28 Effect of Moisturizers on the Structure of Lipids in the Outer Stratum Corneum of Humans

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CONTENTS

28.1 INTRODUCTION

Lipids in the stratum corneum (SC) account for only about 15% of its weight, yet they constitute the primary barrier of the skin,[1–5] forming a protective sheath that shields us from desiccation and environmental insults.[6] These barrier lipids exist in the SC intercellular space as highly organized lamellar bilayers that are readily visualized by the marriage of transmission electron microscopy (TEM) with RuO_4 staining.[7,8] The lamellar organization consists of a unique pattern of alternating electron-lucent and electron-dense lamellae forming repeating structures[7–10] that are often referred to as Landmann units.[10] This lamellar structure appears throughout most of the SC thickness,

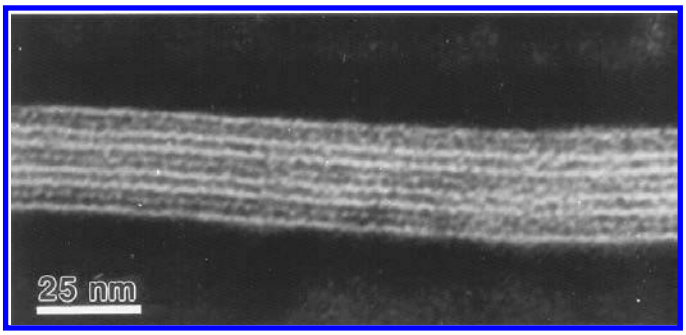

FIGURE 28.1 Normal structure of the lipid lamellae in the intercellular space. Shown are three Landmann units separating the darkly staining corneocytes above and below this intercellular space. Bar = 25 nm.

with variability occurring primarily in the number of Landmann units that bridge the intercellular space.[8,10–12] However, more diverse structures have been described in the outer SC,[11–14] perhaps reflecting environmental impact[12,14] or inherent differences in lipid composition.[15–18]

This chapter focuses on the lipid structure found in the outermost layers of the SC in humans. We present a modified TEM technique to investigate this structure, attempt to systematize and understand the variability in lipid structure observed in the outer SC, and explore the effect of moisturizers on the outer SC at microscopic and macroscopic levels.

28.1.1 Inner Stratum Corneum Lipids

The Landmann-unit structure of intercellular lipid lamellae is illustrated in Figure 28.1. This structure is found throughout nearly all of the normal SC. Swartzendruber et al. proposed a plausible molecular model that accounts for the electron-lucent and electron-dense lamellar structure of the Landmann unit.[10] These Landmann units are dynamic in nature. At least in the inner and middle SC they are altered by age,[19] disease,[8,11,20–22] and hormonal status[23,24]; by experimental solvent treatment[25–27] and topical inhibitor treatment.[28,29] They are known to reform spontaneously following solvent extraction,[5,26] and topical application of certain lipids is also reported to effect lamellar repair and barrier improvement.[29–33]

28.1.2 Outer Stratum Corneum Lipids

In contrast to the more extensive studies of the intercellular lipids of the inner and middle SC, there are few studies of the lipid structure in the outer SC. Evidence suggests that the intercellular lipid composition in the uppermost layers of the SC differs from that found in the lower layers.[34] The outer SC lipids also exhibit structural variability compared to the inner and middle stratum corneum, both with regard to lipid ordering and lateral packing[16,35] and the number of intercellular lamellae, which increases from the usual two or three to in excess of 100 bilayers.[13] For normal skin with little or no visible dryness the outer SC intercellular space is filled with an amorphous lipid material, whereas in soap-treated skin with pronounced visible dryness this space is filled with numerous disorganized lamellae.[14] A separate *in vitro* study using human skin substrate also showed disordered lipid lamellae in the outer SC following soap treatment, less lipid disruption following treatment with a soap/glycerin/oil bar, and normal lamellae following treatment with an isethionate-based bar.[12]

To the extent that lipids are involved in corneocyte cohesion,[36–39] the lipid structure in the outer SC is presumably very important for proper desquamation. However, because the outer SC interfaces with the surrounding environment, its lipids are the most susceptible to structural alterations caused by environmental insult or consumer products that often contain surfactants or solvents.[5,12,30] While

the quantity of SC lipid is apparently not a primary determinant of dryness in normal skin,[40] there may be a functional relationship between the lipid structure of the outer SC and skin dryness.

If consumer products containing soaps or solvents can damage the outer SC lipid structure, then products like moisturizers might also have an impact on this structure. For example, glycerin is reported to increase water binding in the SC and act as a corneodesmolytic,[41] inhibit humidity-induced SC lipid crystalline phase transitions,[42] and speed barrier recovery.[43] Maleated soybean oil inhibits crystalline phase transitions and reduces water loss in model SC lipid systems.[42] And petrolatum, which is often viewed as a gold standard for moisturization, can permeate the upper layers of the SC, affect SC lipid structure, and accelerate barrier repair.[29,44] Conversely, there is evidence that single components of physiologic lipid mixtures and some moisturizers interfere with recovery following experimental barrier disruption.[31,45,46]

Studies employing mixtures of physiological lipids provide important insights into how topical application of these products can impact SC lipids. However, moisturizers sold in the mass market are often quite different from these specialty formulations, being based on more common moisturizing ingredients. Although commercial moisturizers typically improve skin condition, relatively little is known about *how* they effect this improvement. These products appear to provide a continuum of effects ranging from the purely cosmetic, such as temporarily camouflaging visible dry flakes, to more functional effects such as abetting biological repair processes.[47] As noted previously, one mechanism by which the latter might occur is by aiding the digestion of desmosomes that are abnormally retained in the outer SC, thereby enhancing the desquamation process.[41,48] Another mechanism, however, might involve the SC lipids. Moisturizers often contain lipophilic materials, and lipids play a very important role in skin barrier properties,[49,50] so it is reasonable to assume that moisturizers in some way interact with the SC lipids to improve the skin barrier and thereby enhance SC hydration by a mechanism other than simple occlusion.[44,45,49–51]

This chapter investigates alterations in the lipid structure of the outer SC that are induced by moisturizing ingredients and commercial moisturizing products. As a preface to this investigation, we also examine the normal variability in the lipid structure of the outer SC and how it is affected by factors such as age, level of visible dryness, and personal cleanser use.

28.2 TAPE STRIP PROTOCOL

The outer SC was sampled by tape stripping (Scotch Magic Tape 810, 3*M*) using a modification of a previously reported procedure.[14] The tape was applied to the lateral leg surface using gentle pressure and carefully removed after approximately 30 sec. Under stereomicroscope observation, regions of the tape having large clusters of skin flakes were cut out and placed in 0.25% RuO_4 in a 0.1 *M* cacodylate buffer for 1 h at 4°C, rinsed briefly in 0.1 *M* cacodylate buffer, and then dehydrated through a graded acetone series prior to Epon embedding and overnight polymerization at 65°C. Thin sections were cut on an ultramicrotome, counterstained with uranyl acetate and lead citrate, and analyzed in a Philips CM12 at 100 keV. The lipid structure of the SC improves as a function of depth into the SC; by the third tape strip, lipid structure has normalized to the typical Landmann pattern.[14] To focus on the superficial SC only one tape strip was taken, and whenever possible micrographs were obtained only from the outermost 3 to 4 corneocytes, adjacent to the tape. Similarly, to minimize possible artifacts resulting from the mechanical process of tape stripping or from previously uplifted scale, whenever possible micrographs were taken from closely apposed intercellular regions, thus minimizing potential problems of physical trauma or interference from the tape adhesive or applied materials. Since the assessments of lipid structure were qualitative and subjective, tape strip samples were blinded until the analysis completed.

Although this tape-stripping approach is a useful procedure, it does have limitations. For example, there are limitations inherent to RuO_4 staining due to its poor penetration and high reactivity, as discussed previously.[22] These staining limitations are superimposed on the problems of

representative sampling associated with the tape stripping procedure. Only limited areas within a tape strip meet the analysis criteria for TEM inspection, and lipid structure varies even within a single tape strip. Nevertheless, this normal variation in lipid structure is relatively small compared to the large structural changes that are encountered in the outer SC, as will be seen. In our experience, the outer SC lipid structure of an individual's skin is relatively constant over large areas, so that their outer SC lipid structure is quite consistent over an entire leg and similar between legs. The variation that does exist, however, limits the ability to detect small changes in lipid structure. In particular, it is difficult to detect improvements in lipid structure due to the use of moisturizing products when the skin is already in good condition.

Another important limitation is the labor-intensive nature of TEM investigations; the number of samples that can be analyzed in a reasonable time period is small. In the background studies presented here, a minimum of three SC samples were analyzed for each treatment except for mineral oil, where a single sample was analyzed.

