulose cores (2 mm compared to 7–9 mm, respectively). The three AGM pads were only 2 mm thick, whereas those with cellulose cores ranged from 7 to 9 mm thick.

A second test product series (Test Product Series II) was constructed to compare a number of product differences, including the cellulose and AGM cores, pad thickness, and lotion concentrations and stripe configuration (10,19). The three cellulose core products constructed for the test had concentrations of lotion of 63.7, 60.0, and 57.0 mg/pad. The three AGM products contained 40.0, 33.9, and 30.1 mg/pad lotion. These products were tested in both the BTK and a clinical in-use study to measure lotion transfer.

In the BTK, pads with an AGM core transferred more lotion than those with the more traditional cellulose absorbent core. Figure 57.4a shows data from both the 3-hour and 6-hour collection. As shown, even though the cellulose pads had more lotion applied to the surface, they tended to transfer less lotion to the collection tapes than the AGM pads.

The clinical in-use study results showed a similar trend, although the data were less consistent (Figure 57.4b). No particular trend was obvious at the 3-hour collection point. However, after 24-hour exposure, the AGM pads released similar amounts of lotion compared to the cellulose pad, even though the starting amounts on the AGM pads were greatly reduced compared to the cellulose pads.



**Figure 57.4** Lotion transfer as a function of absorbent material. Lotion transfer from Test Product Series II was evaluated using the BTK and the clinical in-use protocol. The figure shows the amount of lotion transferred (in µg/cm<sup>2</sup>) compared to the starting amount of lotion on the pad (in mg/pad) for both types of absorbent cores: Cellulose and AGM. The three cellulose core products (S, Q, and E) had pad thicknesses of 7, 9, and 6 mm, with lotion concentrations of 63.7, 57.0, and 57.0 mg/pad. The three AGM products (C, B, and K) had pad thicknesses of 2 mm, with lotion concentrations of 40.0, 33.9, and 30.1 mg/pad. This study has been detailed in earlier publications (10,19). (a) Lotion transfer after 3 and 6 hours in the BTK protocol. Pairwise comparisons resulted in the significant differences shown. (b) Lotion transfer after 3 and 24 hours in the clinical in-use protocol. Pairwise comparisons resulted in no significant differences. (Only Phase 1 of the cross-over study is reported for the 3-hr timepoint.)

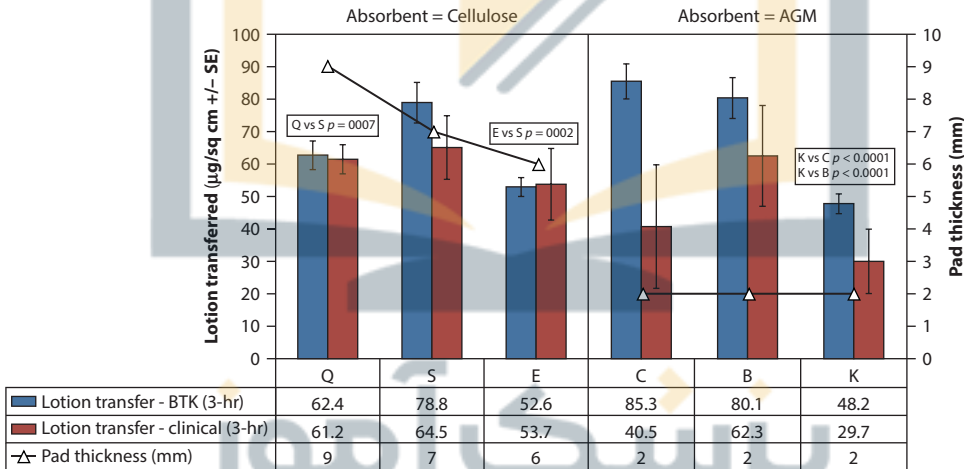
**Influence of Pad Thickness on Lotion Transfer**

The most likely explanation for the results shown in Figure 57.4 is the chemical differences between the AGM and cellulose materials. However, an alternative possibility is that the AGM pads transfer more lotion because they are thinner. If this were the case and thinner pads transfer lotion better, one would expect that transfer from the cellulose core pads would be smallest from pad Q (at 9 mm thick), intermediate from pad S (at 7 mm thick) and greatest from pad E (at 6 mm thick). This was not the case, as shown in the Figure 57.5. Among the cellulose core products (S, Q, and E), the one with the most lotion on the pad to start (pad S with 63.7 mg lotion and a thickness of 7 mm) transferred directionally more lotion to the skin in both the BTK and clinical, although the differences were not statistically significant in either protocol. The thicker cellulose pad Q (9 mm) and the thinner cellulose pad E (6 mm) both transferred an amount that was directionally less than pad S.

**Comparison of the Two Test Protocols for Measuring Lotion Transfer: BTK vs. Clinical In-Use**

When the two test methods for measuring lotion transfer were compared, the BTK and the clinical in-use studies yielded similar results; however, the BTK was more consistent. This

is undoubtedly due to the greater number of variables that impact the in-use studies that do not impact the BTK protocol (reviewed in Table 57.1). First, for the 24-hour test time, the number of pads used differs for different panelists. Differences in daily activities may also influence transfer amounts. In addition, even though the panelists all wear the identical type of panties issued by the test facility, the amount of pressure on the test site would vary depending on the fit of the panties (tight or loose) and the clothing preferences of the panelists (i.e., pantyhose, tight jeans, or skirts). Further, the pressure would change throughout the 24-hour period as the panelist changes from regular clothing to loose-fitting sleepwear. The protocol used in the BTK would result in a more consistent degree of pressure for individual panelists throughout the course of the study. Finally, the clinical protocol design results in a high potential for removal of transferred lotion from the tapes during bathroom visits and other activities. Obviously some external factors, such as sweating, must have had an effect on the amount of lotion either transferred to the tape or the amount remaining on the tape at the 3-hour time point in the clinical study. Supporting this notion is the observation that with some individual panelists, the amount of lotion transferred after 24 hours was actually less than that transferred after 3 hours (data not shown).



**Figure 57.5** Lotion transfer as a function of pad thickness. Lotion transfer (in  $\mu\text{g}/\text{cm}^2$ ) from Test Product Series II, as measured in the BTK and clinical in-use studies after 3 hours of collection, is shown as a function of the thickness of the test pads (in mm). The test pads were described in Figure 57.4 caption. This study has been detailed in earlier publications (10,19). Pairwise comparisons of lotion transfer after 3 hours in the BTK protocol resulted in the significant differences shown. Lotion transfer after 3 and 24 hours in the clinical in-use protocol. Pairwise comparisons in the in-use clinical resulted in a significant difference between products Q and S ( $p=0.0017$ ). This study has been detailed in earlier publications (10,19).

**Table 57.1** Characteristics of the Two Clinical Test Protocols

BTK -Lotion Transfer Clinical Test	In-Use Clinical
Varying number of applications over the 24 hour test period. Conducting different activities during the test would have minimal effects due to the method of product application. Application pressure of the sample is similar for all panelists due to the use of elastic knee braces in application. Application pressure of the sample for each panelist is consistent throughout the duration of the test.	Varying number of applications over the 24 hour test period. The effects of different activities on lotion transfer are unknown.  Application pressure of the sample will vary among panelists depending on the fit of panties and clothing choices. Application pressure of the sample for each panelist throughout the test as the panelists change clothing, particularly as the panelists dress in loose-fitting sleepwear.
There is no potential for accidental removal of the transferred lotion by the panelists.	There is potential for accidental removal of the transferred lotion during bathroom visits and other activities.

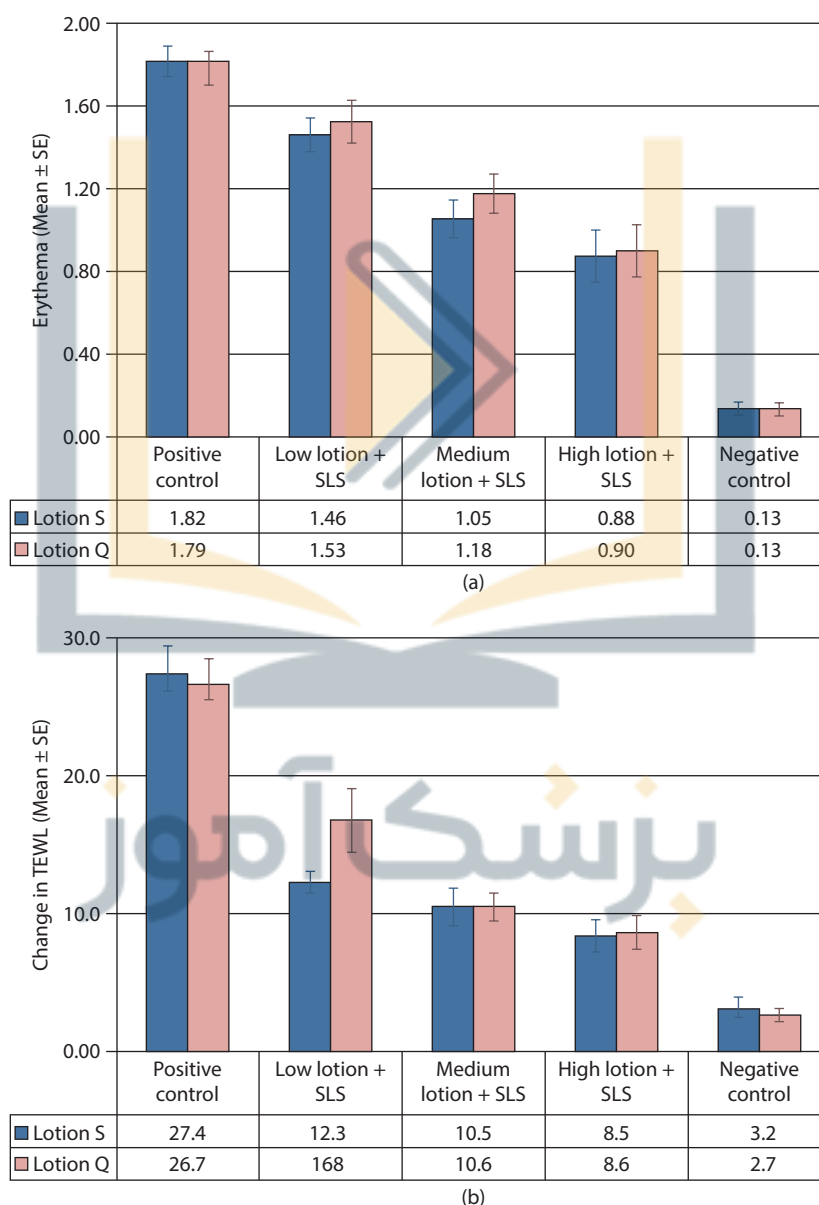


### Skin Benefits of Lotion at Transferred Concentrations

Lotion transfer is measured by the transfer of lotion from an absorbent product to the surface of the collection tape. In order to evaluate the potential benefits of lotion at these quantities, we conducted arm patch tests using a model irritant (SLS) on skin sites pretreated with lotion in various quantities. In the first study, the effect of lotion as an irritant barrier was evaluated. Two different lotion formulations were applied to demarcated skin sites on the upper arm at concentrations of 165, 495, and 825  $\mu\text{g}/\text{cm}^2$  (14). Sites were then patched with 0.3 mL of

0.5% SLS for 24 hours. The resulting erythema and change in TEWL are presented in Figure 57.6. Both lotions produced a dose-dependent decrease in the effects of the irritant as assessed by both the erythema (Figure 57.6a) and the change in TEWL (Figure 57.6b).

In the second study, the effect of lotion as a moisture barrier was evaluated. A prototype test lotion was applied at four concentrations; 170, 140, 110, and 80  $\mu\text{g}/\text{cm}^2$ . One site was left untreated with lotion to serve as a control. After lotion treatment, patches consisting of pantiliner cutouts saturated with saline were applied for 3 hours. TEWL measurements were



**Figure 57.6** Effects of different lotion amounts on lotion barrier effects. Arm patch studies were conducted on the lotion formulations Q and S. Test sites were pretreated with three concentrations of lotion (low = 165  $\mu\text{g}/\text{cm}^2$ , medium = 495  $\mu\text{g}/\text{cm}^2$ , high = 825  $\mu\text{g}/\text{cm}^2$ ), then patched with 0.3mL of 0.5% SLS. Control sites received no pretreatment. The positive control was patched with SLS. The negative control was patched with saline. Sites were evaluated for erythema (a) and TEWL (b). The average of all scores post baseline are plotted. (a) Erythema: Means for all treatments with lotions S and Q (high, medium, and low) were significantly lower than the mean for the positive control ( $p \leq 0.05$ ). (b) Change in TEWL: The change in means for all treatments with lotions S and Q (high, medium, and low) were significantly lower than for the positive control ( $p \leq 0.05$ ). This study has been detailed in an earlier publication (14).

conducted prior to any treatment (baseline), immediately after patch removal. A reduction of the TEWL value reflects reduced water uptake by the skin and thus protection against over hydration. The results shown in Figure 57.7 indicate that all lotion concentrations provided protection against moisture uptake by the skin. TEWL measurements taken immediately after patch removal (0 minutes) at all lotion-treated sites showed a significantly lower increase in TEWL compared to the non-lotion-treated site. Subsequent measurements taken at 5, 10, and 15 minutes after patch removal (data not shown) indicated that the TEWL had recovered substantially at all sites, including the non-lotion-treated sites. However the site treated with 170  $\mu\text{g}/\text{cm}^2$  continued to have a significantly lower TEWL than the non-lotion control site.

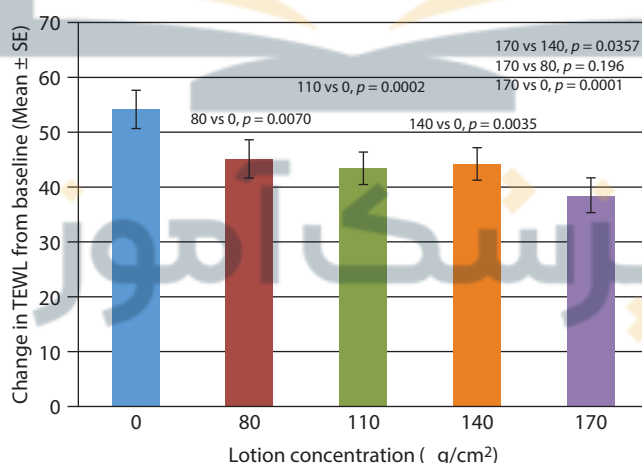
The potential of lotioned products to have healing effects on irritated skin were evaluated using an mFCAT protocol (20). Identified sites on the forearm were patched with a model irritant (0.1% SLS) for 24 hours on day 1. On days 2 and 3 patching, the test sites treated by swiping lotioned feminine pads on the patched sites in a manner described in the “methods” section. Figure 57.8 presents resulting evaluations for erythema, dryness, and TEWL. Prior to any treatment there was no evidence of erythema or dryness at any of the test sites (baseline values). After application of the SLS irritant patch for 24 hours, the skin sites showed a low level of irritation, as reflected by increases in mean scores for erythema, dryness, and TEWL (shown as post-irritation values). Test sites patched with saline instead of SLS showed no evidence of irritation. The first treatment with either test product (post-treatment 1 values) did not reduce the level of erythema at the test sites (Figure 57.8a), but

did reduce the level of dryness (Figure 57.8b). TEWL was not affected by the first treatment (Figure 57.8c). With the second treatment both test products reduced the levels of erythema and dryness, indicating that the application of lotion promoted a return toward baseline values (shown as pre-treatment 2 and post-treatment 2 values). TEWL was not demonstrably affected by the second treatment.

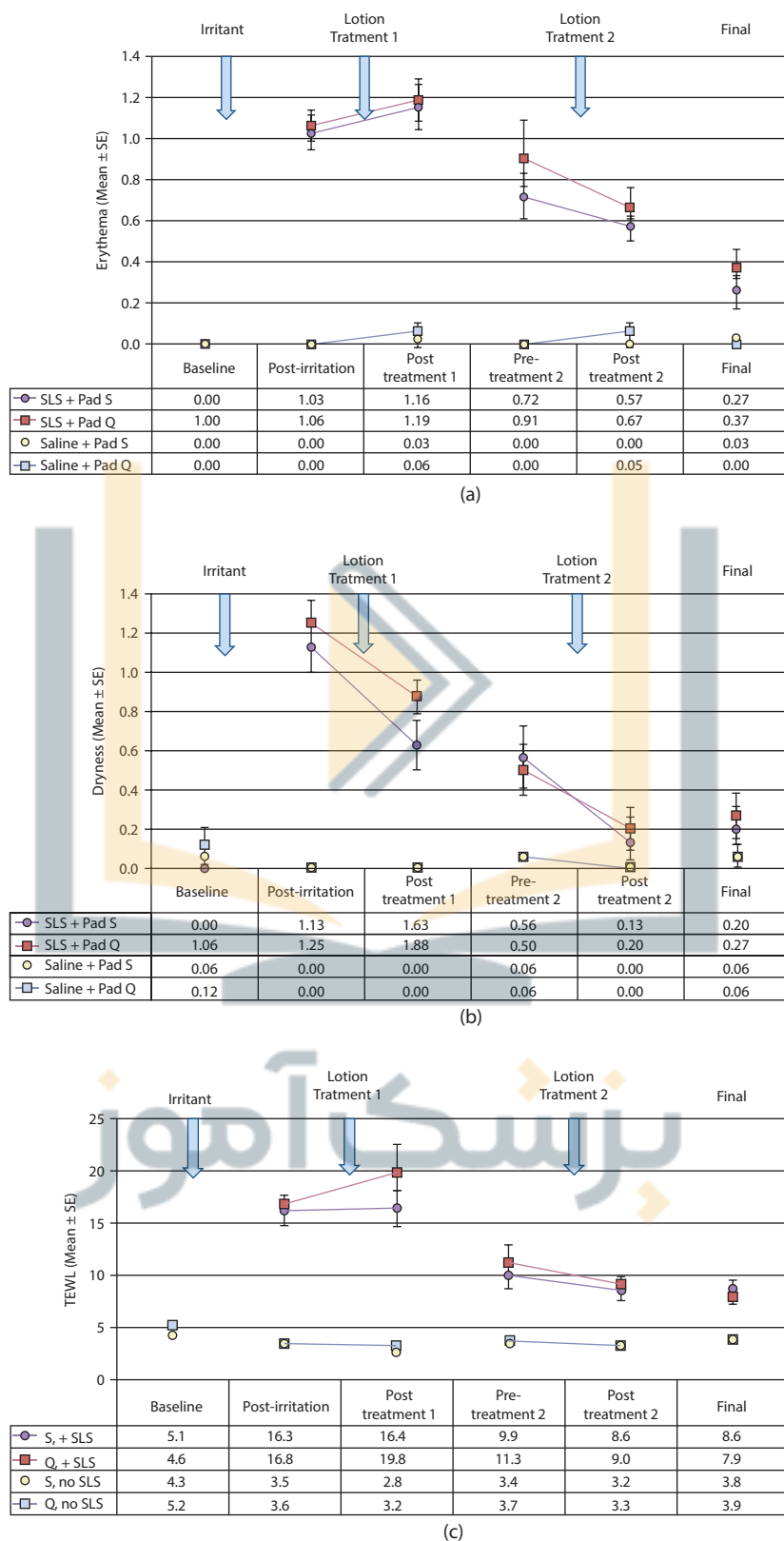
Other investigations have provided evidence of skin benefits from topically applied lotion. Feminine pads were used in forearm application where pads were swiped across the surface of the skin in a controlled fashion (8). The coefficient of friction was evaluated as an indication of the resulting skin moisturization at 5 and 10 minutes after treatment. Compared to two control sites (i.e., no treatment or treatment with a non-lotioned test pad), sites treated with lotioned pads showed a significant increase in skin moisturization at both time points, as indicated by an increase in the coefficient of friction.