28.3 NORMAL LIPID STRUCTURE OF THE OUTER STRATUM CORNEUM

The objective of this study was to observe the lipid structure of the outer SC in a population of people engaged in their usual personal care practices. Accordingly, in this study of normal lipid structure healthy female participants were selected at random without advance knowledge of their usual body skin care practices and without any preconditioning or product use restrictions. The ages of the selected individuals ranged from 22 to 52. Leg dryness was evaluated by an expert grader prior to tape strip sampling.[52]

28.3.1 Young Skin

The lipids of young skin (individuals in their early twenties) with little or no visible dryness typically have a good Landmann unit structure even at the surface of the SC, as shown in Figure 28.2(a). Youthful skin in good condition is invariably associated with closely apposed corneocytes, narrow intercellular spaces, and distinct bilayer structures. In contrast, young individuals with dry skin do not have Landmann units in their outer SC. A variety of intercellular lipid morphologies is observed in different individuals with poor skin grade including fibrous, mesh, and amorphous structures. Usually the intercellular spaces are considerably widened. An example of the latter is shown in Figure 28.2(b), in which the intercellular spaces are filled with an amorphous material having a variety of textures.

28.3.2 Old Skin

Focal domains that are depleted or devoid of lipid bilayers are reported in aged (>80 years) skin.[19] The oldest subject who participated in the present work was considerably younger than this, but we typically did not observe intercellular lipids with a Landmann unit structure in the outer SC in individuals over 40 years of age, regardless of skin condition. It thus appears that loss of SC lipid structure begins much earlier in life than was previously reported, and on this basis we define "old skin" to be skin from a person greater than age 40. An example of lipid structure from an "old" person with good skin condition is shown in Figure 28.3(a). It is common to find lamellae, but these lamellae are seldom present as fully formed Landmann units. Often lamellae are present at the periphery of corneocytes separated by a central band of nonlamellar amorphous/fibrous material as shown in Figure 28.3(a). Other intercellular spaces are simply filled with nonlamellar material (not shown). As with more youthful skin, the corneocytes are nevertheless typically closely apposed. In older individuals with dry skin the intercellular spaces can become spectacularly abnormal. Very widened intercellular spaces are common, usually filled with amorphous material that can contain a great

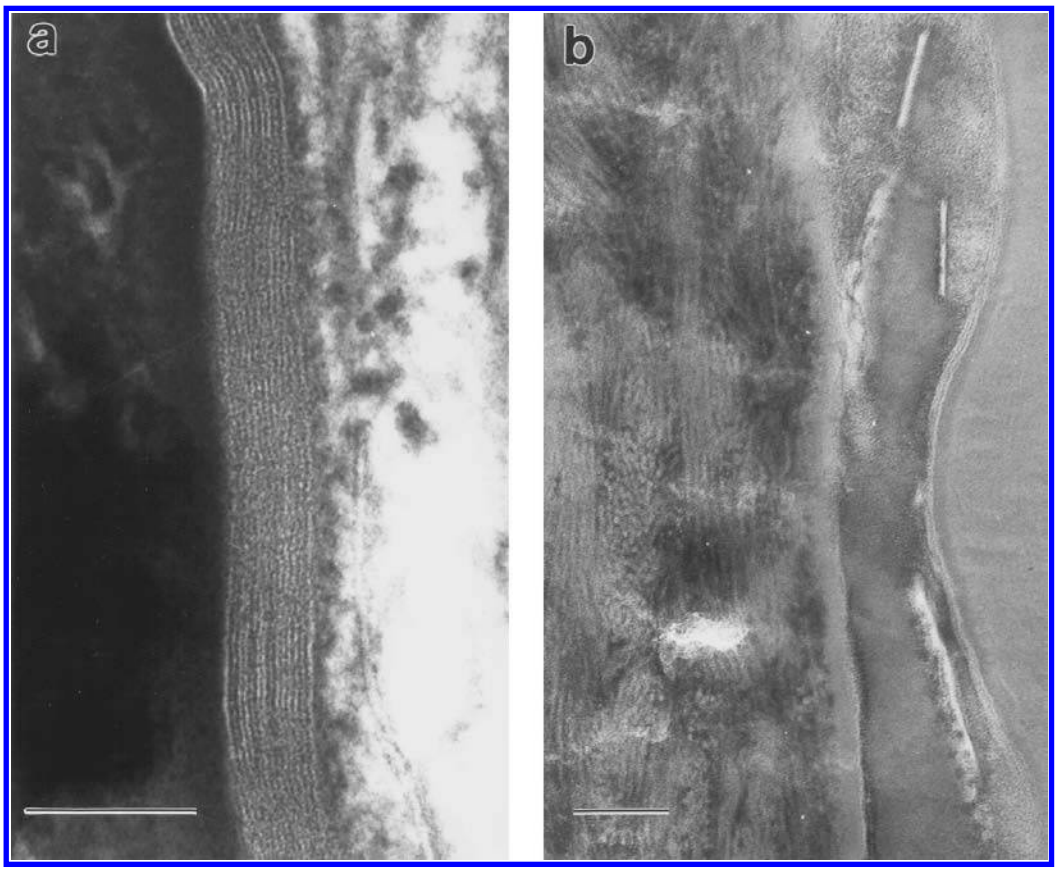

FIGURE 28.2 (a) Landmann units from the outer SC of a person 24-years old, skin grade 0.5. (b) A mixture of amorphous materials with different textures in the intercellular space of the outer SC from a person 28 years old, skin grade 5.0. Bar = 100 nm.

diversity of lipid structures. An example is shown in Figure 28.3(b); the outermost intercellular space appears to consist of a two-phase system, the noncontinuous phase being membrane-bound. Vesicles are apparent. The intercellular spaces are generally widened, many apparently filled with an amorphous material. There is no organized lamellar structure.

28.4 THE EFFECT OF SURFACTANT-BASED CLEANSERS

Surfactants are natural emulsifiers of oils and lipids. This property makes them effective cleansers but also contributes to their ability to impact SC lipids, whether through lipid extraction or lipid compositional changes. Controlled washing of the leg with soap for two weeks results in a worsening of dry skin appearance and produces alterations in the lipid structure of the outer SC. Two distinct altered intercellular structures are observed. In one form, intercellular spaces appear "invaded" by heavily staining globules of a variety of sizes, as shown in Figure 28.4(a). A more frequently observed response to soap use is the formation of profuse disorganized lamellae within widened intercellular spaces, as illustrated by Figure 28.4(b) and as reported previously.[14] In this latter figure, although localized domains of ordered lamellae exist over short dimensions, the lamellae are visualized as single electron-dense and electron-lucent lines with no evidence of the distinct substructure of the Landmann unit.

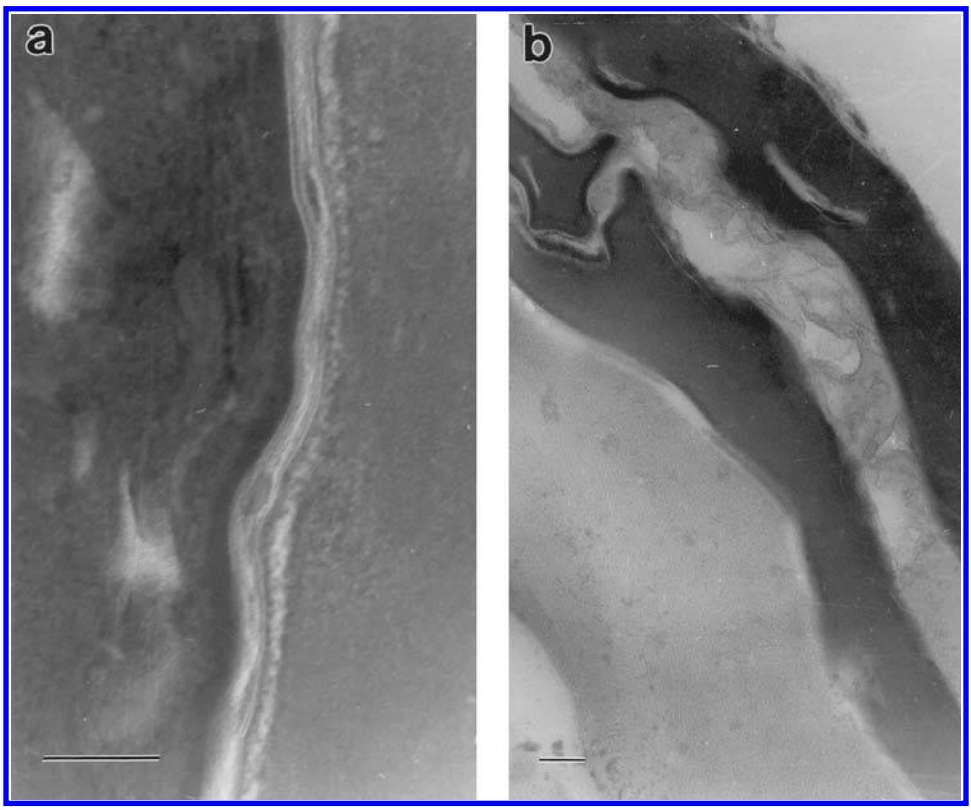

FIGURE 28.3 (a) Lipid structure in the intercellular space from a person 45-years old, skin grade 1.0. The corneocytes are closely apposed and lamellae are frequent, but these lamellae appear somewhat disorganized and do not form Landmann units. The core of the intercellular space is filled with nonlamellar material that is amorphous and fibrous with interspersed granular deposits. (b) Lipid structure from a person 49 years old, skin grade 3.5. The outermost intercellular space contains vesicular structures and membrane-bounded phases. Inner intercellular spaces appear to contain largely amorphous material. Bar = 100 nm.

Synthetic, that is, nonsoap surfactants often exhibit better skin compatibility than soap and are found in a range of personal care products. Cleansers based on these surfactants generally produce less visible irritation and dryness than soap; however, they can still remove significant quantities of lipid from the skin during washing.[53] Effects on SC lipid bilayer structure consistent with those we observed following soap washing were recently reported following controlled washing with cleansers based on "mild" synthetic surfactant systems.[54] Thus, it appears that surfactant-induced changes in the lipid structure of the outer SC are possible with a wide range of cleanser types, not just with soap.

28.5 THE EFFECT OF MOISTURIZERS

The results presented thus far show that the lipid structure of the outer SC varies with age and dry skin condition, and that cleansing products can degrade this lipid structure. We now return to questions raised earlier: do moisturizing ingredients enter the SC, and if so, can they alter the outer SC lipid structure? To address these questions we investigated the effect of neat moisturizing ingredients, reduced-concentration (i.e., "formulated") moisturizing ingredients, and fully formulated commercial products on the lipid structure of the outer SC of the leg following two or three weeks of product use.

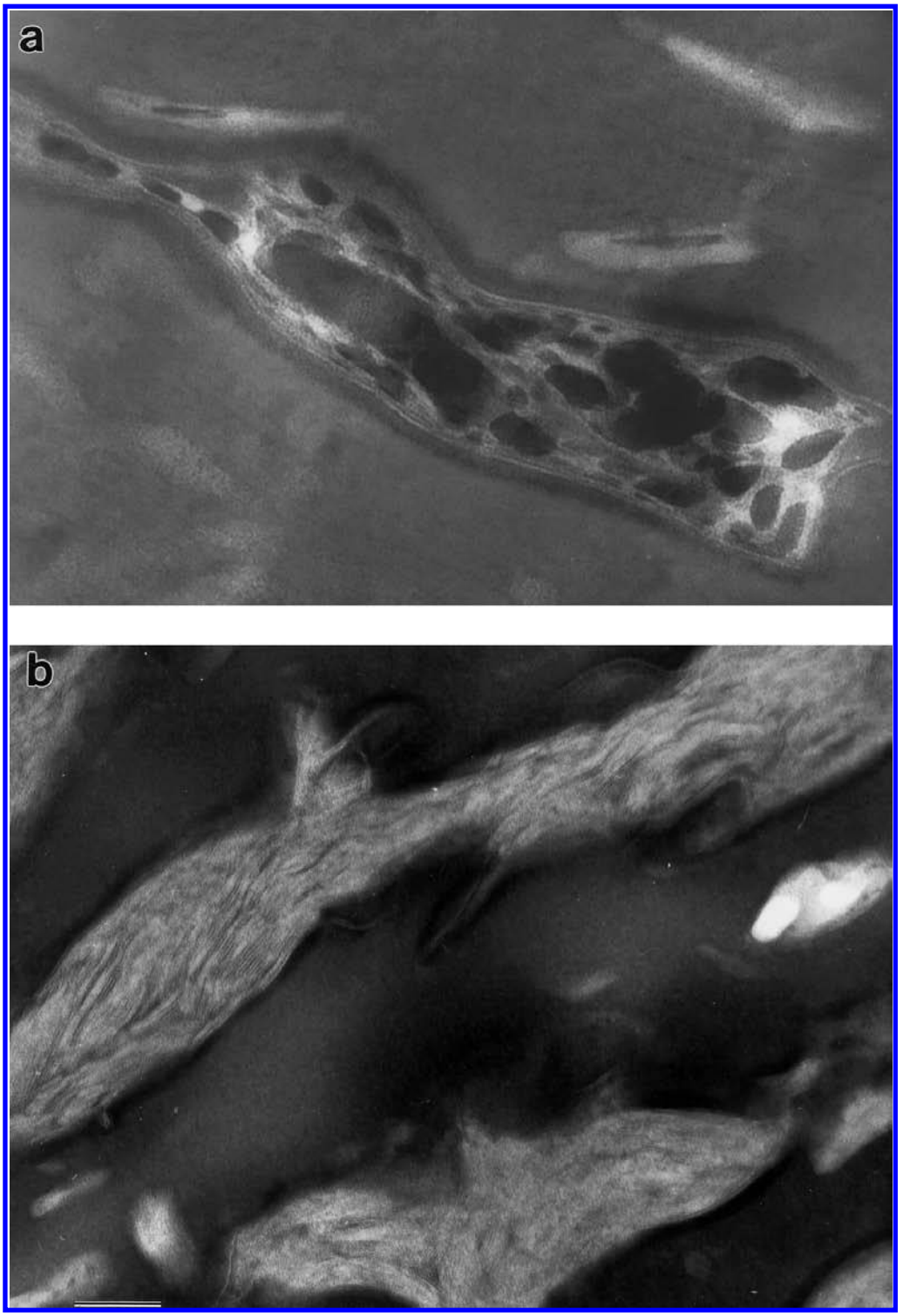

FIGURE 28.4 (a) Soap treatment frequently results in the formation of many darkly staining globular bodies in an amorphous matrix. (b) The signature pattern of soap use is the presence of widened intercellular spaces that are filled with numerous disorganized lamellae without a Landmann pattern. Bar = 100 nm.

For maximum comparative value the focus of this discussion is on results generated in matched studies conducted on a 42-year old male, though similar results were obtained from other subjects. Mineral oil, petrolatum formulated at 10% in an oil-in-water emulsion vehicle containing high levels of humectants, and sucrose esters of fatty acids (SEFA, The Procter & Gamble Company, Cincinnati, OH) formulated at 2 and 10% in the same vehicle, were applied at 3 mg/cm^2 on the lower leg twice a day for two weeks. Neat petrolatum and neat SEFA were applied ad lib twice a day for two weeks. In all cases the final product application was 12 h before tape stripping. All subjects used a syndet-based bar for daily personal cleansing, avoiding direct application of the bar or its lather to the treatment areas.

28.5.1 Mineral Oil

The control, nontreated site is shown in Figure 28.5(a), and the mineral oil-treated site in Figure 28.5(b) (same magnification). In the control skin, the outermost layers contain amorphous material and darkly staining globules. Lamellar structures are found in lower layers but the lamellae do not appear to form Landmann units. Following use of mineral oil the intercellular space is uniformly filled with a smooth-appearing amorphous material, presumably the mineral oil. Intercellular spaces were occasionally focally dilated. There seemed to be little effect of the mineral oil other than as a "spacer" separating corneocytes.