## CONCLUSIONS

Both the BTK and the clinical protocol provide a means of evaluating the transfer of lotion formulations from feminine protection pads. However, this adaptation of the BTK test method provides more consistent results at a fraction of the cost. Further, we have developed a number of simple test protocols to determine the potential benefits of lotion on the skin at amounts comparable to the transfer amounts determined in the BTK. This includes protocols to evaluate moisture barrier effects, visible signs of irritation, and healing effects after application of an irritant.



**Figure 57.7** Effects of lotion on moisture barrier. Areas on the volar surface of the forearm of adult, female volunteer subjects were treated with four levels of a prototype test lotion (170, 140, 110 and 80  $\mu\text{g}/\text{cm}^2$ ). One site was left untreated with lotion to serve as a control. Following the application of the lotion, patches made of full-thickness cutouts from the pantiliner were pre-wetted with water and applied to the skin test sites. The wet patches were kept in place for 3 h. TEWL measurements were conducted prior to any treatment and immediately after pantiliner removal. The change in TEWL from baseline for each lotion concentration is plotted. Statistical analyses were conducted using generalized linear mixed models (GLMM) with subject as a random effect and baseline TEWL as a covariate. Results were adjusted for a small sample bias (21).



**Figure 57.8** Healing effects of lotion on skin compromised with an irritant in the mFCAT. Modified FCAT studies were conducted on two products with different lotion formulations (Q and S). Test sites on the volar forearm were pretreated via 24-h patch with 0.1 percent SLS. Control sites were treated with saline. On days 2 and 3, the forearms were wiped in a controlled manner with test pads Q or S, as described in the methods sections. Reactions were evaluated for erythema (a), dryness (b), and TEWL (c). The graph shows the mean scores for each day before and after treatment with lotioned product. There were no significant differences between the two test pads in mean erythema, dryness, or TEWL. This study has been detailed in an earlier publication (14).



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# Assessing the Efficacy of Moisturizers

Whitney Hannon

## INTRODUCTION

This chapter gives an introductory background to practitioners in cosmetic dermatology who wish to better understand the science behind efficacy claims for moisturizers. In an age of evidence-based medicine and savvy consumers, it is increasingly important to be able to understand, critically evaluate, and summarize the experiments used to evaluate the efficacy of moisturizers.

One of the first challenges to the clinician is actually obtaining information about moisturizer efficacy. Most of the studies are not published because they are proprietary information. Information can be requested from companies but can be time-consuming to obtain. The information that is readily available is often incomplete (for example, only available in abstract form) or in difficult or expensive to access journals or books. Once an article is obtained, the clinician is faced with the second challenge of understanding a study that is often filled with technical jargon and unfamiliar instruments. Once the study is deciphered, the third challenge is for the clinician to critically appraise the validity of the information. For example, the clinician needs to ask, "Was the experimental design of this study of sufficient quality to warrant trusting the conclusions?" Examples of recommended moisturizer study designs are summarized by Grove (1). Fourthly, complex machines are often used to make skin measurements. The clinician needs to ask, "Was the machine used properly in this experiment?" and on a more advanced level, "did this machine really accurately

measure the variable claimed during the experiment?" A checklist of other important questions to ask when assessing the performance and validity of a machine has been developed by Serup (2).

Besides the difficulties mentioned, there are other significant but more subtle issues that need to be taken into account. For example, moisturizers are often complex mixtures of ingredients that may have interactive properties. In other words, the composition of a moisturizer is more complex than simply the sum of its independent ingredients. Interactions may occur between water-binding substances (3) and theoretically also between any of the other ingredients including the vehicle. In addition, it has been observed that the individual component has different properties when measured individually than when measured as part of a combination (4).

In summary, the quest to obtain reliable, useful information on the efficacy of moisturizers encounters three main categories of difficulties: problems with the availability/accessibility of information, problems with the experimental design, and problems with the measurement technologies. Table 58.1 lists specific issues in each of these categories, and the implications that these problems could have on the interpretation and validity of the information. This chapter attempts to assist the busy clinician to overcome these challenges by reviewing the literature, translating some of the jargon and identifying some of the most useful and comprehensive evidence-based references.

**Table 58.1** Problems with Moisturizer Efficacy Studies

	Issues	Implications
<i>Information access</i>	Journals/books not easily accessible; full details not published	Missing data, publication bias
<i>Experimental design</i>		
Patient selection	Volunteers Histories not clearly stated, age/sex not always stated	May not be representative of consumer population. Population in unknown; biased population; clinical extrapolation is difficult
Study size	Tends to be small	May not be enough subjects to satisfy study objective
Controls	Often inadequate Most studies only measured on one side rather than contralateral side Often not stated whether or not other moisturizers/beauty products were used	Cannot account for changes during experiment Need to control for variation on different sides of body Effects may be due to other moisturizers
Materials studied	Often studies not blinded and no placebo group Materials used or concentrations not always stated Range of concentrations often was not studied Complex mixtures studied, not broken down into parts so that they could be compared and evaluated	Potential for bias Cannot compare studies easily; moisturizer effects are presumably dose-responsive No information on dose-response Unable to separate effects

(Continued)

**Table 58.1** Problems with Moisturizer Efficacy Studies (*Continued*)

	Issues	Implications
Measurements	Not enough time points Often did not assess both TEWL and SC at the same time Three-prong approach often not used: panelist self-appraisal, expert grader evaluation and relevant instrumental measures	Gaps in information about time-course Cannot make conclusions about hydration state
Statistics	Statistics not always used to analyze data If statistics used, <i>p</i> values not always stated	Comparisons have no scientific basis No knowledge of level of significance
General	Not enough studies Few materials studied	No verification of findings Large amount of materials have unknown efficacy
Bioengineering methods		
Operator-dependent	Potentially improper use of machines Some studies have no statement of ambient conditions	Misleading data Misleading data

Source: Hannon W, in *Textbook of Cosmetic Dermatology, Fourth Edition*, Robert Baran and Howard I Maibach, eds., London: Informa Health Care, 2009. Also some information in original table was derived from text of Grove GL, in *Clinical Safety and Efficacy Testing of Cosmetics*, C Waggoner, ed., New York: Marcel Dekker, 1990, 121-48.

## SUMMARY OF BIOENGINEERING TECHNIQUES

A variety of bioengineering techniques have been used to assess the efficacy of moisturizers on the human stratum corneum. These techniques can be divided into measurements of:

1. Skin surface contour
2. Desquamation
3. Elasticity (parallel to skin plane)
4. Elasticity (perpendicular to skin plane)
5. Other mechanical techniques
6. Indirect electrical properties
7. Spectroscopy or thermal transfer
8. Transepidermal water loss (TEWL)
9. Stratum corneum imaging
10. Optical characterization of skin properties

The Appendix to this chapter gives details of the techniques, some of the researchers involved in the development of the technology, names of different machines, variables measured, principles behind the technologies, and their respective advantages and disadvantages.

Many authors (5-7) have reviewed and compared these technologies. Marks (8) took a unique approach by using an arbitrary scale to compare the reproducibility, sensitivity, and directness of measurement, capability for quantization, standardization, cost-effectiveness, ease of use, and convenience.

## SUMMARY OF EFFICACY DATA BASED ON STUDY TYPE, BIOENGINEERING TECHNIQUE, AND MOISTURIZER TYPE

Gabard (9) proposed a useful classification for studies on the efficacy of moisturizers. He divides the studies into five main types:

1. Single application to normal skin
2. Multiple applications of moisturizers over time to normal skin
3. Moisturizer applied to experimentally irritated skin (one large irritant insult)

4. Moisturizer applied to experimentally irritated skin (mild irritant applied repeatedly over time)
5. Clinical studies in which moisturizer was applied to groups of patients with various conditions

To allow evidence-based comparisons, studies can be further divided by the bioengineering technique used to assess the moisturizer and types of moisturizer studied. Some of the more comprehensive reviews that include these important variables and their conclusions are summarized in Table 58.2 and the paragraphs that follow.

Agner (10) reviewed the literature on moisturizer efficacy studies and summarized the findings. Studies reported were of Gabard study experimental design type 1, 2, 3, 4, and 5. The bioengineering technique used in these studies was predominantly TEWL. Various proprietary formulations of moisturizers were reported in the studies.

Hannon (11) reviewed moisturizer efficacy data as measured by the indirect electrical techniques (capacitance, conductance, impedance) in detail and identified 20 studies of Gabard study design type 1 (single application of moisturizer to normal skin). The conclusions are summarized as follows. Glycerol, urea, and petrolatum were the best-studied substances. One application of urea, glycerol, petrolatum, hyaluronic acid, or hydrogenated phosphatidylcholine was capable of increasing stratum corneum hydration for at least several hours: urea for at least 6 hours even if washed off, glycerol for at least 6 hours even if wiped off, and petrolatum for at least 2 hours (but not if it is wiped off). Water itself had a hydrating effect in the short term but eventually resulted in dehydration. The studies had many limitations, especially in the area of controls. For example, many studies did not control for vehicle type (a very important variable) or ingredient interactions. Others failed to control for the following complexities associated with indirect electric measurements: for example, that 1) the capacitance ratio (moisturizer-treated-skin capacitance/untreated-skin capacitance) varies over time depending on type of moisturizer, or 2) electrical readings are not always proportional to the water present depending on the substance, or 3) each component of a moisturizer has its own electrical properties that can be a source of false positive results (12).

Sivamani (13) reviewed moisturizer efficacy data as measured by various mechanical (tribological) techniques



**Table 58.2** Selected Evidence Based Reviews of Moisturizer Efficacy Data

Review Author	year	Gabard Classification of Studies Reviewed	Bioengineering Techniques Utilized In Studies	Moisturizers Studied
Agner	2002	1,2,3,4,5	TEWL, electrical conductance, electrical capacitance, laser Doppler flowmetry, colorimetry	Various proprietary formulations and others
Hannon	2004	1	Indirect electrical techniques (capacitance, conductance, impedance)	Glycerol, urea, petrolatum, hyaluronic acid, hydrogenated phosphidylcholine and others
Agache	2006	1,2	Torsional skin elasticity	Glycerol, lactic acid, petrolatum
Sivamani	2006	1,2	Mechanical (tribological)	Various
Yokota	2006	3,4	TEWL, electrical capacitance, laser Doppler, colorimetry	Various proprietary formulations, Dimethicone, xanthan gum, petrolatum and others
Fluhr	2008	5 (psoriasis)	TEWL, capacitance, evaporimetry	Urea, alpha-hydroxyacids (glycolic acid, lactic acid) Omega fatty acids niacinamide
Crowther	2009	1,2,3,4	Confocal Raman microspectroscopy	Various proprietary formulations and barrier creams
Bauer	2010	5 (OIHD)	TEWL	Various proprietary formulations
Crozier	2010	2,3,4	TEWL	Urea, ammonium lactate, lactic acid, petrolatum, various proprietary formulations and others
Greenfield	2012	1,2,3,4,5 (hand eczema and ichthyosis)	TEWL, electrical capacitance, confocal Raman spectroscopy	Rosmarinic acid and various proprietary formulations
Hon	2012	5 (atopic dermatitis)	TEWL, D-squame, evaporimeter, stereoimage optical topometer	Lipids, borage oils, fish oil, petrolatum, sunflower oil, canola oil, urea, glycerin, mineral oil, lactic acid, ammonium lactate, PCA, various proprietary formulations and others
Loden	2012	1,2,3,4,5 (atopic dermatitis, ichthyosis)	TEWL	

and found that there were limited studies for Gabard type 1 and type 2 experimental designs. Sivamani concluded that the water and moisturizers had similar effects on the friction coefficient except the effects of the creams/emollient lasted for hours and the water effects only lasted 5–20 minutes. Important variables in studies included the temperature of creams, anatomic location, age of patient, and design of the test apparatus.

Agache (14) reviewed moisturizer efficacy data as measured by torsional measurements of skin elasticity (twistometry) for both Gabard type 1 and type 2 experimental study designs. The conclusions were based on a limited number of studies. Some of the results were somewhat contradictory. In most studies, glycerol and lactic acid had longer lasting effects than petrolatum. In one study, glycerol demonstrated increased elasticity up to 1 week. Other important variables noted were an increase in skin temperature with increased elasticity.

Yokota (15) systematically reviewed the literature published between 1992 and 2006 on the efficacy of the moisturizers in prevention and treatment of irritant dermatitis on normal skin. Results were ten studies with Gabard type 3 experimental design. The studies included outcomes measured by the following bioengineering techniques: TEWL, electrical capacitance, and laser Doppler colorimetry. The studies involved moisturizers of various proprietary formulations, dimethicone, and others, as well as vehicles and controls. Conclusions from the quantitative analysis were that direct comparisons between studies were difficult due to study designs that involved different anatomic sites and exposure durations. Two of the most comparable studies, however, by different authors, reported contradictory results.

Fluhr (16) reviewed the literature for moisturizer efficacy data in psoriasis (Gabard type 5 studies). Bioengineering

methods used in the studies were TEWL and capacitance. He summarized the known studies for the following moisturizers: urea, alpha-hydroxyacids (glycolic acid and lactic acid), and omega-fatty acids.

Crowther (17) reviewed moisturizer efficacy data as measured by confocal Raman microspectroscopy for Gabard study design types 1, 2, 3, 4. Conclusions were that little difference was observed in moisturization on day 1. Over a 2-week period, use of moisturizers containing niacinamide was associated with increased total skin hydration. Important variables to control included osmotic changes in skin based on moisturizer properties and changes in stratum corneum thickness based on different types of moisturizers.

Bauer (20) conducted a Cochrane systematic review on occupational irritant hand dermatitis (OIHD) treatments including moisturizers and barrier creams. Four randomized controlled trials (RCTs) were identified. Bioengineering techniques for measurements were predominantly TEWL. Moisturizers studied were various proprietary formulations. Of the four identified RCTs, one RCT of Gabard type 5 is relevant to the current review. This crossover design RCT showed that during the moisturizer use phase of the study, there were no cases of OIHD. However in the control phase of the study (no moisturizer use), 19 out of 93 participants developed OIHD.

Crozier (18) conducted a structured systemic review of the literature published from 2000 to 2010 on skin care regimes for normal term infants. Nine studies of Gabard types 2, 3 and 4 were identified. One study by Bartels (19) of 64 full term newborns divided into four groups reported lower TEWL in newborns that were treated with a proprietary moisturizer compared with those treated with a control of no moisturizing

cream. The other eight studies involved washing products only without use of moisturizers and therefore were not of relevance to the current review.

Greenfield (23) reviewed moisturizer efficacy data for Gabard type 1, 2, 3, 4, and 5 studies. The Gabard type 5 studies reviewed included the conditions of hand eczema and ichthyosis. Bioengineering techniques used to assess efficacy were TEWL, electrical capacitance, and confocal Raman spectroscopy. The following moisturizers were reviewed: petrolatum, urea, lactic acid, and ammonium lactate and as well as some proprietary combinations.

Hon (21) reviewed moisturizer efficacy data for atopic dermatitis (Gabard type 5). The following bioengineering techniques were used to assess efficacy: TEWL, D-squame, evaporimeter, and stereoisage optical topometer. Various proprietary moisturizers and rosmarinic acid were studied.

Loden (22) reviewed moisturizer efficacy data for Gabard type 1, 2, 3, 4, and 5 studies. The Gabard type 5 studies that were reviewed included conditions such as ichthyosis and atopic dermatitis. The bioengineering technique used to assess efficacy in the majority of studies was TEWL. The following moisturizers were reviewed: borage oil, fish oil, petrolatum, sunflower oil, canola oil, urea, glycerin, mineral oil, lactic acid, ammonium lactate, and PCA as well as some proprietary formulations.

In summary, there have been some detailed reviews of moisturizer efficacy studies that employ bioengineering techniques for assessment. More work is needed to fully summarize and analyze the extent of available information.

## APPENDIX: BIOENGINEERING TECHNIQUES FOR ASSESSING MOISTURIZER EFFICACY

1. Skin surface contour evaluation (Table 58.3)
2. Desquamation (Table 58.4)
3. Mechanical bioengineering techniques to measure elasticity (parallel to skin surface plane) (Table 58.5)
4. Mechanical bioengineering techniques measuring elasticity (perpendicular to skin surface plane) (Table 58.6)
5. Other mechanical properties (Table 58.7)
6. Indirect electrical properties (Table 58.8)
7. Spectroscopy or thermal transfer (Table 58.9)
8. Transepidermal water loss (TEWL) (Table 58.10)
9. Stratum corneum imaging (Table 58.11)
10. Optical characterization of skin properties (Table 58.12)

The Tables that follow (Table 58.3 through Table 58.12) describe each technique, developer/machines, parameters measured/calculated, principles, advantages and disadvantages.

**Table 58.3** Bioengineering Techniques for Skin Surface Contour Evaluation

Technique	Developer/ Machines	Parameters Measured/ Calculated	Principles	Advantages	Disadvantages
Low-power surface magnification (24)	8× lens magnifier	Skin surface contour	Place mineral oil on skin; cover with coverslip; observe skin under low power.	Can visualize epidermis, epidermal–dermal junction and papillary dermis. Easy, non-invasive method. Augments naked-eye observation skills.	Technique has learning curve. Hard to visualize dry, scaly skin with this technique.
Profilometry (Mechanical)	Perth-o-Meter (1971) (25) Surfometer (1975) (26) Surfcom (1979) (27) Talysurf (1979) (28) Anaglyphographe (1982) (29)	Skin surface contour; roughness parameter	Cast replica of skin in silicone rubber is measured with a computerized stylus instrument, which produces plots of data. Valley and peak profile of SC flattens with hydration.	Replica measurements give absolute data. Can evaluate hydration status.	Complex and slow process. The application of silicone rubber may disrupt the surface; fine lines may be effaced when rubber cools; scales may be removed from subject. Needs a smooth, even surface (too many hair follicles, scars, tattoos, detergents, skin damage, or scaling can increase error). Stylus geometry can introduce errors (30). Sources of inter-observer variability are high-pass filters, low-pass filters and sampling intervals (30). Expensive (31). Profilometry can identify products that decrease amount of wrinkles but does not reveal mechanisms or safety of these products (irritants, for example, decrease wrinkling). Results in 2D only; show topography in one direction only. Acquisition time = 8 minutes Accuracy <10 <sup>−3</sup> mm

(Continued)

**Table 58.3** Bioengineering Techniques for Skin Surface Contour Evaluation (*Continued*)

Technique	Developer/ Machines	Parameters Measured/ Calculated	Principles	Advantages	Disadvantages
Profilometry (optical)	Corcuff (1981) (32)	Skin surface contour; roughness parameters; wrinkle	Cast replica of skin in silicone rubber is measured with an optical scanner (laser beam).	Gives absolute data; operating time reduced over mechanical method; non- contact sensor; 3D data possible; fast acquisition time = < 1 minute.	Complex and slow process. The application of silicone rubber may disrupt the surface. Needs a smooth, even surface (too many hair follicles, scars, detergents, skin damage, or scaling can increase error); also unable to measure soft, fragile, liquid, high temperature objects; does not measure in real time; availability is limited due to sophistication. Accuracy = $10^{-3}$ mm
Laser profilometry with densitometry	Gormley (1985) (33) Barton (1987) (34)	Contour; roughness parameters; wrinkle quantification	Photographic negative of skin taken under standard light (oblique illumination with incident angle of $25^\circ$ ). Shadows formed are scanned microdensitometrically by a computer and gray level assigned. The relief is reconstructed indirectly from gray level and using appropriate algorithms, slopes and roughness parameters of relief can be calculated.	Rapid measurement of skin surface relief without cumbersome equipment (35). Good for following clinical progression of scaling disorders. Most accurate of all profilometry techniques ( $10^{-5}$ mm). Can plot $10^5$ points.	Only provides a reconstruction and not an exact image, so smaller features may be overshadowed by larger ones and omitted from analysis (35). Less sensitive in screening normal volunteers. Very slow acquisition time = 10–30 minutes. Cannot measure soft, fragile, liquid objects and objects at high temperature.
Proliferometry (interference)	Altmeyer (1995) (119) Lagarde (2001) (120) Dermatop (Eotech)	Surface contour	Calculates a phase image from the interference fringe image projection.	Can determine altitude at each point. Plots more points ( $10^6$ ) than any other proliferometry method. Fast acquisition = 1 minute.	Accuracy = $5 \times 10^{-3}$ mm
Transparency (transmission) proliferometry	De Paepe (2001) (121) Skin visiometer SV600	Thickness, surface contour	Measures the variation of absorbance which is related to transparency and therefore thickness of the replica according to Beer–Lambert's law.	Measures small plane area of 1 $\text{cm}^2$ . Can plot $10^5$ points. Very fast acquisition = <1min,	Very shallow depth of field (500 micrometers) Accuracy = $10 \times 10^{-3}$ mm
In vivo image analysis (digital image processing)	Picton (1976) (36) Taylor (1978) (37) Quantimet (36) Magiscan (37)	Surface contour	Using video camera, can record skin surface features directly. Signal is digitized using a high-speed analog/ digital converter and arranged into an array of picture points. The picture points are introduced into a digital image processor that interfaces with a minicomputer. Filters (mathematical sieves) can be used to enhance detail.	More objective, quantifiable images (shape, color) than clinic notes. Interactive; can be queried, altered, analyzed automatically and rapidly in real time. Permanent record; data easily stored. In vivo, direct measurement of surface possible. Good for evaluation of low to moderate dryness.	Inconvenient. Technique less useful for very dry skin.

(Continued)



**Table 58.3** Bioengineering Techniques for Skin Surface Contour Evaluation (*Continued*)

Technique	Developer/ Machines	Parameters Measured/ Calculated	Principles	Advantages	Disadvantages
Scanning microdensitometry of macrophotographs	Marshall (1983) (123)	Surface contour; roughness parameters	Low magnification photomicrographs are taken under standardized light scanned with microdensitometer which records shadows and highlights and produces a contour line similar to profilometry.	Good for the assessment of clinical progression in patients with scaling disorders.	Not so good for normal skin assessment; still needs additional improvements.

*Note:* Topography measurements can be used to demonstrate changes in amount of wrinkling and state of stratum corneum hydration as noted by attenuation of the relief due to increase in turgor.

**Table 58.4** Bioengineering Techniques to Measure Desquamation (38)

Technique	References	Principles	Advantages	Disadvantages
Squametry of tape strippings	Wolf (1936) (39) Jenkins (1969) (40)	Tape pressed against skin; outermost portion of skin sticks to tape and keeps topographical relationship and desquamation pattern. Tapes are processed. Scales are sized and counted. Samples stained and viewed with microscope (visual scoring).	Simple, non-invasive, painless, more reproducible, objective and consistent than traditional grading systems.	Need to assure clean conditions. Tapes not necessarily well characterized in terms of component properties. Need to precut tape under clean conditions; all squametry techniques are better as a screen for dry skin than as a quantitative method to assess skin moisturizers (106).
Sticky slide (38)	Goldschmidt (1967), Dermatology Lab and Supply Co	Prepare slide by coating with adhesive solution and allow organic solid to evaporate. Press on skin, leave on skin for a few seconds, remove and process.	More reproducible, objective and consistent than traditional clinical grading systems. Quantification/standardization of desquamation possible. More quantitative than skin scraping because fixed area is sampled and loss of material to air currents is more controlled.	Prepared slides have limited life due to gradual air oxidation of adhesive surface. Need skill and practice to perform. Need careful storage and handling to prevent contamination.
Skin surface biopsy with microscopy	Marks (1971) (42)	Cyanoacrylate glue is spread on a flexible plastic slide and applied firmly to skin for 30 sec. Three to five layers of corneocytes are detached, stained, viewed under microscope, and classified into one of 5 xerosis classifications.	Simple, non-invasive, painless. Removes more stratum corneum than pressure-sensitive adhesives.	More difficult to standardize. Skill involved.

(Continued)

**Table 58.4** Bioengineering Techniques to Measure Desquamation (38) (Continued)

Technique	References	Principles	Advantages	Disadvantages
D-Squame® (CuDerm Co, Dallas, TX) analysis using light transmission	Serup (1989) (103)	Small transparent tape discs are pressed against the skin; analysis of discs using light transmission.	Simple, non-invasive, painless; standardized, easy to use.	Small disc size prone to sampling error. May need to delipidize skin to remove scales.
D-Squame (CuDerm Co, Dallas, TX) analysis using image analysis	Schatz (1993) (105)	Small transparent tape discs are pressed against the skin; analysis of discs using image analysis.	Chromometry may add additional precision.	Image analysis can be expensive but more cost-effective machines are being developed (107).
Adhesive disc squametry combined with Chromameter (Minolta) and image analysis	Pierard (1992) (104)	Small transparent adhesive discs are pressed against skin. Corneocytes stained and viewed under microscope, and intensity of stain measured with a chromameter. Quantitative xerosis (based on stain intensity). Image analysis reveals number, thickness and size of squames.	Simple, non-invasive, painless. Allows quantitative assessments of xerosis. Eliminates many of difficulties involved with tape and sticky slides because it is specially formulated and readily available. Three standard sizes. Easy storage and use.	Small disc size more prone to sampling error. May need to delipidize skin to remove scales more effectively. Image analysis is expensive/technical luxury (41).

**Table 58.5** Mechanical Bioengineering Techniques to Measure Elasticity (Parallel to Skin)

Technique	Parameters Measured	Machines Available/ Developer	Principles	Advantages	Disadvantages
Extensometry	Material constants	Extensometer® (Thacker 1977) (43) Gunner (1978) (44)	The arms of two strain gauges are stuck to the skin surface using adhesive tape. By means of a lead screw and carrier, a motor and gear combination moves one arm away from the other at a constant rate, stretching the skin between the tabs. The separation of tabs is measured with a linear variable differential transformer (LVDT) transducer, and the force developed in the skin is measured by strain gauges attached to the reduced sections of the arms. Recoil apparatus can be installed to measure extension–time characteristics of skin when deforming force is removed (44).	Can be hand-held. In vivo measurements possible.	Strain gauges are stiff, and may impose frictional forces. Some systems are bulky and not convenient for clinical use. Technique deforms fiber network in skin so sequential measurements may be increasingly effected by prior measurements.

(Continued)

**Table 58.5** Mechanical Bioengineering Techniques to Measure Elasticity (Parallel to Skin) (*Continued*)

Technique	Parameters Measured	Machines Available/ Developer	Principles	Advantages	Disadvantages
Gas-bearing electrodynamicometer (GBE)	Dynamic spring rate (DSR) (analogous to Young's elastic modulus); loss angle (stiffness, softness, and compliance)	Hargens (1977) (45)	GBE measures displacement of skin in response to a rapidly oscillating force placed next to its surface. Dynamic stress-strain loop appears on oscilloscope, which can be analyzed. Application of moisturizer to the skin surface results in a decrease in the DSR and a concomitant increase in the loss angle.	Good for quantifying stiffness in surface plane of skin, i.e. SC. High degree of correlation between elastic modulus measurements and visual assessments of skin by a trained grader. Sensitive enough to measure changes in SC induced by topically applied agents or mechanical disruption (45). Can apply small forces. Measurement is direct rather than implied from inference, as is the case with electrical conductivity or sonic propagation.	May measure dermal components as well. Changes perceived by trained subjects may not correspond to GBE measurements. Manual stretching of skin can change baseline. Thickness of SC, size, and geometric arrangement of corneocytes, and chemical composition differences may influence measurements (45).
Linear skin rheometer (LSR)	DSR	Matts et al (1998) (86)	Has the same measuring principles as GBE but none of the components. Instead of a magnet/solenoid as in the GBE, there is a force-controlled miniature DC servo, gearing, and lead screw. Instead of the linear variable differential transformer, there is a calibrated load beam. The machine interfaces with a portable computer containing user-friendly software.	More compact, efficient with greater inherent accuracy than the GBE and reduced service requirements. It can differentiate between varying degrees of SC hydration (87).	Not readily available. Technique deforms fiber network in skin so sequential measurements may be increasingly effected by prior measurements.

*(Continued)*



**Table 58.5** Mechanical Bioengineering Techniques to Measure Elasticity (Parallel to Skin) (*Continued*)

Technique	Parameters Measured	Machines Available/ Developer	Principles	Advantages	Disadvantages
Torque meters (disproportional strain measurements)	Torque and phase angle-extensibility (resistance to stretch), viscoelastic properties	Vlasblom (1967) (46) Finlay (1970) (47) Twistometre® (Leveque, L'Oreal); Dermal Torque Meter® (Dia-stron Ltd, Andover, UK) Barbenel (1977) (48)	Disc attached to skin with adhesive. Weak, constant torque applied to rotating disc. Movement of disc monitored by rotational sensor. Fixed guard ring delineates area. When distance between disc and guard ring is less than 1 mm, extensibility reflects SC resistance to stretch. Microprocessor computes main parameters. Immediate rotation corresponds to immediate extensibility, followed by slow increase corresponding to "creeping" of the viscous and plastic skin characteristics.	Sensitive in both short- and long-term studies rating hydrating efficacy. Clear correlation between SC extensibility and severity of dryness. Measurements made parallel to skin surface, so effect of links between dermis and hypodermis are minimized. Can be used to describe mechanical changes in skin with aging, sun exposure, and scleroderma (49). Weibull or extreme-value distribution is more accurate and sensitive than other torsion methods (47).	Standardization not yet complete (47). Technique deforms fiber network in skin so sequential measurements may be increasingly effected by prior measurements.
Mechanical impedance	Point impedance	Franke (1950) (50) Von Gierke (1952) (51) Swept-frequency viscoelastometer (51)	Impedance head is mounted on an electromagnetic actuator or shaker, which is driven by a swept sinusoidal voltage. Corrected force and velocity signals are inputted into RMS circuits and then to a log-ratio amplifier to obtain output proportional to log mechanical impedance. Phase angle between force and velocity signals obtained via a phase meter. Phase angle and log-impedance used as vertical drive signals to a multichannel display multiplexor on an XY storage display oscilloscope. Horizontal drive obtained from frequency to voltage converter and log-amplifier. Thus real-time plots of log Z vs log of frequency can be obtained.	Can study elastic tissues or viscous parameters in living soft tissue.	Technical difficulties still need to be overcome. Technique deforms fiber network in skin so sequential measurements may be increasingly effected by prior measurements.

**Table 58.6** Mechanical Bioengineering Techniques Measuring Elasticity (Acting Perpendicular to Plane of Skin Surface) (53)

Technique	Parameters Measured/ Calculated	Machines/ Developer	Principles	Advantages	Disadvantages
Suction chamber (disproportional superficial strain)	Stiffness (distensibility, resilient distensibility, hysteresis); elasticity (relative elastic retraction [RER])	Cutometer (SEM 474 (Courage and Khazaka); Cutometer® 580MPA (134)	A suction probe is applied vertically on the skin with a constant pressure. The amount of skin elevation is measured using an optical system, which measures the decrease in intensity of an infrared beam. The instrument interfaces with an IBM personal computer, and standard software provided allows the storage of data concerning important variables and graphical display of stress-vs-strain and strain-vs-time curves.	More useful for cosmetological purposes, which aim to measure mechanical properties of epidermis and papillary dermis. Measurements can be enhanced by use of optical elastography (132). Cutometer® 580MPA (134) was developed for use on the foot.	Type of strain measured may be irrelevant to common practice. May still measure mechanical properties of the deeper layers of dermis and subcutis to an unknown extent; limited in range of viscoelasticity; complex measuring units; data can be difficult to interpret; cannot measure viscoelasticity of more rigid skin; cannot evaluate skin anisotropy; seforms skin making sequential measurements difficult.
Suction chamber (proportional full-thickness strain)	Material constants (stiffness, resilience, distensibility); hysteresis; elasticity (RER)	Grahame (1970) (54); Gniadecka, Serup (Dermaflex A, Denmark) (88), Serup (Dermalab) (89)	A suction probe is placed directly on the skin. An electronic sensor in the probe measures the amount of skin elevation by measuring the electric capacitance between the skin surface and the electrode placed in the top of the suction chamber. The data are collected and can be visualized. Skin distensibility and hysteresis increase slightly after epidermal moisturizing (55).	Larger probe is more useful for medical and dermatological applications, for example in scleroderma and chronically inflamed skin; Dermalab can be mounted with special probes to measure TEWL and skin hydration as well.	Correlation of separate parameters of skin mechanical properties with structural elements of skin not fully elucidated. Must control for numerous biological and environmental variables (56). Must avoid repeated measurements at same site for at least 1 hour due to skin deformation.
Dermaograph	Distensibility, relaxation, and elasticity	Sclerimeter® (90); Dermaograph (91)	A vacuum probe is placed on skin. Constant vacuum is applied for 6 sec and the amount of aspirated skin (mm) is measured (distention phase). There is then a 4-sec phase of slow relaxation (elastic retraction).	Good intra- and interrater reliability. Able to measure accurately and rapidly different areas relevant to patients with scleroderma.	Not readily available, some anatomic areas more difficult to measure reliably; normal skin more difficult to measure reliably. Deforms skin, making sequential measurements difficult.

(Continued)

**Table 58.6** Mechanical Bioengineering Techniques Measuring Elasticity (Acting Perpendicular to Plane of Skin Surface) (53)  
(Continued)

Technique	Parameters Measured/ Calculated	Machines/ Developer	Principles	Advantages	Disadvantages
Levarometry, tonometry	Index of deformability; skin extensibility (skin slackness); biological elasticity	Levometer: Dikstein (1979) (57) and Gartstein (1990) (58) Tonometry; Pierard (1980) (59)	The skin is attached (using Perspex disc and double adhesive tape (Dikstein), or cyanoacrylate (Pierard), or vacuum (Gartstein) with (Pierard) or without (Dikstein) a guard ring) to a counterbalanced measuring rod. Different weights can be applied, elevating the skin. For Dikstein's levarometer, the rod is attached to a linear variable differential transformer, and this output is recorded graphically.	The method is sensitive and reproducible. Topical applications or environmental conditions probably do not affect measurements (60). Highly discriminating between old and young skin and old female and old male skin.	Not currently commercially available. Deforms skin, making sequential measurements difficult.
Ballistometry (61)	Coefficient of restitution (amount of energy returned to the tissue)	Tosti (1977) (62); Ballistometer Integrated Dynamic Rebound analyzer (IDRA) PC-based ballistometer (94)	Measurement of a drop impact of a body onto the skin	Non-invasive. Easy to use. No probes attached to skin. Instrument is cheaper than dynamometer. Good for measuring elastic parameters in deeper dermal structures. Can measure differences in elastic modulus between young and old, various body sites and changes after pharmaceutical treatment. Can obtain a lot of data quickly.	Cannot obtain data on status of stratum corneum, as one can from shear measurement. Gives only an indirect indication of underlying tissue changes. Deforms skin, making sequential measurements difficult.
Indentometry (63)	Skin compressibility	Schade (Elastometer, 1912); Kirk (1949); Tregear (1965); Robertson (1969); Daly (1974); Pierard (1984); Dikstein (1981)	A circular piece of plastic material attached to a weighted metal rod is applied perpendicularly to the skin to indent the skin. The rod is counterbalanced so that the net pressure in the system is a given value. The measuring rod is loaded with specially constructed weights increasing the baseline pressure to the desired level (57). The rod is attached to a linear variable differential transformer, and the output of the deformation curve can be plotted using various methods.	Good for measuring water state of ground substance—elastin network in dermis; most useful in evaluating edematous skin conditions and altered water handling of the dermis.	Not the best method to discriminate between old and young skin or female and male skin. Deforms skin, making sequential measurements difficult.



**Table 58.7** Other Mechanical Bioengineering Techniques

Technique	Parameters Measured/ Calculated	Machines/ Developer	Principles	Advantages	Disadvantages
Coefficient-of-friction devices	Coefficient of friction (oiliness/greasiness)	Rotating wheel (Teflon Newcastle friction meter); resolving ground glass disc; sliding sled, modified viscometer	Friction of human skin in vivo can be measured by determining how much force is required to drag object across skin surface; smoother or drier skin theoretically needs less force.	Good for screening topicals for after-feel greasiness (64). Some machines are portable. Measurements with Newcastle machine can correlate with sensory scores of smoothness.	Interpretation of differences in frictional properties between products are very complex. Moisturizers can increase friction as a result of increased contact area. Lubricants make skin more slippery.
Scratch-resistance test	Hardness, softness	Prall (1973) (95)	A stylus just visibly scratches skin; measure lowest pressure load.	Can reveal underlying defects not seen at first glance.	Somewhat invasive.
Durometer (92)	Hardness	Durometer model 1700 type 00 (Rex Gauge) (93)	A calibrated gauge with spring loaded interior that senses hardness by placing an indentation load on the specimen. It registers linearly the relative degree of hardness on a scale of units 0–100.	Very simple to use, portable, hand-held; highly reproducible. Good for measuring scleroderma, lipodermatosclerosis, and neuropathic foot hyperkeratosis.	Measurements should be made in the supine position. May be insensitive in areas with decreased subcutaneous tissue.
Microindentometer	Pliability, hardness	Indentometer (96) (Guibarra, 1979); Microindentometer (97) (Graves, 2002)	Indentation of the SC by a needle is opposed by the horny layer, and this force of reaction is monitored by a force transducer.	One of the few devices available to measure pliability and hardness of SC.	Must immobilize area that is being measured since movement or vibration will alter measurements. Needs further development to eliminate inertial artifacts.
Acoustic spectrometer (65)	Softness, hydration level; energy loss of viscous component of skin; elastic modulus	Tronnier (1952) (66); Potts (1985) (67); Torgalkar (1981) (65)	Vibration device in audible range gives small-amplitude oscillations normal to skin surface with second stylus as comparison. Spectrum analyzer can calculate time for shear waves to travel and degree of amplitude dampening; resonance frequency can be measured. The more SC hydration, the lower the resonance frequency. Energy loss can also be calculated. Can be used as predictive measure. Indirect measure of hydration state. Correlated with subjective assessments of moisturization.	Can be used as predictive measure. Indirect measure of hydration state. Correlated with subjective assessments of moisturization.	Thickness of horny layer, thickness and tension of the skin and nature of underlying tissues can be sources of error.

(Continued)

**Table 58.7** Other Mechanical Bioengineering Techniques (*Continued*)

Technique	Parameters Measured/ Calculated	Machines/ Developer	Principles	Advantages	Disadvantages
Viscoelasticity skin analyzer (VESA)	Elasticity, shear waves propagation (SWP)	Sarvazyan (1990) (112); Vexler (1999) (113)	Probe consists of three piezoelectric transducers (central transmitter with two receivers equidistant on either side). Transmitter produces a tangential oscillatory deformation on the surface of the skin (SWP) in the acoustic frequency range. SWP is calculated from the time of flight of signal to transmitter. Average reading displayed on LCD.	Allows anisotropic measurements; compact; portable, user-friendly; high accuracy; reproducible readings; measurements using this technique do not deform the skin as with distortion, rotation, stretch or suction so sequential measurements are possible; allows measurements of upper skin layers without influence of subdermis.	Not readily available.
Tactile sensor (Venustrom®) (100)	Changes in resonant frequency ( $\Delta f$ ) correlate with spring constant $k$ and stiffness; elasticity	Omata (1999) (99); Sakai (2000) (100)	Sensor with resonant frequency is pressed and released from the skin at a constant rate. Depth and pressure are determined allowing hysteresis curve $\Delta f$ calculation.	Simple; allows rapid determination of multiple stiffness parameters; correlated with firmness (dehydration of skin), ratio of acidic amino acids, and elasticity.	Significance of correlation needs further investigation.
Cohesography	Intracorneal cohesion measurements	Nicholls (1971); Marks (1977) (31)	After hydrating SC, there is a drop in intracorneal cohesion. Drop follows same magnitude as flattening in surface contour, and changes are of same order of magnitude (31).	Able to assess hydrating agents.	Not generally commercially available (31).
Reviscometer® (133)	Resonance running time (RRT)	RVM600	RRT is inversely related to skin stiffness (133).	Better able to discriminate between experimental treatments than cutometer. Measurements most sensitive on transverse forearm (133).	Sensitivity may differ depending on anatomic site (133).