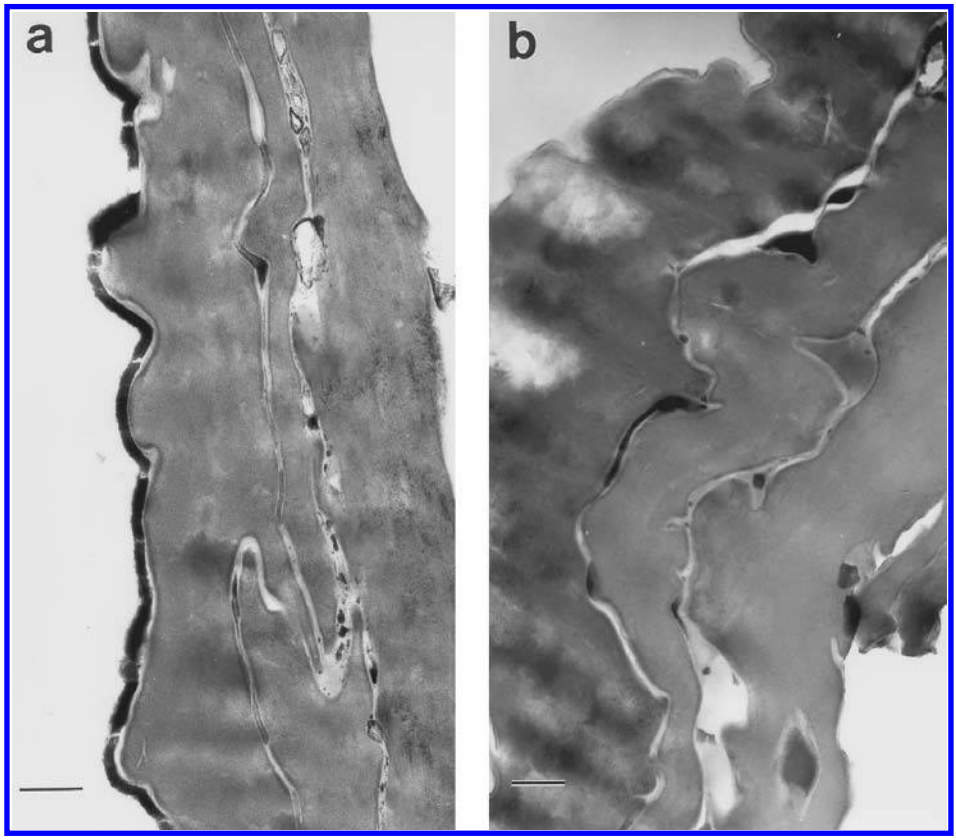

FIGURE 28.5 (a) Control, nontreated site from a 42-year-old male. The outermost (right) layers contain darkly staining globules in an amorphous matrix. Lamellae are present in deeper corneocyte layers, but Landmann units are rare. (b) Treatment with mineral oil results in the formation of large amorphous phases containing some darkly staining material. Bar = 200 nm.

28.5.2 Petrolatum

28.5.2.1 Neat Petrolatum

Petrolatum comprises a complex hydrocarbon mixture that is about 60 to 70% mineral oil, the remainder consisting primarily of paraffin and microcrystalline wax. Despite this composition, the effect of petrolatum on outer SC lipids is distinct from that of mineral oil. As shown in Figure 28.6(a), neat petrolatum forms lamellar-like "streamers" in the intercellular space, as seen previously.[44] The streamers appear to be suspended in a nonstaining or empty intercellular medium,

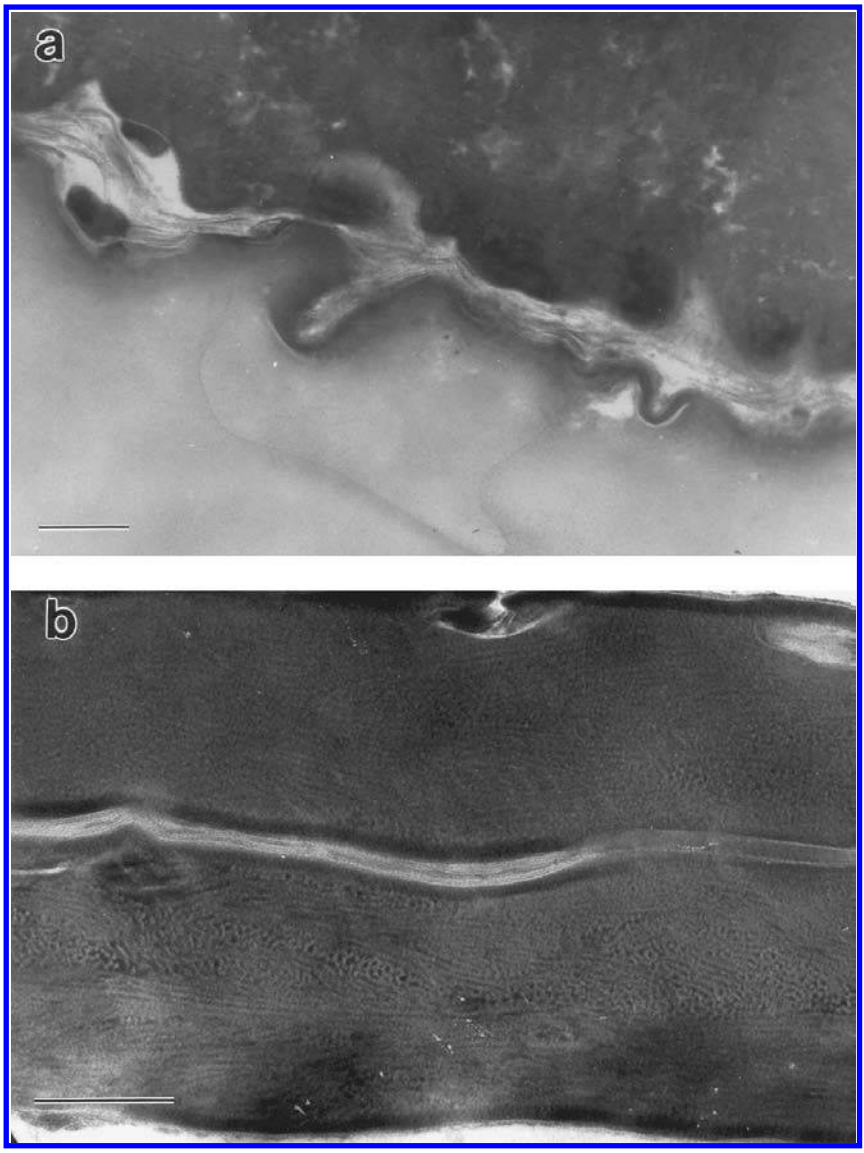

FIGURE 28.6 (a) Neat petrolatum site from a 42-year-old male. Flocculent/fibrous material existing as "streamers" or bands is present within an otherwise empty-appearing intercellular space. (b) Formulated (10%) petrolatum site. Lamellae, occasionally forming Landmann units, are sometimes separated by a thin layer of more darkly staining amorphous material. Bar = 200 nm.

or possibly water. In other areas, petrolatum forms a more continuous amorphous phase, also reported previously.[45] Intercellular structures intermediate between these two appearances are also formed (data not shown).

In other studies, similar streamer and amorphous structures were observed in a young female with dry skin following the above treatment protocol, although the amorphous phase was less prominent. In contrast, the streamer phase was less obvious in older individuals treated with 2 mg/cm^2 twice a day for three weeks.

28.5.2.2 Formulated Petrolatum

The "streamer" phase observed with neat petrolatum (Figure 28.6[a]) was not observed, but amorphous material was common (data not shown). Reasonable lamellae were occasionally encountered, as shown in Figure 28.6(b). Often these lamellae were separated by thin expanses of amorphous material, as shown in the center of Figure 28.6(b). In general, treatment with "formulated" petrolatum resulted in an appearance of the intercellular lipids that was much improved over that of neat petrolatum or mineral oil. The corneocytes were more closely apposed, and Landmann units were more common.

28.5.3 Sucrose Esters of Fatty Acids

28.5.3.1 Neat SEFA

Treatment with SEFA resulted in a very characteristic appearance of the intercellular space, shown in Figure 28.7(a), which we describe as the "SEFA look." The corneocytes are relatively closely apposed, single Landmann units are present at corneocyte margins, and the slightly expanded intervening space is "plugged" with an amorphous material, presumably SEFA. Unlike the other products above, multiple Landmann units are occasionally present, although the multiple units are usually present in short regions within the SEFA "plug," as shown in Figure 28.7(b). Very similar results were obtained in a young female with dry skin.

28.5.3.2 Formulated SEFA

The structure of the lipids in the intercellular space is overwhelmingly the "SEFA look" for both the 2 and 10% concentrations, as shown in Figure 28.8(a). With the 10% concentration, extra Landmann units within the SEFA phase were occasionally seen, as shown in Figure 28.8(b).

In a separate study, 2 mg/cm^2 of 2 or 10% SEFA in a humectant vehicle were applied to the lower leg twice a day for three weeks. The control nontreated site of a 52-year-old female subject, shown in Figure 28.9(a), is characterized by numerous disorganized lamellae characteristic of soap use. Numerous darkly staining globular deposits were also common (data not shown). The humectant vehicle alone resulted in substantial improvement in the outer SC lipid structure of this subject, but Landmann units were not common and many intercellular spaces contained indistinct or amorphous material (data not shown). Notably, the vehicle did not produce the "SEFA look." Following treatment with the 2% SEFA preparation, the "SEFA look" was commonly observed (Figure 28.9[b]), but so too were Landmann units, which is an unusual finding for a person of this age. Following treatment with the 10% SEFA preparation, the "SEFA look" was less common, and Landmann units more common (Figure 28.9[c]).

To conclude this section, previous reports on the beneficial effects of topically applied moisturizing preparations have often focused on optimizing their physiological lipid composition. The results found for the "formulated" SEFA and petrolatum in this work, when viewed relative to the neat materials, suggest that proper formulation of even nonphysiological moisturizing agents will enhance the beneficial effect these materials have on outer SC lipid structure.

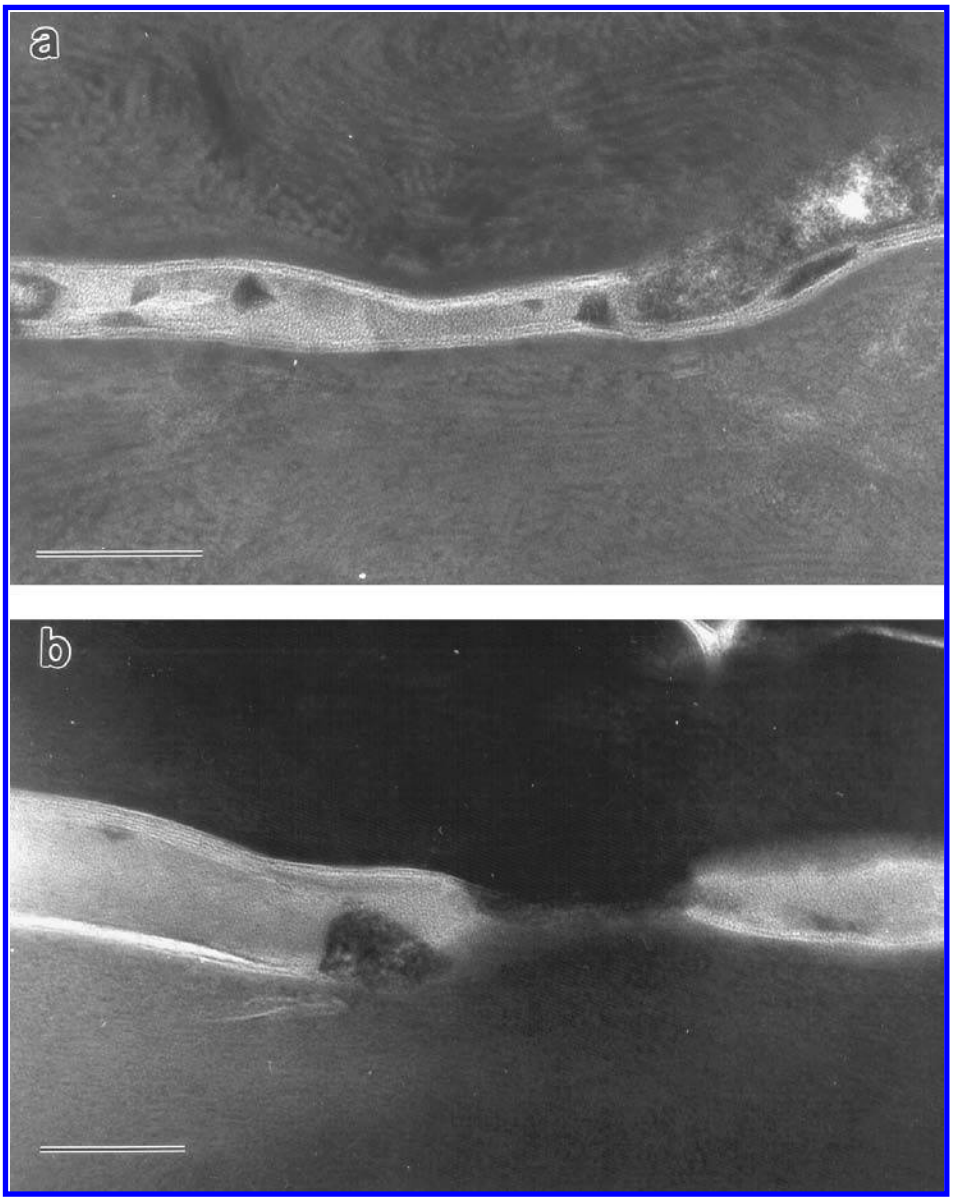

FIGURE 28.7 Neat SEFA site from a 42-year-old male. (a) Corneocytes are closely apposed, with well-formed lamellae at the corneocyte surface. Between the lamellae is a relatively uniform layer of an amorphous material. This pattern is referred to as the "SEFA look." (b) Occasional multiple Landmann units are present in the intercellular space. The length of the double Landmann units is always relatively short. Bar = 100 nm.

28.5.4 Product Comparisons from Clinical Studies

28.5.4.1 Neat Petrolatum versus Neat SEFA versus Glycerin-Based Moisturizing Lotion

Products were applied at 2 mg/cm^2 to the lower leg twice a day for three weeks. Typical results are presented from a 52-year-old female panelist. Petrolatum use resulted in an intercellular space containing diverse intercellular structures including darkly staining globular material, amorphous

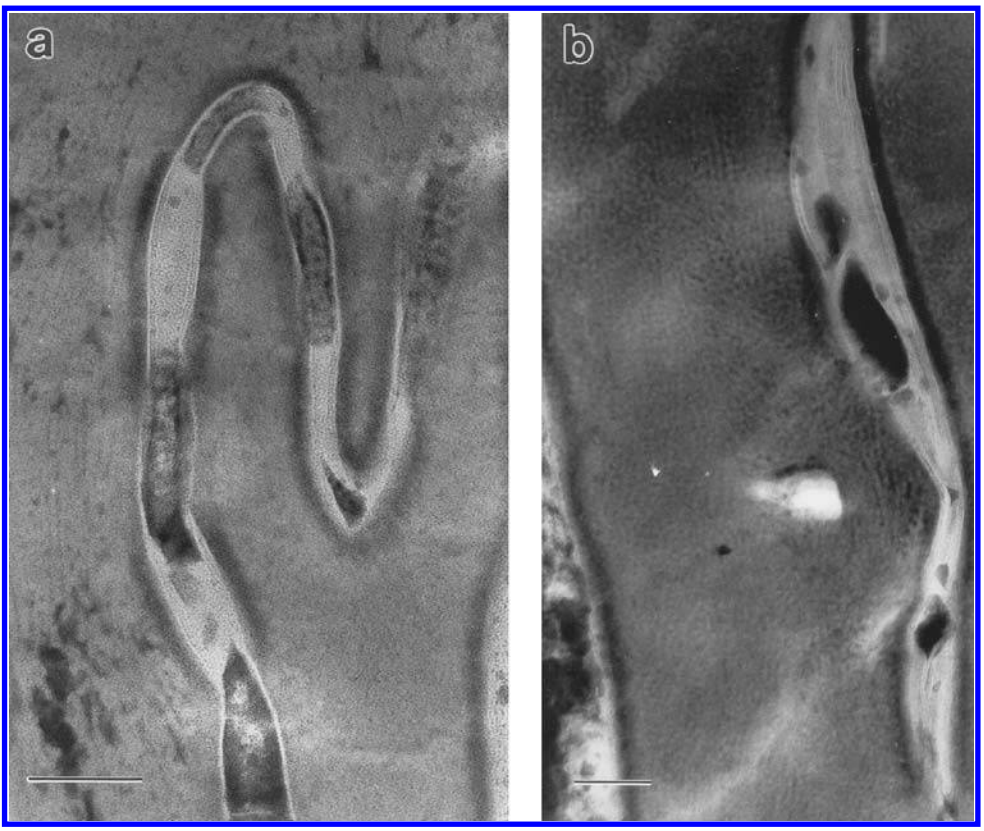

FIGURE 28.8 (a) Formulated (2%) SEFA site from a 42-year-old male. The characteristic "SEFA look." (b) Formulated (10%) SEFA site. In addition to the SEFA look, an extra Landmann unit is present within the amorphous material. This extra Landmann unit is slightly separated from the peripheral lamella, which is common. Bar = 100 nm.

regions, and some lamellae, but few Landmann units, as shown in Figure 28.10(a). SEFA substantially improved the intercellular structures, as shown in Figure 28.10(b), including the occasional formation of multiple Landmann units characteristic of younger skin (insert, Figure 28.10[b]). In striking contrast, a glycerin-based lotion yielded a diverse and unusual lipid structure that included mixtures of amorphous and fibrous material (Figure 28.11[a]), phase-separated amorphous lipids (not shown), and frequent bizarre vesicular structures (Figure 28.11[b]). Landmann units were rarely observed. Other workers have reported that glycerin, under open or occlusive application, speeds transepidermal water loss (TEWL) recovery in SC whose barrier function is compromised by tape stripping or surfactant washing.[43] This seeming disparity with the present work could be a result of the higher doses of glycerin applied or the different treatment forms used. Or the effect of glycerin on SC barrier function, as measured by TEWL, might occur deeper in the SC than in the topmost layers assessed in this work.

The blinded TEM studies rated the test materials' potential to improve lipid ultrastructure as: neat SEFA > neat petrolatum > lotion. However, the blinded expert scoring in this study ranked the test materials' ability to improve dry skin oppositely: lotion > neat petrolatum > neat SEFA. This reversal illustrates two of the roles a moisturizer can play; the former showing the materials' potential to effect functional improvement and biological repair, the latter showing their potential to cosmetically improve dry skin.[47] Importantly, these results demonstrate that the cosmetic and functional aspects of a moisturizer's action on skin do not necessarily contribute to the same extent or need not even act in parallel for a given material or product.