**Table 58.8** Indirect Electrical Bioengineering Techniques

Technique	Developer/Machines	Principles	Advantages	Disadvantages
Low-frequency impedance (frequency domain)	Clar (1975) (138)	Impedance drops with increasing hydration. Frequency-domain approaches examine the response of skin to sinusoidal stimulating frequencies.	Low frequencies give most informative data about physiological condition of skin overall because charge carriers can travel more time before field reverses (47).	Need liquid junction. Electrodes are occlusive. Long time needed for data collection (>20 min). Agents other than water can lower impedance. Measurements are quantitative rather than qualitative.

(Continued)

**Table 58.8** Indirect Electrical Bioengineering Techniques (*Continued*)

Technique	Developer/Machines	Principles	Advantages	Disadvantages
High-frequency impedance (3.5mHz) (frequency domain)	Tregear (1965) (68)	Impedance drops with increasing hydration. Impedance decreased with increasing frequency. Higher frequency, more skin penetration.	Provides information on deeper levels of skin; can use dry electrodes.	Occludes site. Depth of SC not well defined. Agents other than water can affect readings. Pressure of probe and dermal irritants can influence readings. Cannot measure resistance and capacitance separately at high frequencies.
Impedance (capacitance calculated)	Nova DPM-9003 (Dermal Phase Meter)	Integrates selected measurements at varying frequencies of the applied alternating current. Capacitance is calculated from the signal phase delay using a proprietary chip. Final readout is in arbitrary units related to capacitance.	Good for assessing highly hydrated skin due to low variability of readings (69). Due to monofrequency approach, subject to less error, less confounding variables, and has increased sensitivity and specificity (less false-positives and false-negatives) when compared with single-frequency machines; handling easy due to small dimensions and low weight.	Less sensitive for grading the dry state than the Corneometer (72). Agents other than water affect measurements.
Impedance (surface characterizing)	Surface-characterizing impedance monitor (SCIM) (Servo-Med, Sweden)	Impedance is dependent on tissue hydration, composition, and condition. SCIM measures impedance magnitude and phase at 31 frequencies to five selectable depths.	Uses the intrinsically more informative multifrequency approach, which is independent of changes in sweat gland activity, skin temperature, confounding variables. Allows electrical impedance spectroscopy of selected layers.	Same disadvantages as with many electrical methods. Must use probe correctly (perpendicular, with correct pressure); wait 5s between repeating measurements on same site to avoid occlusion. Measurement failures with wet surface, dirt. Must perform measurements under appropriate ambient conditions (<22°C and = 60% RH).
Capacitance	Corneometer (Courage and Khazaka, Germany)	Capacitance increases with increasing hydration. The Corneometer uses variable frequencies in the low-frequency range (40–75Hz); <75 dehydrated skin; 75–90 skin with tendency to dehydrate, >90 normal skin (arbitrary units) (35).	Easy to operate. Highly reproducible (35,69). Short measuring time (1s). Economical (69). Useful for extremely dry scaly skin. Information can be enhanced and nonhomogeneity of skin can be accounted for by using capacitance images for example SkinChip (129).	Confined to measurement of variation in SC between initial and final states (35). Poor sensitivity to hydration process taking place in SC of normal skin because optimal range of water content in the SC for the capacitance method is much lower than for high-frequency conductance methods.
Conductance	Skicon 100, 200 (Masuda, IBS Co, Ltd)	Uses a fixed frequency (3.5mHz) to measure conductance and capacitance separately.	Dry electrodes can be used. Correlates well with water content of superficial and deep SC layers. Suitable to assess the hydration dynamics of the SC induced in the skin. Not affected by electrolyte-rich solutions (71).	Single-frequency approach subject to more error, confounding variables, decreased sensitivity and specificity (increased false-positives and false-negatives) when compared with multifrequency machines (47). Current must propagate at least 5 mm to obtain reliable values.

(Continued)



**Table 58.8** Indirect Electrical Bioengineering Techniques (*Continued*)

Technique	Developer/Machines	Principles	Advantages	Disadvantages
High-frequency microwave (GHz)	Jacques (1979) (124) Wavetek 1005	Dielectric probe response (DPR) (70) a percentage based on the probe's response to skin versus a drop of water. A signal swept several mHz around a GHz resonates in a cable. Charged grid contacts skin, water absorbs energy and produces a standing wave shift, detection of which is adjusted to be linearly proportional to hydration level.	Detects quantitative differences. Rapid quantitation. Unaffected by topicals. SC probe depth varies. DPR basic unit is useful for comparisons.	DPR is not a true hydration percentage.
Millimeter wave reflectivity	Alexseev, Szabo, Ziskin (2008) (131)	Analysis of reflection of millimeter (mm) wavelength electromagnetic waves. Amount of reflection depends on electric property of skin (permittivity). Permittivity depends on free water content of skin. Free water content can be calculated from permittivity values using skin mode (131).	Good for measuring areas of thick stratum corneum such as palms in vivo.	Not so sensitive for areas of thin skin.

*Note:* The general advantages of these techniques are that they provide easy to measure, continuous data on skin hydration status and are readily available commercially.

**Table 58.9** Bioengineering Techniques Based on Spectroscopy or Thermal Transfer

Technique	Developer/Machines	Principles	Advantages	Disadvantages
Fourier-transformed infrared spectroscopy (ATR-FTIR) (attenuated total reflectance)	1970s	Beam of polychromatic IR light is shone through a zinc or germanium selenide crystal applied to skin surface. Crystal creates 5–20 reflections, and absorption cycles between crystal and skin. Reflected beam is detected by spectrophotometer, Fourier transform of beam gives IR spectrum with bands of absorption in SC. Ratio of areas of amide I and II bands (peaks) provides relative SC water content. Amide I at 1645 cm (44) is overlapped by band of protein-associated water, and thus will change with protein water content, whereas amide II at 1545cm (44) is not influenced by water (35).	In vivo, direct measurement of water. Quantitative, theoretical relationship between measured parameter and water concentration understood.	Expensive. Need signal averaging during time when site is occluded, since water content changes during measurements. Depth of penetration can vary with parameters. Bands from interfering substances could obscure amide bands. IR beam is weak (5–20µm) penetrator. Data only pertain to superficial SC.

(Continued)

**Table 58.9** Bioengineering Techniques Based on Spectroscopy or Thermal Transfer (*Continued*)

Technique	Developer/Machines	Principles	Advantages	Disadvantages
Magnetic resonance spectroscopy (MRS)/ nuclear magnetic resonance spectroscopy(NMR-S)	Foreman (1970s, in vitro) (115); Cuono (1988, in vitro); Klein (1988, in vitro); Zemtsov (1989, in vivo)	Same principles as MRI apply except that the magnetic resonance signal is used to construct a magnetic resonance spectroscopic spectrum. MRS spectra can be obtained from protons as well as <sup>13</sup> C or <sup>31</sup> P. <sup>31</sup> P provides information about intercellular pH, tissue turnover rate, and tissue bioenergetics (ATP, Pi, phosphocreatine).	Gives information about presence of chemical species as well as environment in which these materials exist and how this is changing over time. Quantifies hydration in both epidermis and superficial dermis. Metabolic, functional, and structural information is possible. May be able to quantify specific tissue composition of hemoglobin, melanin, elastin; more precise and reproducible than capacitance or TTT; one of the few direct methods; considered a reference technique.	Still experimental. Expensive. Limited availability. MRI images prone to motion artifacts. Underlying tissue may cause data contamination. Not portable; heavy measuring apparatus limits measurement to forearms only.
Near infrared spectroscopy (NIR)	Putnam (1972) (73), Osberghaus (1978) (74) Rigal (1992) (122) NIRS5000 Spectrophotometer	NIR penetrates deep into skin. Two absorption bands are used at 1100 nm (minimal skin absorbance) and 1336 nm (strong absorption band by water molecules). The difference in absorbance at the two wavelengths is well correlated to clinical scores for skin dryness.	Gives information on molecular constitution of skin. Measures stratum corneum, epidermal and dermal water. Under certain conditions, exact quantitative relationship between IR absorption and water concentration in the stratum corneum (67). Can be used in clinical studies with the fiberoptic probe (Smartprobe™) to calculate changes in % relative humidity (%RH) (127). Permits avoidance of chemometric manipulation in data analysis (needed in most other techniques used). Direct correlation with visual dryness assessment scores. Had better linear regression for %RH scores when compared with conductance and visual dryness scores (127).	Topical agents may introduce error. Complicated and costly. Abrupt relative humidity variations may introduce error.
Multiphoton laser tomography	DermaInspect® Konig, Riemann (2003) (113)	Femtosecond near-infrared (NIR) laser scanning system based on two-photo excited autofluorescence. Nonlinear induced autofluorescence comes from endogenous fluorophores such as NAD(P)H, flavins, elastin, porphyrin, and melanin. Addition of second harmonic generation (SHG) can be used to detect collagen. Fluorescence lifetime imaging (FLIM) allows 4D imaging (3 dimensions plus time) (137).	Noninvasive, ultrahigh subcellular resolution up to 200 μm.1 (37). Compact. More consistent results than cutometer and reviscometer (130).	Expensive.

(Continued)

**Table 58.9** Bioengineering Techniques Based on Spectroscopy or Thermal Transfer (*Continued*)

Technique	Developer/Machines	Principles	Advantages	Disadvantages
Photo-acoustic spectroscopy (PAS)	Rosencwaig (1977) (75); Campbell (1979); Simon (1981)	Skin is exposed to IR radiation (heat). Depth of penetration of a periodic heat wave into a solid depends on its frequency. Radiation is absorbed by water in the SC at that depth. The superposition of thermal waves causes periodic temperature/pressure fluctuations at the surface of the skin, which can be detected as sound by a microphone in a closed photoacoustic cell. Signal produced depends on both optical and thermal properties of a sample.	Can quantitatively measure in vivo, and no contact needed between probe and skin. Good for investigation of the horny layer (76). One of the most depth-sensitive methods.	Not readily available. More technical developments needed.
Optothermal infrared spectrometry (OTIS)	Frodin (1988) (98)	Technique derived from photoacoustic spectroscopy, based on detection of heat generated in a sample due to absorption of periodic monochromatic radiation with a wavelength of 1940 nm, a specific absorption band for water. The heat is conducted to a sapphire plate in contact with the skin and transparent to the radiation directed to the test area. The plate expands and is transformed to an electrical signal by an annular piezo-electric crystal cemented to the plate's edge.	By varying the chopper frequency, possible to measure at different thicknesses of the stratum corneum.	Not possible to determine absolute values for skin water content.
Transient thermal transfer (TTT)	Soumet (1986) (114) Hydrascan (Laboratoire DermScan France)	TTT is the property of one body exchanging heat with another when they are in contact. The skin temperature is measured. A stimulator then generates a thermal pulse that propagates through the epidermis to be picked up by a sensor. The difference in temperature is proportional to water content. The signal is analyzed and processed with electronic and data processing equipment. A series of three successive thermal pulses from three increasing powers provides hydration measurements from three epidermal depths.	Precise measurements at different depths is possible; explores deeper depths than capacitance. Small sensor size allows measurements on lips, eyes, nails which are not possible with either capacitance or MRI.	In vivo repeatability coefficient of variation is not as good as MRI but better than capacitance. Analytic variability is not as good as capacitance or MRI; indirect technique; slow data acquisition because requires a minimum of 10 minutes per depth measured.



**Table 58.10** Bioengineering Techniques to Measure Transepidermal Water Loss (TEWL) (77)

Machine/Developer	Principles	Advantages	Disadvantages
Evaporimeter (ServoMed, Sweden)	Probe with two pairs of humidity transducers and thermistor measures the partial water vapor pressure at two points (3 and 6 mm) above skin. Rate of evaporation ( $\text{g/m}^2/\text{h}$ ) calculated from difference in partial water vapor pressure between these points. Probe has surface area $1.13\text{cm}^2$ . Normal TEWL between 2 and $5\text{g/m}^2/\text{h}$ .	Can evaluate products whose mode of action is occlusion. Accurate. Convenient to use. Inexpensive to operate (31).	Strictly speaking, does not measure skin hydration. Many factors can affect measurements, and they need careful monitoring. Evaporimeter may underestimate water evaporation rate at high TEWL (77).
Tewameter (Courage-Khazaka Electronic, Germany)	Same principle of measurement as Evaporimeter except sensors are at 3 and 8 mm above skin, and probe has surface area of $0.79\text{cm}^2$ .	More recent design; measures probe temperature and graphs TEWL over time; more complete, somewhat more convenient, less sensitive to air turbulence than Evaporimeter (77).	Strictly speaking, does not measure skin hydration. Many factors can affect measurements, and they need careful monitoring. Newer instrument, so less well studied.
Derma Lab® System with TEWL and computerized evaporimetry (Cortex Technology, Denmark, 1999)(108)	Similar to ServoMed Evaporimeter. Probe is open cylinder placed perpendicular to skin site. Sensors at fixed distances. Can be standalone or equipped with personal computer interface.	Convenient monitoring of evaporative loss rates in real time so any undesirable influences due to air currents, probe movements are readily apparent and their impact on measurements instantaneously determined as well as retrospectively analyzed. Increased reproducibility and sensitivity.	Possible additional increased expense and complexity.
VapoMeter (Delfin Technology Ltd, Finland)(125)	Uses unventilated chamber method of measurement.	Closed chamber technology allows more mobile, flexible use of instrument. Less vulnerable to external air movements. Self-contained battery powered.	Problems with water vapor accumulation as with all closed chamber techniques.
Aquaflux (Biox Systems Ltd, UK)(125)	Uses condenser-chamber method of measurement.	Closed chamber technology allows more mobile, flexible use of instrument. Less vulnerable to external air movements. Benchtop; sensors protected from contamination and can maintain measurement geometry.	Problems with water vapor accumulation as with all closed chamber techniques. Somewhat less mobile than VapoMeter.

**Table 58.11** Bioengineering Techniques to Image Stratum Corneum

Technique	Developer/ Machines	Principles	Advantages	Disadvantages
High-frequency (20mHz) ultrasound, A mode	Alexander (1979); Muller (1985); machines: DUB20 (Taberna pro medicum, Germany); Dermascan C (Cortex Technology, Denmark) (78)	A (amplitude) mode can measure the thickness of the skin layers. Adaptations to skin need a strongly dampened high-frequency ultrasound detector with very short impulses produced by ceramic or piezoelectric polymer transducers in order to detect as many echoes generated from as many interfaces as possible. The receptors made up of a device protecting against emitter overcharge, a wide-band radiofrequency amplifier, and a detector of radiofrequency signals. Signals are viewed on an oscilloscope.	Good for whole-skin visualization. Can differentiate epidermis from dermis in some cases. Can follow aging, sunlight damage, scleroderma, steroid atrophy.	Difficult to measure water quantitatively from images. Motion creates artifacts. Encoding process can distort space. Difficult to visualize very thin sites.

(Continued)

**Table 58.11** Bioengineering Techniques to Image Stratum Corneum (Continued)

Technique	Developer/ Machines	Principles	Advantages	Disadvantages
High-frequency (20MHz) ultrasound, B mode	DUB20 (Taberna pro medicum, Germany); Dermascan C (Cortex Technology, Denmark) (78)	In B (brightness) mode, a succession of signal lines in A mode is acquired and reconstructed into a 2D image. B scans are oriented in the x or y direction.	Useful to measure thickness and depth of skin cancers. Appearance of non-echogenic band in upper dermis may be more sensitive marker of aging than skin thickness. Distinguishes skin irritation versus allergic reactions. Ultrasound waves theoretically carry information on elastic properties.	Difficult to measure water quantitatively from images. Motion creates artifacts. Encoding process can distort space. Information on how ultrasound waves carry information on skin elastic properties cannot yet be interpreted. More research needed
High-resolution magnetic resonance imaging (MRI)	1987–1988 Hyde (79), Querleux (80), Bittoun (80); (Skin Imaging Modele, France)	Conventional MRI equipment adapted to reduce field of view and pixel size using surface coils. Small surface radiofrequency coil to improve the signal-to-noise ratio. Bittoun made further advances by using the device with a 1.5T system, obtaining very high-resolution images of normal skin as well as calculations of T1 and T2 (80).	More adapted to visualization of whole skin. Epidermis can be clearly delineated and analyzed to an axial spatial resolution of 35–70µm. Able to measure water directly and quantitatively in vivo. Can study proton-exchange phenomenon. Repeated measurements over time in vivo.	Errors introduced by very short T2, chemical shift and partial-volume effect can overestimate epidermal thickness. Artifacts also caused by motion and spatial distortions introduced by encoding. Clinical utility limited by high cost, cumbersome equipment.

**Table 58.12** Optical Techniques for Characterization of Skin Properties

Technique	Machines/ Developer	Principles	Advantages	Disadvantages
In vivo confocal microscopy/ confocal scanning laser microscopy (CSLM)	Petran (1968); Corcuff (1993); Tandem Scanning microscope (Tracor Northern)	A focused spot of light scans the sample. Reflected light in focal plane passes through a pinhole in front of a photomultiplier/TV camera detector. Images received are perfectly focused because almost all of the reflected light from above and below the plane in focus is blocked. The Nipkow disc has 2000 pinholes arranged in Archimede spirals, and allows lightening spot scanning and reflected-light formation, which can be collected by a TV camera. After computer processing, a volume representation can be obtained.	Excellent axial (spatial) resolution (1µm). Makes horizontal optical sections. Very good at visualizing SC. Preserves natural tonicity of skin, hydration of cells, and contrast of structures. Possible to measure SC thickness in vivo. Sharp focused; allows study for first time of previously elusive stratum lucidum and stratum granulosum. Can visualize RBC in capillaries. Excellent reproducibility. Can work in 4D space (volume and time) at the microscopic level non-invasively.	Artifacts caused by motion and spatial distortions introduced by encoding. Present section thickness that can be imaged is limited to 150 µm (82). Still needs optical improvements to increase signal-to-noise ratio on images of inner epidermis. Optical sectioning is limited by transparency of tissue, scattering and absorption of light in the sample, working distance, and numerical aperture of the sample (82).
In vivo fiberoptic fluorescence laser scanning microscopy	Stratum® (Suihko 2005) (128)	Confocal microscope adapted for study of skin and mucous membranes using fiberoptics and fluorophores. Light source is a 488 laser. Fluorescein sodium used as the fluorophore (intradermal injection or topical skin application) (128).	Flexible hand-held system can be used on any site including mucous membranes. Fluorescein sodium is safe for use in vivo. Magnification 1000x. Produces horizontal (en face) images. Cellular and some subcellular resolution is possible (128). Good for kinetic studies of substances applied on or into epidermis (126).	Same as CSLM. Intradermal injection of fluorophore requires some skill.