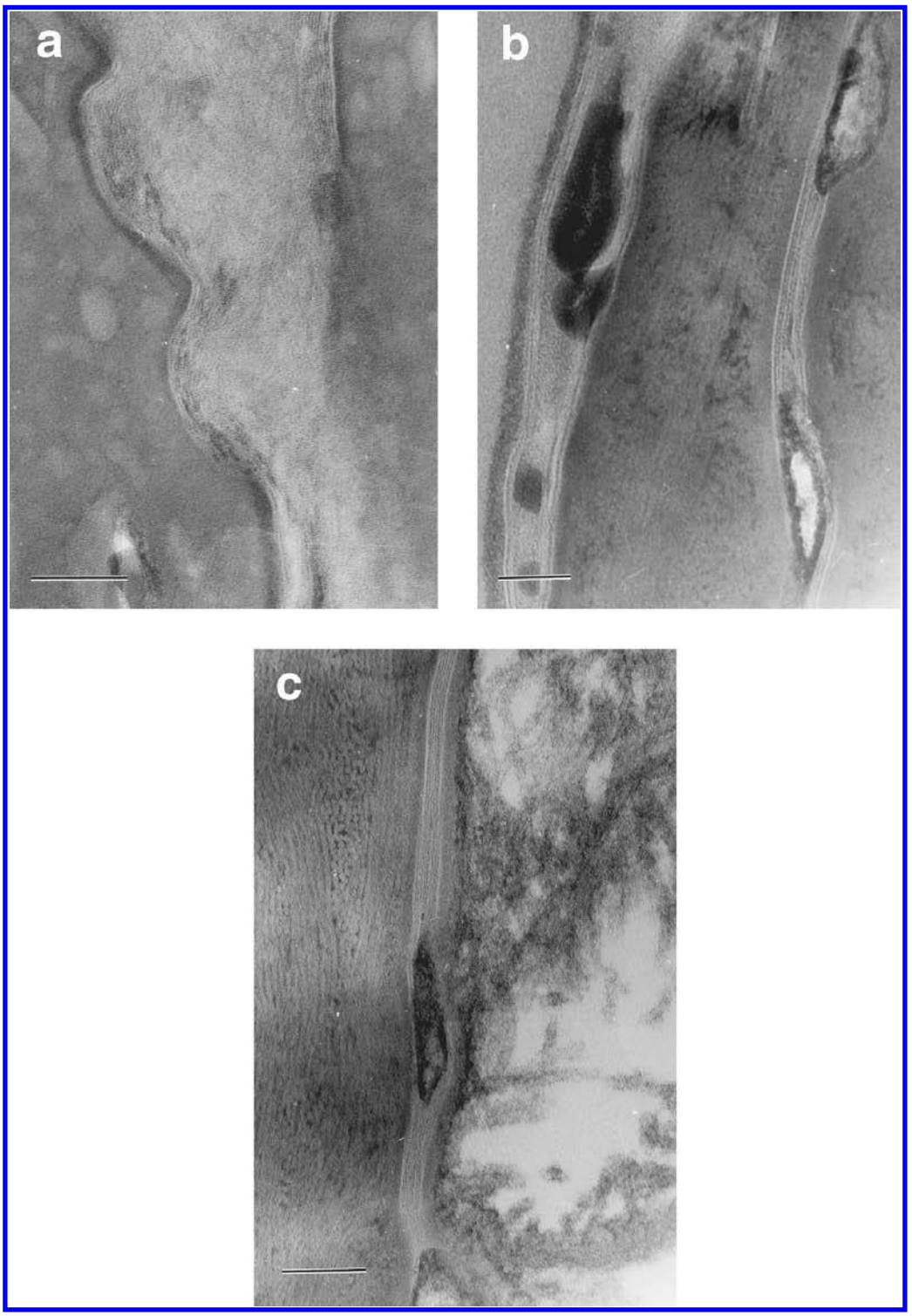

FIGURE 28.9 (a) Control nontreated site from a 52-year-old female. The characteristic lipid structure resulting from soap use (winter xerosis[14]) is evident — compare with Figure 28.4(b). (b) Use of formulated (2%) SEFA results in the SEFA look, as well as Landmann units. (c) With use of formulated (10%) SEFA, Landmann units are commonly observed. Bar = 100 nm.

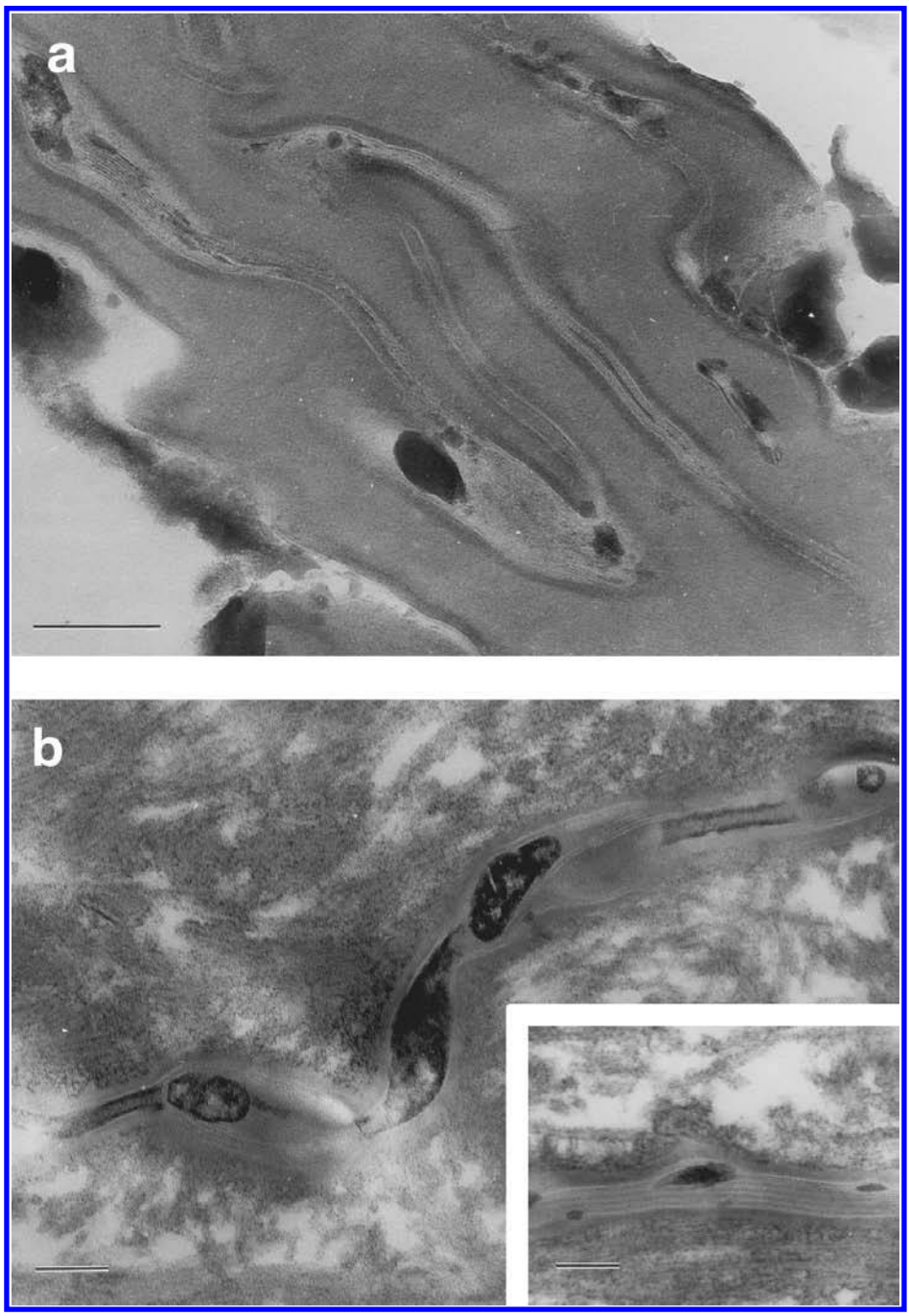

FIGURE 28.10 Neat-petrolatum-treated site from a (different) 52-year-old female. (a) A great variety of intercellular structures are present, but the "streamer" phase typical of petrolatum (Figure 28.6[a]) was not seen. Amorphous regions and expanded intercellular regions containing many darkly staining globular regions are very common, as are lamellae without a Landmann pattern. Landmann units were rare. (b) Neat-SEFA-treated site. The SEFA look is evident. The dark spindle-shaped structures near the center of the micrograph are presumably desmosomes undergoing degradation. In many areas with the SEFA look, multiple, short-length Landmann units are common in the intercellular space, as shown. Normal well-formed Landmann units are relatively common, as shown in the insert. Bar = 100 nm.