(Continued)

**Table 58.12** Optical Techniques for Characterization of Skin Properties (*Continued*)

Technique	Machines/ Developer	Principles	Advantages	Disadvantages
In vivo confocal Raman microspectroscopy	Caspers (1998) (110); (2001) (111)	In vivo optical method based on inelastic light scatter rather than absorption (vibrational spectroscopy). Skin Raman spectrum are measured; signal is analyzed to extract information.	Depth resolution of 5 micrometers; able to measure concentration profiles; quantitatively and qualitatively accurate when compared with gold standard x-ray microanalysis. Only in vivo method to analyze skin molecular composition as a function of distance to skin surface with similar detail and resolution.	Expensive, limited availability.
In vivo optical coherence tomography (102,109) (OCT)	Fercher (1988) (117); Huang (1991) (101)	Technique based on the Michelson principle of interferometry. OCT uses light in the near infrared range. Gel interface couples probe to skin. Light source (LED) emits a broad band light into fiber; coupler directs one portion into reference arm. Diverging light beams are relayed via both objectives to the skin probe and reflecting mirror. Detector signals are converted from optical to electrical signals. Thickness measurements are calculated using software.	Better resolution than MRI or high resolution ultrasound; resolution to cellular level = 10–15 micrometers. Maximum imaging depth is 1.2–2 mm. Lateral resolution is 15 micrometers. Real time imaging. Fiberoptic systems allow better access to normally difficult to access areas of skin; non-invasive so allows monitoring of inflammation over time; can objectively monitor treatment effect.	Expensive; limited availability; not good enough resolution to judge grade of melanocytic tumors; axial and lateral resolution is inferior to CSLM; allows visualization of architectural changes but not single cells. Only able to image stratum corneum on palms and soles due to increased thickness.
Ellipsometry	Jasperson (1969) (83)	Monochromatic light passes through a plane polarizer oriented at 45° with respect to the incidence plane. Polarizer output is fed into a photoelastic modulator composed of a piezoelectric crystal oscillating at a particular frequency. Output of the modulator passes through collimator side of ellipsometer to skin of interest. Reflected light goes to telescopic side of spectrometer and is directed through a second polarizer to a photomultiplier tube (PMT). PMT output and reference signal enter a lock-in amplifier, which gives intensity readings for the ellipsometric parameters. A computer program calculates the refractive index. Measures refractive index.	Changes in refractive index can be used to monitor hydration status, effect of moisturizers.	Topical agents may cause a change in reflectivity. Very indirect method.
Skin critical surface tension (CST)	Jacobi (1949); Schneider (1951); Ginn (1968); El Khyat (1996) (84)	Droplets of standard liquids applied to skin and viewed under microscope. Critical surface tension can be calculated using Zisman technique; also can measure wettability.	Can quantify surface energy phenomenon resulting from sweat, serum, and emulsion application as well as interactions. Can quantify wettability.	Requires some skill on the part of the operator.



## CONCLUSIONS

There are multiple challenges facing the clinician who wishes to understand the scientific basis behind moisturizer efficacy claims, including access to information, understanding the information, and making sure the study design and technologies used are valid. There is an urgent need to perform meta-analyses (quantitative and/or qualitative) of the moisturizer studies categorized by study type, bioengineering technique, ingredients, and clinical population in order to have more clinically relevant information on the efficacy of moisturizers. For those interested in exploring the topic of moisturizers or non-invasive bioengineering techniques more profoundly, some textbook and journal references have been listed in the Additional Reading section.

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پزسک آموز

# Index

- AA, *see* Azelaic acid
- AAG, *see* Androgenogenetic alopecia
- a-Aminolevulinic acid (ALA), 442
- ABA, *see* Aldobionic acids
- Abdominal treatment, cryolipolysis, 542, 544
- Aberrant plasmin activity, in epidermis, 118
- Ablative mesotherapy, 431
- Ablative ultrapulse laser therapy, 217
- Abnormal hairs, 230
- Abnormal keratinization, 56, 167
- AbobotulinumtoxinA (ABO), 459
- Abrasive nail files, 266
- ABS, *see* Acrylonitrile butadiene styrene
- Absorbent core, on lotion transfer, 554–555
- Acacia nilotica*, 155
- Accelerated weathering, 232
- ACDS, *see* American Contact Dermatitis Society
- Acetone, 279–281
- Acid-based primer, 279
- Acid neutralization, 503–505
- Acid strength, of organic hydroxyacid, 59
- Acne
- chemical peeling, 386–387
  - hydroxyacids, 63
  - stratum corneum, 130–131
  - treatment of, 171–172
- Acneogenesis, 302–303
- Acneic inflammatory lesions, improvement of, 108
- Acne keloidalis, 394
- Acne scarring, 387
- Acrylic acid, 278, 290
- Acrylic nail extensions, 273
- Acrylonitrile butadiene styrene (ABS), 276
- Actinic keratoses (AK), 531
- chronicity and secondary prevention, 214–215
  - diagnosis of, 214
  - hydroxyacid, 65
  - multiple, non-hyperkeratotic, 216–217
  - nonablative laser rejuvenation, 531
  - photodynamic therapy, 444–445
  - therapeutic perspectives, 217
  - treatment algorithm for, 215, 216
- Acute angioedema-type hypersensitivity, 475
- Acute formaldehyde reaction, 284
- AD, *see* Atopic dermatitis
- Adapalene, 332, 363
- Adenosine monophosphate disodium salt, 332
- Adherent scalp flaking scores (ASFS), 254
- Adipokine, 308
- Adverse skin effects
- acneogenesis and comedogenesis, 302–303
  - contact urticaria, 303–304
  - photoreactions, 304
  - primary dermal irritation, 301–302
  - sensory irritation, 302
- Affirm™ Multiplex laser, 525
- African hair, 390, 392
- AG, *see* Ascorbyl glucoside
- Aging
- definition of, 143
  - of female reproductive system, 424
  - of hair, 231–232
- AHAs, *see* Alpha-hydroxy acids
- Airborne contact dermatitis, 271, 285
- AK, *see* Actinic keratoses
- ALA, *see* a-Aminolevulinic acid
- 5-ALA, *see* 5-Aminolevulinic acid
- Aldaric acid, 57
- Aldobionic acids (ABA), 58
- Aldonic acid, 57
- Alduronic acid, 57–58
- Alkyl  $\alpha$ -hydroxyacids, 57
- Allergic contact dermatitis, 148–149, 277
- Allergic rhinitis score (ARS), 161
- Alltrans retinoic acid (ATRA), 88
- Alopecia, 426
- Alpha-hydroxy acids (AHAs), 178, 287, 291, 301, 498
- Ambulatory phlebectomy, 318
- American Contact Dermatitis Society (ACDS), 301
- 5-Aminolevulinic acid (5-ALA), 157
- Amphoteric surfactant, 60, 237
- AMPLI RF, *see* Automatic multifrequency and low impedance radio frequency
- Analysis of covariance (ANCOVA), 552–553
- Analysis of variance (ANOVA), 552
- Ancillary skin care products
- astringents, 178
  - face masks, 180–181
  - facial scrubs, 179–180
  - mechanized skin care devices, 180
- ANCOVA, *see* Analysis of covariance
- Androgenetic alopecia, 394, 415
- Androgenogenetic alopecia (AAG), 426
- Anionic emulsifiers, 290
- Anisopoikilocytotic adipocytes, 308
- Anodal electrical stimulation, 489
- ANOVAs, *see* Analysis of variance
- Antagonistic acetoxyacids, 66
- Anticellulite products
- classification of, 309
  - etiopathogenesis, 308–309
  - noninvasive techniques, 309–310
  - therapies, 310–311
- Anti-dandruff shampoo, 237, 252
- Antimicrobial activity, 491–492
- Antioxidants, 65–66, 187
- activities, 104
  - constitutive skin antioxidant network, 80–82
  - dietary components, 188
  - effects of environmental stressors on, 82–83
  - evaluation of, 85
  - nonsteroidal anti-inflammatory drugs, 187–188
  - photoprotection of human skin, 83–85
  - properties, 59
  - reactive oxygen species, 80
  - in skin, 410–412
- Anti-stress activity, 104
- Apoptosis, 536
- Apple acid, 57
- Aralkyl  $\alpha$ -hydroxyacids, 57
- Arborizing telangiectasias, 510
- Arbutin, 154, 332
- Areolar layer, 308
- Argon laser, 512
- Arm patch protocol, 552–553
- ARS, *see* Allergic rhinitis score
- Arteriolar telangiectasias, 510
- Artificial nails, 220, 264, 276–279
- removing sculptured, 279
- ASA, *see* Ascorbic acid
- Ascorbic acid (ASA), 153, 332
- Ascorbyl glucoside (AG), 154
- ASFS, *see* Adherent scalp flaking scores
- Asian hair, 390, 392–394
- Astringents, 178
- Asymmetric smile, 466
- Atopic dermatitis (AD), 130
- pruritus, 158
  - psoriasis, 161–163
- ATRA, *see* Alltrans retinoic acid
- Automated phototrichograms, 416–417
- Automatic multifrequency and low impedance radio frequency (AMPLI RF), 310
- Azelaic acid (AA), 172, 331, 363–364
- Azzalure®, 459
- Bacterial biofilms, 491
- Baphicacanthus cusia*, 162
- Barrier function, stratum corneum, 132–133
- Barrier recovery test, 117
- Basal cell carcinomas (BCCs), 186, 193, 194, 444
- Basal layer peel, 499–500
- Base coats, nail, 269–271
- Basement membrane (BM), in photo-aged skin, 120–121
- BBI, *see* Bowman-Birk protease inhibitor
- BCCs, *see* Basal cell carcinomas
- BCOP, *see* Bovine corneal opacity and permeability test
- BCSO, *see* Blackcurrant seed oil
- Becker nevus, 340
- Behind-the-knee (BTK) test, 51
- lotion transfer, *see* Lotion transfer methods of, 551–553
  - pressure on test samples, 553
  - skin benefits of, 557–558
- Bend of elbow, washing test on, 21
- 1,4-Benzoquinone molecule, 76
- Benzoyl peroxide (BPO), 171–172, 360–361
- Beta-carotene, 71
- BHA, *see*  $\beta$ -Hydroxyacids
- Biochemical action, 62–63
- Biochemistry, hydroxyacids, 59
- Bio-Electric Imager®, 482
- Bioelectricity
- antimicrobial activity, 491–492
  - biomimetic electricity, 483, 492–496
  - bone regeneration, 487–488
  - cellular effects of, 485–487
  - electric air bath, 481
  - innate electrical system, 482
  - ion transport properties, 482–483

- microcurrent therapy, 490–491  
 ranges and cell proliferation, 488  
 skin wounds and skin aging, 483–485  
 tissue engineering, 490  
 transcutaneous electrotherapy and  
 delivery enhancement, 482  
 wound healing enhancement, 489–490
- Bioengineering techniques, 562  
 indirect electrical, 573–575  
 measure desquamation, 566–567  
 measure elasticity, 567–571  
 mechanical, 571–573  
 skin surface contour evaluation, 564–566  
 spectroscopy/thermal transfer, 575–577  
 stratum corneum imaging, 578–579  
 transepidermal water loss, 578
- Biological action, hydroxyacid, 62  
 Biomimetic electricity, for dermatological  
 applications, 483, 492–496  
 Biomimetic electric stimulation, 481  
 Biomimetic complex  
 biomimetic-electricity delivery systems,  
 492  
 clinical safety and tolerability of,  
 493–494  
 skin anti-aging of, 494–496  
 in vitro biological responses of, 492–493
- Bionic acid, 58  
 Bismuth oxychloride, 269  
 BKT, *see* Behind-the-knee  
 Blackcurrant seed oil (BCSO), 160  
 Blood vessel reactivity, 404, 406–407  
 Blowdrying hair, 244  
 BO, *see* Borage oil  
 Bocoture®, 459  
 Body hair, 395–396  
 Body lotions  
 adverse reactions, 304–305  
 advertising, 298–300  
 alleviating dry skin, 291–294  
 consumers' skin condition, 301  
 ingredients for, 288–291  
 instrumental evaluations of, 295–297  
 lotion composition, 300–301  
 predicting adverse skin effects, 301–304  
 preventing primary irritation, 294  
 and skin, 287–288  
 trained expert sensory panel, 298  
 user evaluation of, 297–298
- Bone regeneration, influenced by electric  
 fields, 487–488
- BoNT-A, *see* Botulinum neurotoxin type A  
 Borage oil (BO), 160–161  
 Botanicals, role in cosmeceuticals, 182–183  
 Botulinum neurotoxin type A (BoNT-A),  
 259, 459, 467  
 adjunctive treatments, 466–467  
 contraindications/limitations, 461–462  
 facial asymmetry, 465–466  
 hyperhidrosis, 467–468  
 lower face, 464–465  
 middle face, 464  
 pharmacology and mechanism of action,  
 459  
 platysmal bands and décolleté wrinkles,  
 466  
 reconstitution and dose equivalence,  
 459–461  
 upper face, 462–464
- Bovine collagen, 473  
 Bovine corneal opacity and permeability  
 test (BCOP), 305  
 Bowen disease, 446–447  
 Bowman-Birk protease inhibitor (BBI), 94  
 BPO, *see* Benzoyl peroxide  
 Browning reaction, 174  
 BTK, *see* Behind-the-knee  
 Butylhydroxytoluene, 279
- Café-au-lait macules, 340  
 Caffeine, 188  
 Calcium hydroxylapatite (CaHA), 477–479  
 Calibrated electrical stimulation of skin, 52  
 Camellia sinensis, *see* Green tea  
 Camouflaging, 336  
 CAMVA, *see* Chorioallantoic membrane  
 vascular assay  
 Canfield EpiLume®, 416  
 Carbohydrate metabolism, 59  
 Carbon dioxide (CO<sub>2</sub>) lasers, 512  
 Carotenoids, 188  
*Cassia fistula*, 154–156  
 Cathodal electrical stimulation, 489  
 Cationic emulsifiers, 290  
 Cationic water-soluble polymers, 29  
 Caucasian hair, 390–394  
 Causative ingredients, 149  
 CCCA, *see* Central centrifugal cicatricial  
 alopecia  
<sup>14</sup>C-Ciclopirox penetration, 36, 39–40  
 CCNI, *see* Cysteine-rich protein 61  
 CDLQI, *see* Children's dermatology life  
 quality index  
 Cellular necrosis, 442  
 Cellular retinoic acid binding proteins  
 (CRABP), 88  
 Cellulite, 309, 431  
 Cellulosic polymers, 290  
*Centella asiatica*, 310  
 Central centrifugal cicatricial alopecia  
 (CCCA), 393, 395  
 Ceramide-acid-base, 125  
 Ceramides  
 biosynthesis of, 132–135  
 ethnicity, 132  
 human stratum corneum, 126  
 lipid organization in, 128–129  
 nomenclature, 124  
 normal-phase liquid chromatography, 123  
 nuclear magnetic resonance, 128  
 physical properties of, 129  
 variations in, 129–132
- CES, *see* Cranial electrotherapy stimulation  
 Chamois buffers, 267  
 Chemical depilatories function, 325–326  
 Chemical peeling, 386  
 acid neutralization, 503–505  
 depths of peels, 498–503  
 and fibroblasts, 507–508  
 phenol, 508–509  
 trichloroacetic acid, 505–507  
 Chemical relaxers, 393  
 Chemi-osmotic theory, 487  
 Cherry hemangiomas, 510  
 Children's dermatology life quality index  
 (CDLQI), 161  
 C3H6O3, *see* Dihydroxyacetone  
 Chorioallantoic membrane vascular assay  
 (CAMVA), 305  
 Chromhidrosis, 342  
 Chronic inflammation, atopic dermatitis, 160  
 Chronicity, actinic keratoses, 215–216  
 Chronic low back pain, 491  
 Chronic telogen effluvium, 413  
 CI, *see* Color index  
 Ciclopirox, 155  
 CIT, *see* Cumulative irritation test  
 Citrate cycle, energy production, 59  
 Classical mesotherapy, 432  
 Cleansing cloth, 179  
 Cleansing products, 169  
 Climateric hyperkeratosis, 427  
 Climateric keratodermas, 427  
 Climateric syndrome, 424  
 Climbazole, 253  
 Clinical efficacy, of retinol, 89–91  
 Closed-chamber instruments, 28–29
- Clostridium botulinum*, 459  
 CLSM, *see* Confocal laser scanning  
 microscopy  
 Coal tar, 253–255  
 CO<sub>2</sub> lasers, *see* Carbon dioxide lasers  
 Collagen  
 fibers, 259  
 production, 103  
 synthesis, 490  
 Colorant powders, 264  
 Colorants, *see* Coloration additives  
 Coloration additives, 269  
 Colored polymer powders, 277  
 Colored UV gels, 264, 280  
 Colorimetric assay, 145–146  
 Color index (CI), 269  
 Color stabilizers, 269  
 Color-treated hair, shampoos for, 237  
 Comedogenesis, 302–303, 303  
 Comedolytic agents, 171  
 Compact stratum corneum, 425  
 Condenser-chamber method, 29  
 Conditioners, 237–243  
 Conditioning shampoos, 237  
 Confocal laser scanning microscopy  
 (CLSM), 34, 168  
 Confounding lifestyle factors, 49  
 Congenital dermal hypermelanocytosis, 340  
 Constitutional dry skin, 167  
 Constitutive skin antioxidant network,  
 80–82  
 Consumers' skin condition, role of, 301  
 Contact dermatitis, 385  
 Contact urticaria, 272, 279, 303–304  
 Continuous wave lasers, 512–514  
 Conventional "facelift" procedures, 261  
 Conventional injection technique, 462  
 Cooling, role of, 515  
 Copper-vapor laser, 513  
 Corneocyte counting, 255  
 Corneocyte desquamation, 167  
 Corneocytes, 404–405  
 Corneofix®, 295  
 Corneofix F20, 168  
 Corneometer, 20  
 Corneometer CM 825, 168  
 Corneometer units (CU), 25  
 Corneofixometry, 130  
 Cortex, 228–229, 392  
*Cortex moutan*, 161  
*Cortex phellodendri*, 161  
 Cosmeceuticals  
 botanical role of, 182–183  
 cosmetic labeling standards, 183–184  
 regulatory oversight of, 182  
 Cosmetic cryotherapy  
 acne, 454  
 complications, 456–457  
 lesion selection, 452–453  
 mechanism of action, 451  
 seborrheic keratosis, 455  
 tattoos, 456  
 technique and terminology, 450–451  
 tissue injury, 451–452  
 vascular lesions, 453–454  
 vascular stasis and occlusion, 452  
 viral warts, 454–455  
 Cosmetic product labeling, 183–184  
 Cosmetic skin parameters  
 climatic data, 16  
 laser profilometry, 16–17  
 outcomes, 21–26  
 Rz parameter, 17  
 in vivo topometry of, 17–21  
 Cotton-tipped dipstick, 451  
 Covalentlybound ceramides, 125  
 COX-2, *see* Cyclooxygenase-2  
 CPDs, *see* Cyclobutane pyrimidine dimers



- CRABP, *see* Cellular retinoic acid binding proteins
- Cranial electrotherapy stimulation (CES), 491
- Critical wavelength (CW) test, 186
- Cross-section trichometer, 419
- Croton lechleri, 182
- Crow's feet lines, 260, 262-263
- Cryolipolysis, 539  
assessment and treatment planning, 540  
clinical technique, 537-539  
efficacy, 542  
mechanism of action, 536-537  
patient expectations, 542-543, 546  
pre-clinical studies, 535-536  
side effects, 540-542  
treatment for, 539-540
- Cryoprobe, 451
- Cryosurgery, 354, 453, 457
- Cryosurgical treatments, 450
- Cryotherapy, 215, 217
- CU, *see* Corneometer units
- Cultured keratinocytes, 96
- Cumulative irritation test (CIT), 301
- Curling irons, 244
- Cutaneous appendages, 384-385
- Cutaneous microcirculation, 9
- Cutaneous microvasculature, 7-8
- Cuticle, hair, 228, 392
- Cuticle, nail  
removers and softeners, 272-273  
trimmers, 267
- Cutometer®, 297
- Cutters, nail, 266
- CW, *see* Critical wavelength test
- Cyclobutane pyrimidine dimers (CPDs), 185
- Cyclooxygenase-2 (COX-2), 96, 187
- Cypraea moneta, 386
- Cystamine, 332
- Cysteamine, 332
- Cysteine-rich protein 61 (CCN1), 89
- Cytokinesis biology, 113
- Damaged corneocytes, 296
- Dandruff, 131, 248
- Dandruff, treatment considerations, 255
- Dark skin, 385
- Dark-skinned swine, 94
- DBT, *see* Dibutyl phthalate
- DC, *see* Diflucortolone-21-valerate
- Décolleté wrinkles, 466
- Decoration of nail, 264-266
- Deep dermal collagen remodeling, 263
- Deep venous thrombosis (DVT), 313
- Dehydroepiandrosterone (DHEA), 425
- DEJ, *see* Dermo epidermal junction
- Delayed hair growth, 94-96
- Delta fiber, 52
- Deoxycholate, 432
- Depigmenting agents  
combination therapies, 333-334  
dermabrasion, 334-335  
hydroquinone derivatives, 331  
phenolic compounds, 330-331  
retinoid monotherapy, 332-333
- Depressor angulis oris, 464-465
- Depressor septi nasi m., 464
- DermaCorder™, 486
- Dermaflex®, 297
- DermaLab, 168
- Dermal extracellular matrix production, 493
- Dermal fibroblasts, 507-508
- Dermal hypermelanocytosis, 340
- Dermal matrix enhancement, 96
- Dermal Torque Meter, 297
- Dermaroller®, 437
- Dermatitis papillaris capillitii, 394
- Dermatitis, to dihydroxyacetone, 176
- Dermatological indications, 63-64
- Dermatology treatments  
atopic dermatitis, *see* Atopic dermatitis  
hyperpigmentation, 153-155  
onychomycosis, 155-158
- Dermo-electroporation, 433
- Dermo epidermal junction (DEJ), 387
- DES1, *see* Dihydro CER desaturase
- DES2, *see* Dihydro CER hydroxylase
- Desquamation index (DI), 295
- Detergent, cause of dry skin, 117
- Deutsche Industrie Norm (DIN), 17
- DF, *see* Dissecting folliculitis
- D-gluconic acid, 58
- DHA, *see* Dihydroxyacetone
- DHEA, *see* Dehydroepiandrosterone
- DI, *see* Desquamation index
- Diastron, 417
- Dibutyl phthalate (DBT), 268
- Diclofenac, 216
- Dietary agents, 187-188
- Dietary soy, 93
- Differential scanning calorimetry (DSC), 128
- Diflucortolone-21-valerate (DC), 160
- Dihydro CER desaturase (DES1), 128
- Dihydro CER hydroxylase (DES2), 128
- Dihydrotestosterone, 415
- Dihydroxyacetone (DHA), 20-21, 336  
application of, 175  
chemical structure of, 174  
dermatitis to, 176
- DIN, *see* Deutsche Industrie Norm
- Dipstick technique, 451
- Directional cellular locomotion, 487
- Direct manual hair count, 416
- Disinfection container, 267
- Disinfection, nail, 274
- Dissecting folliculitis (DF), 394
- DNA-based identification techniques, 251
- DNA repair enzymes, 217
- Domain mosaic model, 129
- Dorfman-Charainin syndrome, 130
- Dosing procedures, nail plate, 34, 36
- Double-blind study, 254
- Dried nail, 276
- Drug-induced pigmentation, 341
- Drug-induced sensitivity, 49
- Drug penetration, into human nail, 39
- Dry skin, 63, 117, 167  
alleviate, 291-294  
t-AMCHA methylamide, 119-120  
treatment of, 170-171  
urokinase in, 118
- DSC, *see* Differential scanning calorimetry
- D-Squame®, 168, 295
- DVT, *see* Deep venous thrombosis
- Dyes, 269
- Dyschromia  
camouflaging, 336  
lasers and phototherapy, 335  
pathophysiology of, 330  
surgical approaches, 335-336  
tattoos, 342-343
- Dyskeratosis, 341-342
- Dysport, 459
- Dystrophic nails, with subungual hyperkeratosis, 283
- Earth-based masks, 181
- Econazole concentration, 39
- Ectoin, 170
- Ectopic contact dermatitis, 285
- EFAs, *see* Essential fatty acids
- Efinaconazole, 157
- Eflornithine cream, 326, 426
- EGCG, *see* Epigallocatechin gallate
- Elastic fiber network, 259
- Elastic recovery/extensibility, skin, 407-409
- Elastin enhancement, 103-104
- Electric air bath, 481
- Electrical properties of skin, 168
- Electric fields  
bone regeneration influenced by, 487-488  
tissues/organs affected by, 488
- Electric neurostimulation, 481
- Electric torpedo fish, 481
- Electrodessication, 511
- Electron spin resonance (ESR) spectroscopy, 85
- Electro Pressure Regeneration Therapy (EPRT) device, 490
- Electrospray ionization-mass spectrometry (ESI-MS), 123
- Electrosurgery, 511
- Electrosurgical epilation, 326
- ELM, *see* Epiluminescence microscopy
- Emery boards, 266
- Emollients, 289
- Emulsifiers, 289-290
- Endogenous electric fields, 487
- Endothelin-B receptors, 8
- Endovenous chemo-ablation techniques, 313-317
- Endovenous laser treatment (EVL), 317
- Environmental stressors, 82-83
- Epidermabrasion, 179
- Epidermal cooling devices, 515
- Epidermal hypertrophy, 118
- Epidermal thinning, 425
- Epidermosides, 124
- Epigallocatechin gallate (EGCG), 83-85
- Epilator, 326
- Epiluminescence microscopy (ELM), 416
- EPO, *see* Evening primrose oil
- EPRT device, *see* Electro Pressure Regeneration Therapy device
- ER, *see* Estrogen receptors
- ERT, *see* Estrogen replacement therapy
- Erythema, 50
- ESI-MS, *see* Electrospray ionization-mass spectrometry
- ESR, *see* Electron spin resonance spectroscopy
- Essential fatty acids (EFAs), 159, 289
- Estradiol, 425
- Estril, 425
- Estrogen, in topical treatments, 425-426
- Estrogen receptors (ER), 415
- Estrogen replacement therapy (ERT), 424-426
- Ethnic cosmetics  
aging and rejuvenation, 387  
chemical peeling, 386-387  
cutaneous appendages, 384-385  
skin color, 384  
skin disease, 385-386  
sun protection, 386
- Ethnic skin  
antioxidant status, 410-411  
blood vessel reactivity, 404, 406-407  
corneocyte variability, 404-405  
effects on, 109  
elastic recovery/extensibility, 407-409  
facial pore size, 411  
lipid content, 408-410  
methods, 398  
pH gradient, 408  
transepidermal water loss (TEWL), 398-401  
water content, 399, 401-404
- Ethyl methacrylate monomer, 279
- Eucalyptus citriodora, 156
- Evening primrose oil (EPO), 160
- Evernia prunastri, 149

- EVLT, *see* Endovenous laser treatment  
 Evolence®, 473  
 Excessive hair  
   causes of, 321–324  
   cosmetic procedures for removal of, 324–325  
   shaving, 325–327  
 Exfoliants, 178  
 Exogenous ochronosis, 386  
 Extrinsic skin aging, 64–66  
 Eye irritation, 304–305  
 Eyelashes, 395–396  
 Eyelid dermatitis, 271, 282
- Face masks, 180  
 Facial asymmetry, 465–466  
 Facial hirsutism, 426  
 Facial lentiginosities, reduction of, 105  
 Facial peeling, 454  
 Facial pore size, 411  
 Facial scrubs, 179  
 Facial telangiectasias, 510–512  
 Facial wrinkles, 462, 466  
 Fair Packaging and Labeling Act (FPLA), 183  
 Fast optical in vivo topometry (FOIT), 17–21  
 FD, *see* Folliculitis decalvans  
 FDA, *see* Food and Drug Administration  
 FD&C Act, *see* Federal Food, Drug, and Cosmetic Act  
 FDD, *see* Frequency distribution of depth  
 Federal Food, Drug, and Cosmetic Act (FD&C Act), 183  
 Female hair with aging  
   clinical manifestations of, 413–415  
   efficacy measures of, 416–419  
   mechanisms of, 415–416  
 Female pattern hair loss (FPHL), 413–414  
 Feminine-care products, 50  
 Ferric ferrocyanide, 269  
 Ferulic acid, 202–204  
 Fibered cloths, 179  
 Fibroblastic phase, 353  
 Fibroblasts, 262, 507–508  
 Field of cancerization, actinic keratoses, 214, 215  
 Film-drying accelerant, 269–270  
 Film formers, 268  
 Film modifiers, 268  
 Finasteride, 426  
 Flank treatment, cryolipolysis, 544  
 Flashlamp pumped-pulsed dye laser (FLDP), 512–513  
 Flat irons, 244  
 Flat lesions, 455  
 FLDP, *see* Flashlamp pumped-pulsed dye laser  
*Flos lonicerae*, 161  
 Fluid mosaic model, 128  
 Fluorescent-style UV nail lamps, 221  
 Fluorescent tubes, 220  
 5-Fluorouracil, 216  
 Foam Sclerotherapy, 315–316  
 FOIT, *see* Fast optical in vivo topometry  
 Follicular unit (FU) density, 391  
 Folliculitis decalvans (FD), 395  
 Folliculitis keloidalis, *see* Acne keloidalis  
 Fontana-Mason staining, 94  
 Food and Drug Administration (FDA), 93  
 Forehead lines, 462–463  
 Forehead wrinkles, 90  
 Formaldehyde, 284, 291, 302–303  
 Formalin, 285  
 Fourier transform infrared (FTIR) spectroscopy, 128  
 4-n-Butylresorcinol, 331  
 Four-week laxity clinical study, 495–496  
 FPHL, *see* Female pattern hair loss
- FPLA, *see* Fair Packaging and Labeling Act  
 Fractional 1440-nm laser, 522  
 Fractional 1540-nm laser, 525  
 Fractional 1550-nm laser, 521–522  
 Fractional photothermolysis, 519, 520  
 Fractional Q-switched ruby laser, 526  
 Fragrance allergic patient, 149–151  
 Fragrance ingredients  
   adverse reactions to, 148  
   allergic contact dermatitis to, 149  
 Frail nails, 266  
 Fraxel division, 520  
 Fraxel DUAL 1550/1927 lasers, 522  
 Free fatty acids, in human stratum corneum, 127, 129  
 Freeze-thaw cycle (FTC), 450  
 Frequency distribution of depth (FDD), 19  
 Frozen porcine fat, 538  
 Fruit acid, 57  
 FTC, *see* Freeze-thaw cycle  
 FTIR spectroscopy, *see* Fourier transform infrared spectroscopy  
 FU density, *see* Follicular unit density  
 Functional magnetic resonance imaging, 52  
 Functional skin properties, 48  
 Fungus identification methods, 33
- GAGs, *see* Glycosaminoglycans  
 Galvanic current, 492  
 Gamma-linolenic acid (GLA), 160  
 Gel matrix, 59  
 Genistein, 207–209  
 Genital discomfort, 426  
 Gentian violet (GV), 160  
 Gingival smile, 465  
 GLA, *see* Gamma-linolenic acid  
 Glabellar lines, 462  
*Glabellar m.*, 462  
 Glutathione (GSH), 81–82, 84  
 Gluteofemoral adipocytes, 432  
 Gluteraldehyde, 462  
 Glyceraldehyde, 174  
 Glycerides, 289  
 Glycerol monothioglycolate (GMTG), 394  
 Glycolic acid, 56, 57, 365  
 Glycosaminoglycans (GAGs), 59, 171  
 GMTG, *see* Glycerol monothioglycolate  
 Greasy skin, 167–168, 171  
 Green tea, 83–84, 182  
 Grenz zone peel, 500–501  
 GSH, *see* Glutathione  
 GV, *see* Gentian violet
- HA, *see* Hyaluronic acid  
 Hair  
   biochemistry, 392  
   care and styling, 393  
   characteristics, 392–393  
   conditioners, 237–243  
   cosmetic aspects of, 227–231  
   cosmetic products for, 235  
   count, 107, 327  
   damage, 232–235  
   density, 231, 391  
   diameter and texture, 230–231  
   excessive, *see* Excessive hair  
   gels, 246  
   greying, 415  
   growth, 391–392, 419  
   phenotype, 232  
   shaft changes, 414  
   shampoos, 235–237  
   straightening, 244–245  
   styling, 243, 245–246  
   types of, 231  
   volume, 243–244, 244
- HAIR-AN, *see* Hyperandrogenism, insulin resistance, and acanthosis nigricans syndrome  
 Hair regrowth  
   automated phototrichograms, 416  
   combination measures, 419  
   diameter, 416–418  
 Hairspray, 246  
 Halo nevus, 338  
 Handheld reusable textured cleansing pads, 180  
 Hand lotions, *see* Body lotions  
 Hard keratin, 32  
 HASI, *see* Hyperhidrosis Area and Severity Index  
 HB-EGF, *see* Heparin-binding–epidermal growth factor  
 Hemosiderosis, 341  
 Heparin-binding–epidermal growth factor (HB-EGF), 88  
*Herba menthae*, 161  
 4-hexyl-1,3-phenylenediol, 144–145  
 HGA, *see* Homogentisic acid  
 HH, *see* Hyperhidrosis  
 Hidradenitis suppurativa (HS), 350  
   age and sex distribution, 369  
   cosmetics of, 371  
   treatment of, 369–371  
 High-grit boards, 266  
*Hippophae rhamnoides*, 154–155  
 Hirsutism, 321–322, 426  
 Hollow microneedles, 439  
 Homogentisic acid (HGA), 386  
 Hormonal skin aging, 424–425  
 Hormone replacement therapy (HRT), 425  
 Horny cells, 385  
 Hot combing, 393  
 HPV, *see* Human papilloma virus  
 HQ, *see* Hydroquinone  
 HRIPT, *see* Human repeated insult patch  
 HRT, *see* Hormone replacement therapy  
 HS, *see* Hidradenitis suppurativa  
 Human abdominal tissue, 536, 538  
 Human-derived collagen, 473  
 Human fingernail plates, 34  
 Human forearm biopsy, 73  
 Human papilloma virus (HPV), 215  
 Human periorbital wrinkle  
   aged skin aspect, 259–260  
   aligned collagen layer, 262  
   computer model of, 261–262  
   implications, 262–263  
   local differences in, 260–261  
 Human repeated insult patch (HRIPT), 301–302  
 Human scalp hair  
   care and styling, 393–394  
   characteristics of, 392–393  
   color, 392  
   density of, 391  
   ethnic variations in, 394–395  
   growth, 391–392  
   shape and thickness, 390–391  
 Humectants, 170–171, 289  
 Hyaluronic acid (HA), 167, 216, 473–476  
 Hydrating products, 169  
 Hydrocolloid masks, 181  
 Hydrogen sulfide, 365  
 Hydroquinone (HQ), 76, 276, 282, 330–331  
 Hydroxyacids, 56  
   bioavailable concentration, 59–60  
   biochemistry, 59  
   cosmetic and dermatological indications, 63–64  
   differences of, 66–68  
    $\alpha$ -hydroxyacids, 56–57, 58  
    $\beta$ -hydroxyacids, 57  
   intrinsic and extrinsic skin aging, 64–66

- mechanisms of action, 62–63  
optimal release formulation, 60  
physicochemical properties, 58–59  
polyhydroxy acids, 57–58  
topical actions, 60–62
- $\beta$ -Hydroxyacids (BHA), 57, 178  
 $\beta$ -Hydroxybutanoic acid, 57  
 $\beta$ -Hydroxybutyric acid, *see*  
     $\beta$ -Hydroxybutanoic acid
- Hyperandrogenism, insulin resistance, and  
    acanthosis nigricans (HAIR-AN)  
    syndrome, 321
- Hyperhidrosis (HH), 467–468  
Hyperhidrosis Area and Severity Index  
    (HASI), 467
- Hyperkeratotic actinic keratosis, 215  
Hyperkeratotic wart, 454  
Hyperpigmentation, 94, 105, 153–155, 451  
Hypertonic saline, 315  
Hypertrichosis, 322–324, 426  
Hypertrophic scars, 529  
Hypopigmentation, 385, 529
- Ichthyoses, 129–130  
Idebnone, 77–78, 84  
    skincare delivery of, 78
- Idiopathic guttate hypomelanosis, 341
- IFRA, *see* International Fragrance  
    Association
- I $\kappa$ B kinases (IKK), 143  
IKK, *see* I $\kappa$ B kinases
- Imidazole antimycotic agent, 253  
Imiquimod, 216
- Immediate pigment darkening (IPD), 185  
Impedance microscopy (IS), 33
- INCI, *see* International Cosmetic Ingredient;  
    International Nomenclature of  
    Cosmetic Ingredients
- INCO, *see* IncobotulinumtoxinA
- IncobotulinumtoxinA (INCO), 459
- Indigo naturalis, 162
- Indoor tanning, 189
- Inflammatory phase, 353
- Infraorbital rhytides, 463
- Ingenol mebutate, 216–217
- Ingredient classes  
    emollients, 289  
    emulsifiers, 289–290  
    minor components, 290–291  
    water, 288–289  
    water-soluble polymers, 290
- Injectable silicone, 479
- Inner thigh treatment, cryolipolysis, 544
- Inorganic agents, of sunscreens, 194–195
- In-plane microneedles, 436
- Integumentary system, 261
- Intense pulsed light (IPL), 336, 511, 514–515
- Intensive conditioners, 239
- Intercellular lipids, 28, 45, 250
- International Cosmetic Ingredient (INCI),  
    288
- International Fragrance Association (IFRA),  
    151
- International Nomenclature of Cosmetic  
    Ingredients (INCI), 268, 284
- Intra-arterial injection, 475–476
- Intradermal botulinum toxin, 172
- Intra-epidermal secondary factors, 117
- Intraepidermic peel, 498–499
- Intralesional injection, 354
- Intrinsic antifungal potency, 255
- Intrinsic skin aging, 64–66
- IPD, *see* Immediate pigment darkening
- IPL, *see* Intense pulsed light
- Irritant dermatitis, 7–8
- Irritant test, 45, 47
- IS, *see* Impedance microscopy
- Isoflavones, 101, 426
- Isolated actinic keratosis, 215
- Isolated hyperkeratotic actinic keratosis, 215
- Isolated onycholysis, 285
- Isotretinoin, 362
- KA, *see* Kojic acid
- Keloidalis nuchae, *see* Acne keloidalis
- Keloids, 456  
    clinical aspects, 349–350  
    histologic examination of, 350–352  
    hypertrophic scars *vs.*, 349  
    pathogenesis, 352–353  
    treatment of, 354–358
- Keratinization, 60
- Keratinocytes, 404  
    destruction, 452  
    differentiation, ceramides on, 134–135  
    proliferation of, 117–118
- Keratolytic treatment, of acne  
    azelaic acid, 363–364  
    benzoyl peroxide, 360–361  
    retinoids, *see* Retinoids  
    salicylic acid, 364  
    sulfur, 365
- Ketoconazole, 253, 254
- Kinetin  
    chemistry, 113  
    clinical studies, 114–115  
    exact mechanism of, 113–114
- Kligman formulation, 386
- Kligman's formula, 333
- Kojic acid (KA), 331
- KTP 532nm green lasers, 512
- Kunitztype trypsin inhibitor, 94
- Lactic acid, 171
- Lactone form, 58
- Lamellar bodies, *see* Membrane-coating  
    granules
- Laminin 332, 121–122
- Lanolin, 289
- Lanugo hair, 228
- L-ascorbic acid, *see* Vitamin C
- Laser Doppler flowmetry (LDF), 309
- Laser Doppler velocimetry (LDV), 406
- Laser profilometry, 16–17
- Laser therapy, onychomycosis, 157–158
- Late-onset pain, 542, 543
- Layered haircut, 393
- LCD, *see* Liquor carbonis detergens
- LDF, *see* Laser doppler flowmetry
- LDV, *see* Laser Doppler velocimetry
- LE, *see* Lupus erythematosus
- Leave-in conditioners, 239
- LED-style nail lamps, 278, 280
- Leg telangiectasias, 513
- Lentigines, 340
- L-ergothioneine, 84
- Levator labii superioris m., 464
- Light emitting diodes (LED) UV nail lamps,  
    221
- Linear density diameter, hair, 417
- Linear telangiectasias, 510
- Lipid content, skin, 408–410
- Lipolytic stimulation, 431
- Lipometer, 169
- Lipophilic compounds, 71
- Liquid nitrogen cryotherapy, 334–335
- Liquiritin, 333
- Liquor carbonis detergens (LCD), 160
- Local anesthetics block response, 50
- Local phenol, 508
- Long-lasting fillers, 476–479
- Long nail, 264
- Long pulsed 1064-nm lasers, 513–514
- Long-pulse dye laser, 513
- Lotion composition, 300–301
- Lotion measurement, 552
- Lotion transfer  
    absorbent core on, 554–555  
    durations of exposure, 554  
    on pad samples, 553–554  
    pad thickness on, 556  
    skin benefits of, 557–558
- Lower face, 464–465
- Low-grit boards, 266
- Low intensity electricity, 491
- LPG endermologie, 310
- L-selenomethionine, 205–207
- Luna stain, 103
- Lupus erythematosus (LE), 424
- Lycopene, 188
- Macrophotographic manual hair count, 416
- Magnetic resonance imaging (MRI),  
    309–310
- Magnolignan, 332
- Mahonia aquifolium*, 161–162
- Maillard reaction, 174
- MAL, *see* Methyl aminolevulinate
- Malassezia furfur*, 248
- Malassezia globosa*, 251
- Malassezia* yeast, 168
- Male skin, age-related changes in  
    mechanical properties, 380  
    methods, 377  
    pH value, 378–379  
    sebum excretion, 379–380  
    skin barrier function, 378  
    wrinkle development, 380–381
- Maltobionic acid, 59
- Marionette lines, 464
- Massage treatment, 310
- Mass balance, of radioactivity recovery,  
    36, 37
- Masseteric hypertrophy, 465
- Matrix metalloproteinases (MMPs),  
    88–89, 120
- MBEH, *see* Monobenzyliether of  
    hydroquinone
- MCG, *see* Membrane-coating granules
- MC1R, *see* Melanocortin 1 receptor
- Mechanism of action, self-tanning products,  
    174–175
- MED, *see* Minimal erythema dose
- Medium-grit boards, 266
- Medulla, 229, 392
- MEHQ, *see* Methyl ethyl hydroquinone
- Melanex, 105
- Melanin, 153, 384, 392
- Melanocortin 1 receptor (MC1R), 188
- Melanocytes, 386, 392
- Melanocyte-stimulating hormone (MSH), 13
- Melanoderma, 330
- Melanogenesis inhibition, 493
- Melanoids, 336
- Melanoma, 193–194
- Melanosomes, 392
- Melasma, 153, 387
- Membrane-coating granules (MCG),  
    123–124, 127
- MEND, *see* Microscopic exudative necrotic  
    debris
- Menopausal dry skin, 425
- Menopausal skin thinning, 425
- Menopause, 415  
    hormonal replacement therapy, 424  
    hormonal skin aging, 424–425  
    hormone regimens, 425–426  
    hormone replacement therapy, 425  
    phytoestrogens, 426
- Mentalis, 464–465