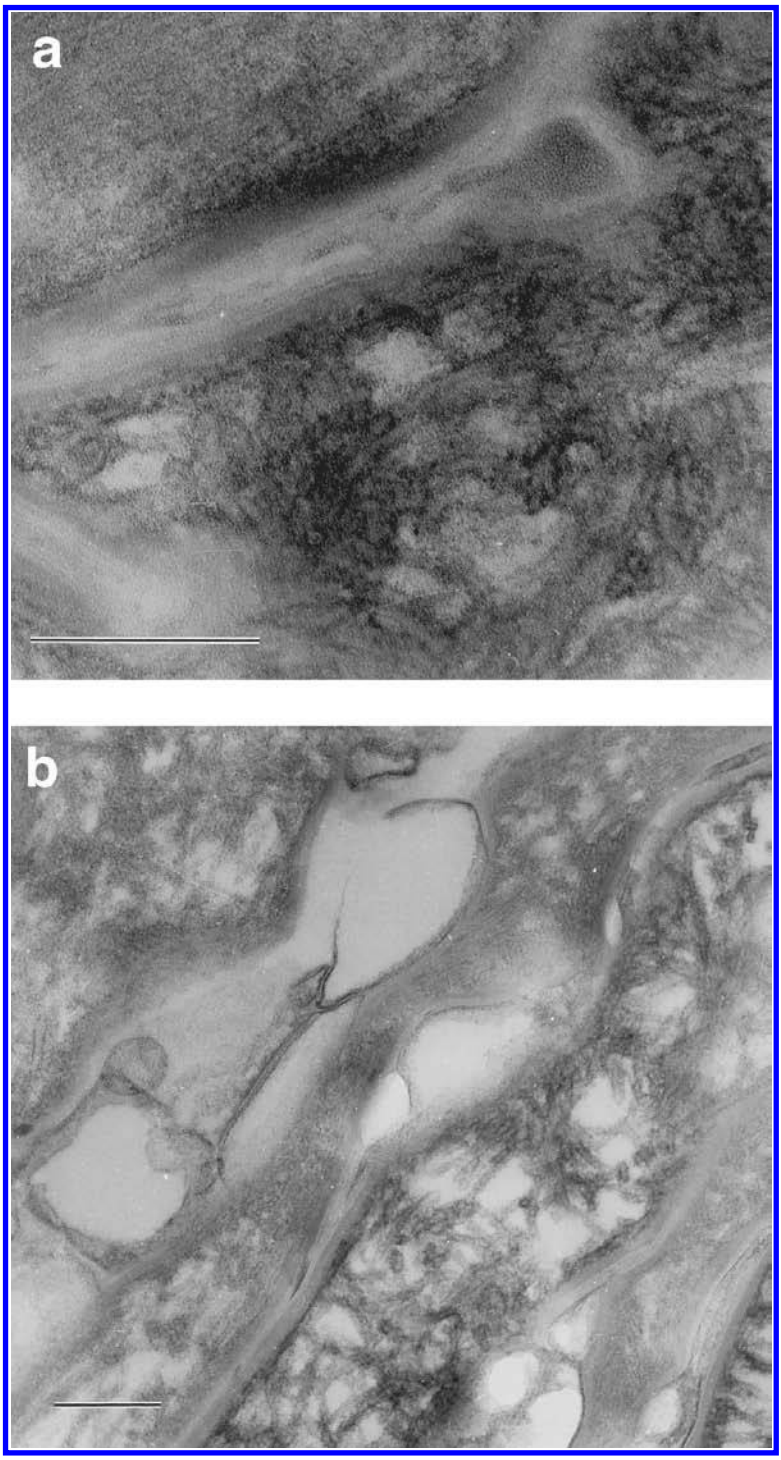

FIGURE 28.11 Site of glycerin-based moisturizing lotion from the 52-year-old female of Figure 28.10. The structure of the intercellular space is unusual. (a) Many areas contain amorphous and fibrous material in the intercellular space. (b) Other areas contain vesicles, membrane-bounded compartments, and a mesh-like material. Bar = 200 nm.

28.5.4.2 Moisturizing Body Wash versus Synthetic Bar + Glycerin-Based Moisturizing Lotion

In a clinical study, a body wash treatment at 10 μl/cm^2 (rinse-off application) and a glycerin-based lotion treatment at 1 μl/cm^2 (leave-on application) were applied to the medial aspect of the legs of female panelists once daily for 25 days. Good repair of the lipids in the intercellular space was routinely obtained with the body wash, which contains 17.5% petrolatum as a skin benefit agent, as illustrated by a particularly dramatic improvement shown in Figure 28.12. The control (water only) treatment site of a 28-year-old panelist, shown in Figure 28.12(a), is characterized by intercellular spaces filled with amorphous material. The effect of the body wash on this subject's outer SC lipids is shown in Figure 28.12(b). The majority of the intercellular space is filled with Landmann units, although amorphous material was occasionally found in some regions. The effect of a syndet bar followed by application of a glycerin-based moisturizing lotion is shown in Figure 28.12(c). This bar/lotion regimen resulted in a clear improvement relative to the control but many intercellular regions are still dilated with amorphous material. Although lamellae are present, Landmann units are relatively rare.

A more typical response produced by these treatments is shown in Figure 28.13, which is from a separate clinical study that used the same treatments applied for only 14 days. The 48-year-old panelist had a moderate amount of skin dryness and the SC at the control site exhibited an intercellular lipid structure similar to that of Figure 28.3(a), that is, a good lipid structure for that age. In this case the body wash resulted in no dramatic change in intercellular lipid structure, shown in Figure 28.13(a), although there was significant improvement in the visual skin grade. The limited improvement in outer SC lipid structure might reflect the shorter treatment period, the decreased dosing compared to the moisturizer study, or possibly the impact of age. Because the aged stratum corneum barrier exhibits a reduced resistance to insult and slower repair than in young skin due to diminished lamellar body secretion,[19] and because the SC turnover rate also typically slows with age, the functional benefits of topical moisturizers might require more time to manifest in "old" skin than in younger skin. Regardless, these results show that while lipid structure was not improved, treatment with the body wash preserved existing lipid structure. In contrast, use of the syndet bar followed by the glycerin-based lotion degraded lipid ultrastructure in the outer SC, as shown in Figure 28.13(b). The intercellular spaces contain amorphous and "fuzzy" material, and prominent disorganized, undulating lamellae. Nevertheless, the visual skin grade was dramatically improved with this latter regimen, again illustrating the distinction between a moisturizers' cosmetic and functional effects.[47]

28.6 CONCLUSIONS

An improved understanding of the structure of the SC barrier is of interest for many reasons such as enhancing percutaneous penetration and, as discussed in this chapter, optimizing topical therapy for the treatment of dry or damaged skin. The results of this TEM work show that the lipid structure of the outer SC is quite variable. Typically, the intercellular spaces in the outer SC are considerably widened and filled with nonlamellar material. These data are consistent with earlier TEM studies[13,14] and with an infrared spectroscopic study that found less structured lipids in the outer SC[16] compared to the middle and inner regions.

Contrary to an earlier report that lipids uniformly have an amorphous structure in the outer SC of normal skin with little or no visible dryness,[14] we instead found considerable variation in this lipid structure among individuals. Intercellular lipids in young skin with little dryness typically had a good Landmann unit structure, even at the surface of the SC. This ideal Landmann unit structure was generally absent in young individuals with dry skin or in individuals over the age of 40 regardless of their dry skin level. In attempting to make sense of this variation, we believe we can generalize and conclude that the outer SC lipid structure is related to an individual's age and dry skin condition.