- Mesotherapy, 311  
     for fat deposits, 431–432  
     skin rejuvenation, 432–434
- Metabolism of retinol, 88
- Methacrylate liquid/powder, 276
- 4-Methoxy Potassium Salicylate (4-MSK), 332
- Methyl aminolevulinate (MAL), 442
- Methylene glycol, 270, 284–285
- Methyl ethyl hydroquinone (MEHQ), 276, 282
- Methylxanthines, 310
- METS, *see* Microcurrent stimulation
- mFCAT, *see* Modified forearm controlled application test
- MIC, *see* Minimum inhibitory concentration
- Microampere current, 490
- Microbiological contamination, 270
- Microcomedo, 360
- Microcurrent electrical therapy, 490–491
- Microcurrent stimulation (METS), 488
- Microdermabrasion, 180
- Microneedle  
     cosmetic uses, 437–439  
     safety of, 439  
     strategies for drug delivery, 437
- Microneedle-based devices, 437
- Microneedle rollers, 436
- Microorganisms, 291
- Microscopic exudative necrotic debris (MEND), 520
- Microthermal zones (MTZ), 520
- Mid-angle sunlight, 223
- Middle face, 464
- Millisecond pulse durations, 516
- Mineral oil, 289
- Miniature needles, 436
- Minimal erythema dose (MED), 185
- Minimum inhibitory concentration (MIC), 33, 38–39
- Minor components, 290–291
- Misuse, body lotions, 301
- Mitochondrial DNA (mtDNA), 80
- Mitochondrial lipid peroxidation, 76
- Mitochondrial theory, of aging, 76
- MMEH, *see* Monomethyl of hydroquinone
- MMPs, *see* Matrix metalloproteinases
- Mode of action, 236, 238–239
- Modern nail art, 264
- modified forearm controlled application test (mFCAT), 553
- MoistureMeter SC-4, 168
- Moisture/smooth hair, 241
- Moisturization balance, in combination skin, 108–109
- Moisturizer efficacy  
     advertising claims made for, 298–299  
     alleviating dry skin, 291–294  
     bioengineering techniques, 562  
     data, 562–564  
     instrumental evaluations of, 295–297  
     problems with, 561–562  
     product evaluation by trained expert, 298  
     user evaluation of, 297–298
- Moisturizing shampoos, 237
- Monobenzylether of hydroquinone (MBEH), 331, 337
- Monomethyl of hydroquinone (MMEH), 331
- Monometric cyanoacrylate, 283
- Mottled pigmentation, 387
- Mousses, 246
- MSH, *see* Melanocyte-stimulating hormone
- 4-MSK, *see* 4-Methoxy Potassium Salicylate
- mtDNA, *see* Mitochondrial DNA
- MTS-Roller™, 439
- MTZ, *see* Microthermal zones
- Mud packs, *see* Earth-based masks
- Multiple hyperkeratotic actinic keratoses, 217
- Multi-wavelength fractional 1440/1320-nm laser, 525–526
- Muscular pumping, 309
- Myroxylon pereirae*, 149
- NAAs, *see* N-acetylamino acids
- N-acetylamino acids (NAAs), 66–67
- N-acetyl compounds, 66–67
- N-acylpeptides (NAP), 67–68
- NAD, *see* National Advertising Division
- NAFL, *see* Nonablative fractional lasers
- Nail  
     base and top coats, 269–271  
     chemical properties, 32–33  
     coloration additives, 269  
     decoration of, 264–266  
     items for, 266–267  
     nail varnish basics, 268  
     potential for injury/infection, 273–274  
     reactions to applied cosmetics, 271–273  
     solvents and diluents, 268–269  
     texture of, 266  
     toiletries and cosmetics, 267  
     treatment of, 283–285
- Nail buffers, 267
- Nail creams, 270–271
- Nail incubation, 34–36
- Nail keratin granulation, 272
- Nail penetration  
     antifungal efficacy coefficient, comparison of, 40  
     <sup>14</sup>C-econazole, profile of, 38  
     nail lacquer formulations, 37  
     nail permeation testing and sampling, 33  
     nail plate and bed, hydration of, 36  
     noninvasive diagnostic tools for onychomycosis, 33–36  
     physical and chemical properties of, 32–33  
     radiolabeled drug penetration, 39
- Nail plate  
     hydration of, 36  
     lipid pathway in, 32  
     staining, 272  
     ventral/intermediate, 35–36
- Nail sampling, 35–37
- Nail varnish, 270, 279  
     basics, 268  
     dermatitis, 271–272  
     diluents and solvents, 268–269  
     formulation, 267
- Nail whitener, 267
- NAP, *see* N-acylpeptides
- Narrow band ultraviolet B (NBUBV) courses, 222
- Nasal tip droop, 464
- Nasal wrinkles, 464
- NASHA, *see* Non-animal-sourced hyaluronic acid gel
- National Advertising Division (NAD), 299
- National Organic Program (NOP), 183
- Natural moisturizing factors (NMFs), 127, 169, 171
- NBUBV courses, *see* Narrow band ultraviolet B courses
- Necrosis, 536
- Netherton syndrome, 130
- Neurogenic causality, 52
- Neurogenic inflammation, 52
- Nevus depigmentosus, 338
- Nevus spilus, 340
- NFκB, *see* Nuclear factor kappa B
- 1927-nm lasers, 522
- Nippers, 266
- Nitrocellulose, 268
- Nitroethane poisoning, 279
- NMFs, *see* Natural moisturizing factors
- NMR, *see* Nuclear magnetic resonance
- NMSC, *see* Nonmelanoma skin cancer
- Nodular basal cell carcinoma, 446
- No-light cyanoacrylate gels, 280, 283
- Nonablative fractional lasers (NAFL), 520  
     cynosure icon family of, 522–525  
     Solta family of, 520–521
- Nonablative infrared lasers, 519
- Nonablative laser rejuvenation, 519  
     cynosure icon family, 522–525  
     fractional 1540-nm laser, 525  
     fractional photothermolysis, 520  
     Fraxel 1550-nm laser, 521–522  
     multi-wavelength fractional 1440/1320-nm laser, 525–526  
     1927-nm lasers, 522  
     nonablative fractional lasers, solta family of, 520–521  
     photodamage, 528–529  
     pigmentary disorders, 531  
     post-care routine, 527  
     scars, 529–530  
     striae, 530–531
- Nonablative photorejuvenation, 387
- Non allergic reactions, nail, 279–281
- Non-amphoteric system, 60
- Non-animal-sourced hyaluronic acid gel (NASHA), 474
- Non-denatured soy, in skin care  
     clinical efficacy of, 105–109  
     composition, 96–102  
     and delayed hair growth, 94–96  
     and dermal matrix enhancement, 96  
     early utilization of, 93–94  
     human health, 93  
     for human topical applications, 102–103  
     preclinical results of, 103–105  
     proteins, 94
- Nonhyperkeratotic actinic keratosis, 215
- Nonhypertrophic acne scarring, 387
- Noninvasive diagnostic tools, for onychomycosis  
     methodology, 34–36  
     results of, 36
- Noninvasive techniques, 391
- Nonionic emulsifiers, 290
- Nonionic shampoo, 237
- Nonkeratinized vulvar skin, 49
- Nonmelanoma skin cancer (NMSC), 188, 221  
     development, 96
- Nonmethacrylic acid primers, 276–277
- Non-nitrated cellulosic, 268
- Nonrepaired damage, of mitochondrial DNA, 76
- Non-scalp seborrheic dermatitis, 252
- Nonsteroidal anti-inflammatory drugs (NSAIDs), 187–188
- NOP, *see* National Organic Program
- Normal-phase liquid chromatography (NPLC), 123
- Normal skin, 167  
     treatment of, 169–170
- Nova Dermal Phase Meter DPM 9003, 168
- Nova™, 297
- NPLC, *see* Normal-phase liquid chromatography
- NSAIDs, *see* Nonsteroidal anti-inflammatory drugs
- Nuclear factor kappa B (NFκB)  
     activation under skin aging, 144  
     homeostasis, 143–144  
     signaling, 143
- Nuclear magnetic resonance (NMR), 128
- Oats, 159
- Occlusive moisturizing ingredients, 171

- Occupational irritant hand dermatitis (OIHD), 563
- Ochronosis, 341
- OCT, *see* Optical coherence tomography
- OFDA, *see* Optical fiber diameter assessment
- OIHD, *see* Occupational irritant hand dermatitis
- Oil inclusion, conditioning products in, 239
- Omega-hydroxy ceramides, 126
- OnabotulinumtoxinA (ONA), 459
- Onycholysis, 264, 273, 277–279, 282, 284
- Onychomycosis, 64, 447
  - alternative treatments for, 159
  - Ciclopirox, 155
  - noninvasive diagnostic tools for, 33–36
  - tavaborole, 157
  - tea tree oil, 155–156
  - topical therapy for, 32
  - Vicks® VapoRub, 156–157
- Onychoschizia, 266
- Open-chamber instruments, 28
- Open spray liquid nitrogen, 454
- Optical coherence tomography (OCT), 33–34, 168, 387
- Optical devices, 310
- Optical fiber diameter assessment (OFDA), 417–418
- Optical techniques, for skin
  - characterization, 579–580
- Optimal metal ions, 102
- Optimal release formulation, 60
- ORAC, *see* Oxygen radical absorbance capacity
- Orbicularis oris* m., 464
- Osler-Weber-Rendu syndrome, 510
- OTC products, *see* Over-the-counter products
- Oval-shaped nails, 264
- Over-the-counter (OTC) products, 385–386
- Oxidation, 59
- Oxygen radical absorbance capacity (ORAC), 85
- Pain sensation for skin, 11
- Palomar Icon Aesthetic System, 525
- Panniculitis, 535
- Papillary dermis peel, 501–502
- Papular telangiectasias, 510
- PAR-2, *see* Protease-activated receptor-2
- Paronychia, 277, 282, 284
- Partial neutralization, 60
- Paste masks, *see* Earth-based masks
- Pathological nails, cosmetics for
  - adverse reactions, 281–283
  - allergic reactions, 277–279
  - artificial nails, 276–277
  - nail hardeners and treatments, 283–285
  - non allergic reactions, 279–281
  - removable UV gels, 281
- PCOS, *see* Polycystic ovary syndrome
- PCPC, *see* Personal Care Products Council
- PDD, *see* Photodynamic diagnosis
- PDT, *see* Photodynamic therapy
- Pedicure implements, 267
- Pentaherbs (PTH), 161
- Percutaneous water loss, 425
- Perfumes
  - allergic contact dermatitis, 148–149
  - applications of, 148
  - causative ingredients, 149
  - fragrance allergic patient, 149–151
  - interventions and regulations, 151
- Perifolliculitis capitis abscedens et suffodiens, *see* Dissecting folliculitis
- Perimenopause, 415, 424
- Perioral area, 464
- Periorbital feet lines, 463
- Permanent fillers, 479
- Peroxisome proliferator-activated receptors (PPAR), 134, 310
- Personal Care Products Council (PCPC), 288
- Pesticide residues, 289
- Petrolatum, 289
- PGE2, *see* Prostaglandin E2
- PHA, *see* Polyhydroxy acids
- Phagocytosis, 536
- Pharmacological agents, 310
- Phenol, 508–509
- Phenotype, 232
- pH gradient, skin, 408
- Phloretin, 203–204
- Phospholipids, 101
- Phosphatidylcholine, 432
- Photoaging, 88–91, 120, *see also* Antioxidants
  - clinical manifestations of, 510
- Photocarcinogenesis, 76, 193–194
- Photodamage, 199, 528–529, *see also*
  - Sunscreen
    - antioxidants and dietary agents, 187–188
    - education and prevention of, 189
    - ferulic acid, 202–203
    - genistein, 207–209
    - phloretin, 203–204
    - protection from visible light and infrared radiation, 188–189
    - selenium, 204–206
    - signs, 90
    - solar exposure, 185–186
    - sunscreen, 186–187
    - ultraviolet radiation mechanisms, 185
    - vitamin C, 199–200
    - vitamin D, 187
    - vitamin E, 200–202
- Photodynamic diagnosis (PDD), 446
- Photodynamic therapy (PDT), 157, 217, 437, 531
  - effects on tissues, 442
  - indications in dermatology, 444–447
  - light source, 443–444
  - oxygen, 444
  - and pain, 448
  - photosensitizing agents, 442–443
  - in practice, 447–448
- Photoinitiators (PI), 220
- Photoplethysmography (PPG), 406
- Photoprotection
  - of human skin, 83–85
  - physical methods of, 187
- Photoprotective products, 170
- Photoreactions, ingredients, 304
- photosensitizing agents, 442–443
- Phototherapy treatment, of pigmentary disorders, 335
- Phototrichograms
  - hair, 417
  - scalp hair, 391
- pH values, 378–379
  - in skin surface, 384
- Physicochemical properties, 58–59
- Phytoestrogens, 426
- Phytosterols, 101
- PI, *see* Photoinitiators
- Picosecond lasers, 516
- Picotage, 432
- Piebaldism, 338
- PIF, *see* Product information file
- Pigmentary disorders, 531
- Pigmentary lesions, treatment of, 515–516
  - dark rings, 340–341
  - dyskeratosis, 341–342
  - hypopigmented striae and iatrogenic leukoderma, 341
  - melasma, 336–337
  - solar lentigines, 338–339
  - vittiligo, 337–338
- Pigmentary mosaicism, 340
- Pigmentation, 269
  - hair shaft, 415–416
- PIH, *see* Post-inflammatory hyperpigmentation
- Pilocarpine iontophoresis, sweat test, 5
- Pilosebaceous unit, 11–13
- Pineal hormone melatonin, 84
- Pityrosporum*, 248
- PL, *see* Polypodium leucotomos
- Placebo cream, 425
- Plantar warts, 454
- Plasmin
  - in dry skin, 117–120
  - in skin aging, 120–122
- Plasminogen activator, 118, 119
- Plasticizers, 268
- Plastic tips, 276
- Platysmal bands, 466
- PLE, *see* Protein-losing enteropathy
- Plicometry, 309
- PLLA, *see* Poly-L-lactic acid
- PMH, *see* Progressive macular hypomelanosis
- PMMA, *see* Polymethylmethacrylate
- PMS, *see* Polymultichrome stain
- Poikiloderma of civatte, 340
- Polidocanol (POL), 315
- Polycarboxy  $\alpha$ -hydroxyacids, 57
- Polycystic ovary syndrome (PCOS), 321
- Polydatin, 170
- Polyhydroxy acids (PHA), 57–58
- Poly-L-lactic acid (PLLA), 476
- Polymer-based microneedle roller, 437
- Polymerization of nail coating, 220–221
- Polymer nails, 279
- Polymethylmethacrylate (PMMA), 479
- Polymultichrome stain (PMS), 295
- Polyols, 171
- Polyphenols, 83–84, 96
- Polypodium leucotomos (PL), 188
- Polyunsaturated fatty acids (PUFAs), 171, 188
- Pomades, 246
- Pomegranate extract, 170
- Popliteal fossa, 551
- Porcine collagen, 473
- Porcine fat, 538
- Porcine tissue, 535–536
- Porosity, of hair, 392
- Porphyrias, 424
- Post-inflammatory hyperpigmentation (PIH), 109, 334, 385–386, 387
- Postinflammatory pigmentation, 341
- Postmenopausal alopecia, 427
- Postmenopausal facial hirsutism, 426
- Postmenopausal vulval atrophy, 426
- Postmenopausal women
  - parallels bone loss in, 425
  - skin aging in, 426
- Postmenopause, 424
- Postpartum telogen effluvium, 413
- Post-peel period, 509
- Postulated activation, of antioxidants, 81
- Potentiated pyrrithione zinc formulas, 253–254
- PPAR, *see* Peroxisome proliferator-activated receptors
- PPG, *see* Photoplethysmography
- Preformed artificial nails, 281–283
- Premarin, 425
- Premenopause, 424
- Pre-peel conditioning, 498
- Preservatives, 290–291, 302
- Press-on nail extender, 273
- Prevelle® Silk, 474
- Primary dermal irritation, 301–302
- Primary irritation, 293–294

- Product information file (PIF), 299  
 Progressive macular hypomelanosis (PMH), 341  
 Proliferative phase, *see* Fibroblastic phase  
 Prolonged paresthesia, 277, 279  
 Pro Mechanica® software, 261  
 Pronounced idiosyncratic edema, 456  
 Proper curing, of nail coatings, 220  
*Propionibacterium acnes*, 167, 303, 360, 361  
 Proprietary gamma irradiation, 99  
 Prostaglandin E2 (PGE2), 187  
 Prosthetic nail, 277  
 Protease-activated receptor-2 (PAR-2), 94, 384  
 Protective property of sunscreen, 186–187  
 Protein-losing enteropathy (PLE), 188  
 Proteoglycans, 167  
 Protoporphyrin IX absorption, 444  
 Pro urokinase-type plasminogen activator (Pro-uPA), 118  
 Pruritus, 64, 158, 162–163  
 Pseudoceramides, 291  
 Pseudochromhidrosis, 342  
 Pseudofolliculitis barbae, 387, 394  
*Pseudomonas*, 273  
 Psoriasis, 129–130, 161–163  
 Psoriatic skin, 118  
 Psychotherapy, 427  
 Pterygium inversum, 284  
 PUFAs, *see* Polyunsaturated fatty acids  
 Purpura, 512  
 Pushers, 267  
 Pyrithione zinc (ZPT), 252–253, 254–255  
 Pyrrolidone carboxylic acid, 171
- QoL, *see* Quality of life  
 QRA, *see* Quantitative risk assessment  
 QSPR model, *see* Quantitative structure–activity relationship model  
 Q-switched lasers, 515–516  
 Quality of life (QoL), 298–299, 369  
 Quantitative risk assessment (QRA), 302  
 Quantitative structure–activity relationship (QSPR) model, 33
- Rabbit ear model, cosmetic ingredients in, 304  
 Racket nails, 276  
 Radiation therapy, 357–358  
 Radiesse®, 477–479  
 Radio frequency (RF), 387  
 Raman spectroscopy, 128  
 Randomized controlled trials (RCTs), 369–370, 563  
 Randomized double-blind study, 255  
 Ra parameter, 17  
 RAR, *see* Retinoic acid receptors  
 RARs, *see* Retinoic acid receptors  
 RBP, *see* Retinol-binding protein  
 RCTs, *see* Randomized controlled trials  
 Reactive nitrogen species (RNS), 80, 82, 84  
 Reactive oxygen species (ROS), 65, 80, 185, 231  
 Refsum disease, 130  
 Rel homology, 143  
 Repeated insult patch test (RIPT), 102  
 Repeated nasal flare, 464  
 Repeated short-term occlusive irritation test (ROIT), 294  
 Repigmentation, 452  
 RES, *see* Resveratrol  
 Research Institute for Fragrance Materials (RIFM), 302  
 Resistance, water, 401  
 Resorcinol, 365  
 Restylane injection, 475
- Resveratrol (RES), 170, 188  
 Reticular dermis peel, 502–503  
 Retinoic acid receptors (RARs), 71, 361–362  
 Retinoid metabolic pathways, 72  
 Retinoids, 71, 88–91, 355, 361–362, 385  
   adapalene, 363  
   tazarotene, 362–363  
   tretinoin, 362  
 Retinoid-sensitive models, 74  
 Retinol  
   biological effect on skin, 88–89  
   clinical aspects of, 89–91  
   metabolism of, 88  
 Retinol-binding protein (RBP), 88  
 Retinol-cytoplasmic retinol-binding protein, 71  
 Retinol esters, molecular biology of, 71–72  
 Retinyl esters  
   biochemistry and molecular biology of, 71–72  
   potential to break retinoid efficacy to irritation correlation with, 72–74  
 Retinyl propionate, and related retinoids, 71–74  
 RF, *see* Radio frequency  
 Rhinophyma, 455  
*Rhizoma atractylodis*, 161  
 RIFM, *see* Research Institute for Fragrance Materials  
 RIPT, *see* Repeated insult patch test  
 RNS, *see* Reactive nitrogen species  
 ROIT, *see* Repeated short-term occlusive irritation test  
 Rooster comb–derived fillers, 474  
 ROS, *see* Reactive oxygen species  
 Rosacea, 63  
 RRR- $\alpha$ -tocopherol, 200–202  
 Rubber-based masks, 180–181  
 Rz parameter, 17
- SA, *see* Salicylic acid; Senescent alopecia  
 Salicylic acid (SA), 253, 364, *see also*  $\beta$ -Hydroxy acids  
 Sanitation, nail, 274  
 Savin patterns of hair loss, 414  
 SC, *see* Stratum corneum  
 Scars, 371, 529–530  
 SCC, *see* Squamous cell carcinoma  
 Sclerotherapy chemo-ablation techniques, 313–317  
 Scoring for atopic dermatitis (SCORAD) scale, 159–161  
 Scoring system, 77  
 Scrubbing pads, 180  
 Sculpted nail, 277  
 Sculptra®, 476–477  
 Sculptured artificial nails, 277, 278  
 SDA, *see* Spectrum descriptive analysis  
 SE, *see* Sebum excretion  
 Seborrheic dermatitis, 168, 248  
   treatment of, 172  
 Seborrheic keratosis, 339, 455  
 Sebufix F16, 169  
 Sebumeter SM 810, 169  
 Sebum excretion (SE), 379–380  
   measurement of, 169  
 Sebum output, quantification of, 169  
 Sebum production, 108–109  
 Sebum regulator, 172  
 Sebum secretion, 167  
 Sebutape®, 51, 169  
 Secondary prevention, actinic keratoses, 215–216  
 Selenium, 204–206  
   with vitamin E, 206–207  
 Selenium sulfide, 253, 254–255  
 Self-tanning products  
   application of, 175  
   indications, 175–176  
   mechanism of action, 174–175  
   safety, 176  
 Semi-automated phototrichograms, *see* Automated phototrichograms  
 Semipermanent fillers, 476–479  
 SENEb, *see* Subepidermal nonechogenic band  
 Senescent alopecia (SA), 413, 415  
 Senescent signals, 415  
 Senile xerosis, 131  
 Sensitive skin  
   factors in, 47–50  
   sensory effects and objective signs, 50  
   zeroing in on biological origin, 50–52  
 Sensitization-delayed (Type IV) contact hypersensitivity, 302  
 Sensitization testing, 302  
 Sensory functions of skin  
   autonomic function, 11  
   thermal response to stimulation, 10–11  
   thermoregulatory, 8–10  
 Sensory irritation, 302  
 Serine palmitoyl transferase (SPT), 129  
 Sesamol, 170  
 Seven-irritant panel, 47  
 Shampoos, 235–237  
 Short microneedles, 437  
 Siderosis, 341  
 Silicone oil, 269  
 Silicones, 239, 289  
 Silicon gel, 354  
 Single gel phase model, 129  
 Sjögren-Larsson syndrome, 130  
 Skicon®, 297  
 Skicon 200, 168  
 Ski-jump nails, 276  
 Skin aging, 88–89, 387, 424–425  
   new target for, 144–146  
   NF $\kappa$ B activation under, 144  
 Skin barrier function, 378  
 “Skin battery,” 483  
 Skin biopsies, 90  
 Skin brightness measurement, 169  
 Skin cancer, *see* Sunscreen  
 Skin care additives, 291  
 Skin care devices, 180–181  
 Skin care products  
   acne treatment, 171–172  
   care of dry skin, 170–171  
   care of greasy skin, 171  
   care of normal skin, 169–170  
   different types of skin, 167–168  
   methods for evaluating skin characteristics, 168–169  
   sebum regulator, 172  
   soybeans in, *see* non-denatured soy, in skin care  
 Skin color, 11, 12, 384  
 Skin conductance, 399  
 Skin disease  
   exogenous ochronosis, 386  
   irritation and stinging, 385  
   post-inflammatory hyperpigmentation (PIH), 385–386  
 Skin dryness, 295  
 Skin elasticity, 297  
 Skin firming, enhancement in, 108  
 Skin glycosaminoglycan composition, 259  
 Skin homeostasis, 143–144  
 Skin hydration measurements, 168  
 Skin innervation, 411  
 Skin irritation, 385, 399  
 Skin lipids, 384  
 Skin maximization study, 78  
 Skin peeling, 60–62



- Skin physiology  
   biochemical composition of, 3–5  
   functional differences, 5–7  
   sebaceous glands, 11–13  
   sensory functions, 8–11
- Skin radiance, 90
- Skin rejuvenation, 387
- Skin renjuvenation, mesotherapy for, 432–434
- Skin smoothing, 63
- Skin stinging, 385
- Skin surface morphology, evaluation of, 168
- Skin surface texture, treatment with *t*-AMCHA methyamide, 120
- Skin thickness, 60, 425
- Skin xerosis, 132
- SLS, *see* Sodium lauryl sulfate
- Smell, 371
- SOD, *see* Superoxide dismutase
- Sodium dependent vitamin C transporter (SVCT), 82
- Sodium lactate, 171
- Sodium lauryl sulfate (SLS), 117, 398
- Sodium morrhuate, 315
- Soft keratin, 32
- Soft tissue augmentation  
   evolution of fillers and, 473  
   long-lasting/semipermanent fillers, 476–479  
   permanent fillers, 479  
   temporary fillers, 473–476
- Solar exposure, 185–186
- Solar keratosis, 65
- Solar lentigines, 338–339, 387
- Solid crystal, 289
- Solid microneedles, 436, 437–439
- Solta lasers, 521
- Sonicating electric toothbrushes, 180
- Soybean oil, 93
- Soybean plant, 93
- Soybean trypsin inhibitor (STI), 94
- Soy isoflavones, 102
- Soy proteins, 94  
   isolate/hydrolysate, 93
- Spectroscopic methods, for skin microstructure, 296
- Spectrum descriptive analysis (SDA), 298
- SPF, *see* Sun protection factor
- Sphingolipids, 134–135
- Sphingosine, 128
- Spider nevi, 453
- Spider telangiectasias, 510
- Spider veins, treatment of, 313
- Spinocellular carcinoma, *see* Squamous cell carcinoma
- Spray-on tanning formulations, 175
- Spritz, *see* Hairspray
- SPT, *see* Serine palmitoyl transferase
- Squamometry, 255
- Squamous cell carcinoma (SCC), 186, 193, 194, 214, 446  
   lesions, 96
- Ssurface erythema, 248
- Standard pyrrhione zinc formulas, 253–254
- Staphylococcus aureus*, 160, 273, 395
- Staphylococcus epidermidis*, 273
- Static electricity generators, 481
- Stearalkonium hectorite, 269
- Stereoisomers, 58
- Sterilization, nail, 274
- STI, *see* Soybean trypsin inhibitor
- Stick-on nail dressings, 273
- Straight Caucasian hair, 390
- Straightening, 244–245
- Stratum corneum (SC), 28, 32, 38, 250, 302, 436  
   barrier function, 59–60, 117  
   ceramides, *see* Ceramides
- hydration, 378, 379  
   lipid composition, 132  
   of microneedles, 439  
   sphingolipid, 4  
   structure, 384  
   urokinase activity in, 118
- Stratum corneum's surface, 295–296
- Streptococcal-derived fillers, 474
- Streptococcus thermophilus*, 134
- Striae, 530–531
- Styling problems, 243
- Styling products, 245–246
- Subcutaneous fat, 535–536, 539, 546  
   layer, 259
- Subepidermal nonechogenic band (SENEB), 387
- Sulfur, 365
- Sunburn cells, 64–65
- Sun protection, 386
- Sun protection factor (SPF), 186, 195
- Sunscreens, 186–187, 194–196  
   activity, 175  
   human factors in, 196–197  
   ultraviolet radiation, 193–194
- Superficial basal cell carcinoma, 445–446
- Superficial peelings, 365
- Superficial thrombophlebitis, 316–317
- Superoxide dismutase (SOD), 80–82, 84  
   in kinetin, 113
- Suppuration, 371
- Suprabasal keratinocytes, 88
- Surface corneocyte integrity, 296
- Surface nail damage, 281
- Surface washing procedures, nail plate, 34, 36
- Surgical ligation, 317–318
- Surgical subcision, 311
- SVCT, *see* Sodium dependent vitamin C transporter
- Sweat secretion rates, 5–7
- Syncope, 456
- Synergistic compositions, 62
- t*-AMCHA, *see trans*-4-(aminomethyl) cyclohexane carboxylic acid
- t*-AMCHA methylamide, 119–120
- Tantalizing clues, 50
- Tape strippings, stratum corneum, 124, 131, 133
- TAPS, *see* Tetracytlyphytosphingosine
- Tartaric acid, 57
- TAS, *see* Terminal amino silicones
- Tattoos, 456
- Tavaborole, 157
- Tazarotene, 332, 362–363
- TCHM, *see* Traditional Chinese herbal medicine
- TE, *see* Telogen effluvium
- Tea tree oil, 155–156
- Teflon nail, 276, 277
- Telangiectasia vein, 510  
   epidemiology, 312  
   sclerotherapy/endovenous chemo-ablation techniques, 313–317  
   venous anatomy and physiology of, 313
- Telangiectatic matting, 316
- Telogen effluvium (TE), 413
- Temporary fillers, 473–476
- Tensile strength, of hair, 393
- TEP, *see* Transepithelial potential
- Terminal amino silicones (TAS), 238
- Terminal hair shaft, 228
- Test products of behind-the-knee test, 551
- Test reactions to ingredients, 303
- Tetracytlyphytosphingosine (TAPS), 134
- TEWL, *see* Transepidermal water loss
- Textured cloths, 179
- TF resin, *see* Tosylamide/formaldehyde resin
- Thermal sensation for skin, 11
- Thermocouple device, 451
- Thermography, 309
- Thermoplastic resin, 271
- Thigh circumference measurement, 309
- Thimble-shaped finger, 279
- Thinning of nail plate, 278
- Thioctic acid, 333
- Thiol antioxidants, 84
- Thiol retention activity, 104
- Thixotropic systems, 269
- Thulium 1927-nm wavelength, 522
- Tight braids, 393
- Timed spot freeze technique, 450–451
- Tissue engineering, 490
- Tissue-type plasminogen activator (tPA), 118
- $\alpha$ -Tocopherol, 81–85
- Tocopherols, molecular structures of, 201
- Toenails, 267
- Toluenesulfonamide/formaldehyde resin (TSFR), 268
- Top coats, 269–271
- Topical antibiotics, 172
- Topical keratolytics, 360
- Tosylamide/formaldehyde resin (TF), 268
- Toxicology, of body lotions  
   adverse reactions, 304–305  
   adverse skin effects, 301–304  
   lotion composition, 300–301  
   skin condition, 301
- tPA, *see* Tissue-type plasminogen activator
- Traction alopecia, 393
- Traditional Chinese herbal medicine (TCHM), 161
- Traditional pulsed dye laser, 512
- Tranexamic acid, 332
- trans*-4-(aminomethyl) cyclohexane carboxylic acid (*t*-AMCHA), 117–118
- TransScreen-N™, 33
- Transcutaneous electrotherapy, 482
- Transdermal drug delivery, 436
- Transepidermal penetration, 506
- Transepidermal water loss (TEWL), 378, 398–401, 552  
   biological background, 28  
   measurement of, 28–29, 169  
   parameter in skin research, 30  
   skin, 29
- Transepithelial potential (TEP), 483
- Traumatic injuries, nail, 273–274
- Traumatic thermal burn scars, 529
- Treatment algorithm for actinic keratosis, 215, 216
- Tretinoin, 172, 332, 362
- Trichloroacetic acid, 505–507
- Trichophyton rubrum*, 155–158
- TrichoScan®, 391, 416
- Triethanolamine, 273
- Triglycerides, 289
- TRP-1, *see* Tyrosinase-related protein
- Trypsin inhibitory activity, of non-denatured soy, 103
- Tryptophan fluorescence, 90
- TSFR, *see* Toluenesulfonamide/formaldehyde resin
- Twelve-week placebo-controlled anti-aging study, 494
- Tyndall effect, 475
- Tyrosinase activity, potent inhibitors of, 144
- Tyrosinase-related protein (TRP-1), 331
- Ultra-low intensity electricity therapy, 482
- Ultrasensitive vibrating probe technique, 484
- Ultrasonography, 309
- Ultrasound (US), 309, 387

- Ultraviolet A (UVA), 193–196  
 Ultraviolet B (UVB), 193–196  
 Ultraviolet-induced damage, reduction of, 106  
 Ultraviolet (UV) radiation, 193–194  
 Ultraviolet radiation (UVR), 80, 82–84  
   mechanisms of, 185  
 Under-eye wrinkles, 90  
 Ungual drug permeability, limitations of, 33  
 Unilateral treatment, cryolipolysis, 544  
 United States Department of Agriculture (USDA), 182  
 United States, hand and body lotions in, 287, 288  
 Unstable electron transfer, 76  
 uPA, *see* Urokinase-type plasminogen activator  
 Upper arm treatment, cryolipolysis, 544  
 Upper face, 462–464  
 Urea hydrates, 171  
 Urokinase activity  
   in dry skin, 117–120  
   in skin aging, 120–122  
 Urokinase-type plasminogen activator (uPA), 118  
 Urticaria, 313  
 US, *see* Ultrasound  
 USDA, *see* United States Department of Agriculture  
 Use of testing, assess adverse reactions, 304–305  
 UVA, *see* Ultraviolet A  
 UVA/UVB protection, 106  
 UVB, *see* Ultraviolet B  
 UVB-induced skin damage, reduction in, 104  
 UV bulbs, 278  
 UV-cured (meth)acrylic nails, 281  
 UV-curing gels, 279–280  
 UV gel polish/manicure, 270, 280–281  
 UV gels, 281  
 UV nail lamps, 220  
   proper polymerization, 220–221  
   safety of, 221–223  
 UV nail lights *vs.* natural sunlight, 223  
 UV penetration, 278  
 UVR, *see* Ultraviolet radiation  
 UV radiation, *see* Ultraviolet radiation  
 Vaginal dryness, 426  
 Validated assessment scales (VAS), 380–381  
 Varicose vein  
   historical aspects, 312  
   modern minimally invasive surgical approaches for, 317–318  
   venous anatomy and physiology of, 313  
 VAS, *see* Validated assessment scales  
 Vascular endothelial growth factor (VEGF), 104  
 Vascular lesions, 453–454, 510–511  
 Vascular stasis, 452  
 Vasoactive response, 49  
 Vasovagal symptoms, cryolipolysis, 542  
 VEGF, *see* Vascular endothelial growth factor  
 Velasmoor, 310  
 Vellus hair, 228, 391  
 Venule-derived telangiectasias, 510  
 Verrucae vulgares, *see* Warts  
 Very long chain fatty acids (VLCFA), 127  
 Vicks® VapoRub, 156–157  
 Videodermoscopy, 391  
 Villus hair, 396  
 Vinyl-based masks, 180–181  
 Viral infection, actinic keratoses, 214  
 Viral warts, 454–455  
 Viscosity modifiers, 269  
 Visible light-induced skin oxidative stress, reduction in, 105  
 Visible light, protection from, 188–189  
 Visioscan® VC98, 168, 296  
 Vistabel®, 459  
 Vistabex®, 459  
 Vitamin B12, 158–159, 163  
 Vitamin C, 83, 199–200  
   with ferulic acid, 203–204  
   with vitamin E, 202–203  
 Vitamin D, 187  
 Vitamin E, 83, 200–202  
   selenium with, 206–207  
   vitamin C with, 202–203  
 Vitamins, 170, 188  
 VLCFA, *see* Very long chain fatty acids  
 Vocal anesthesia, 448  
 Volume control  
   of hair health, 241, 243–244  
 Vulva, 49  
 Vulvar atrophy, 426  
 Warts, 63–64, 273  
 Water-based nail varnishes, 270  
 Water content (WC), 399, 401–404  
   of skin, 297, 384  
 Water-soluble polymers, 290  
 Water vapor, 29  
 Wattage, 220–222  
 Waxes, 246  
 Wax masks, 180  
 WC, *see* Water content  
 Weathering, process of, 232  
 Wettability, skin, 169  
 Winter xerosis, 131–132  
 Wound healing enhancement, 489–490  
 Wounding epidermis, plasmin, 118  
 Wrinkle, 380  
   development, 380–381  
 Wrinkles, 65  
   aspect of aged skin, 259–260  
   compared to surrounding skin, 260–261  
   implications for treatment of, 262–263  
 Xenon halogens, 443  
 Xerosis, *see* Dry skin  
 Zeatin, 113  
 ZPT, *see* Pyrithione zinc