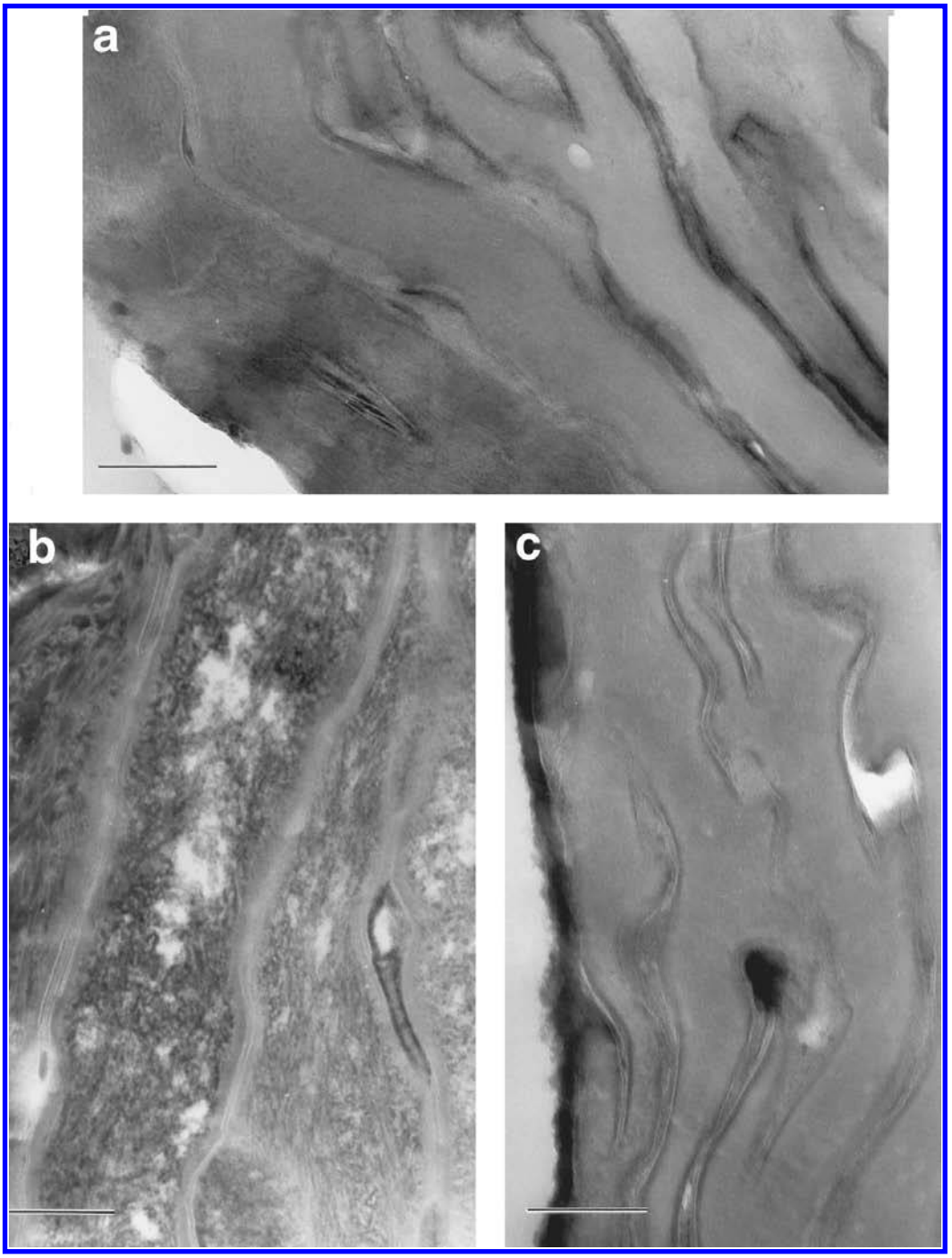

FIGURE 28.12 (a) Control nontreated site from a 28-year-old female. The intercellular spaces are completely filled with amorphous material. Lamellar structures are rare. (b) Site of application of a commercial moisturizing body wash containing petrolatum. Lamellae are common, as are Landmann units. (c) Site of application of a mild synthetic bar followed by a glycerin-based moisturizing lotion. Lamellae are present, but few have the Landmann unit structure. Amorphous material is still common. Bar = 200 nm.

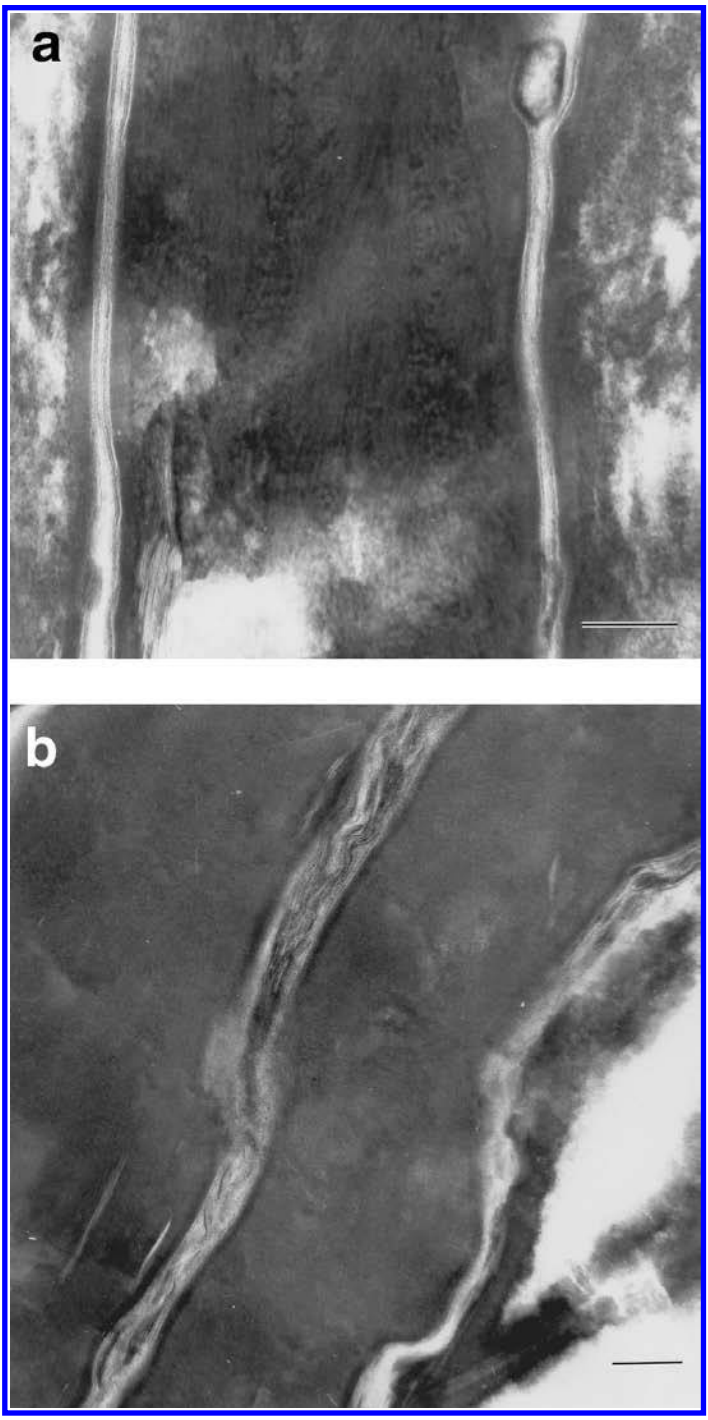

FIGURE 28.13 Tape strips from a 48-year-old female. (a) Site of application of a commercial moisturizing body wash containing petrolatum. The appearance is typical of a person over age 40 with good skin condition. The corneocytes are closely apposed and lamellae are frequent, but the lamellae appear somewhat disorganized and amorphous/fibrous material is present between lamellae. Landmann units are nevertheless easy to find, as shown in the left intercellular space. (b) Site of application of a mild synthetic bar followed by a glycerin-based moisturizing lotion. The intercellular spaces contain amorphous and fibrous material as well as prominent wavy lamellae without a Landmann pattern. Bar = 100 nm.

The outer SC lipid structure of older individuals is altered compared to that found in young skin, the older SC having fewer lipid lamellae and more open intercellular clefts.[19] This paucity of lamellae may be due to a decrease in SC lipid synthesis or lamellar body secretion, resulting in a decreased SC lipid content.[19,51] These results are from observations made on individuals of advanced age, 80 years old. However, we observed changes in the outer SC lipid structure of individuals as young as 40 years old. This is not entirely unexpected since literature data support age-associated changes in SC lipids in relatively young individuals. For example, there is a sharp decrease in SC lipid content by age 45 and a nearly constant SC lipid profile afterwards.[55] Total SC ceramides undergo a sharp drop in concentration around age 40,[40] and levels of individual ceramide species also change with age and female hormonal status.[23,56] A challenge for skin moisturizers is to arrest, and ideally reverse, the age-related decline in the SC lipid barrier that accompanies these changes.

The blinded assessment of changes in SC lipid structure generally corresponded well with the independent, expert assessments of dry skin appearance. However, a number of striking outliers show that lipid structure is not the major determinant of dry skin appearance, which is not surprising given the complexity of SC homeostasis. A good example was the neat petrolatum/SEFA/lotion comparison mentioned earlier, in which neat SEFA improved lipid structure but produced only a marginal reduction in dry skin appearance, whereas the glycerin-based lotion apparently degraded lipid structure but yielded skin with minimal visible flaking. This suggests that another mechanism, such as desmosomal breakdown, is a more important determinant of the skin's dry appearance. However, a healthy, nondry SC may ultimately rely more on the integrity of the lipid barrier than on the state of desmosome degradation in the outer SC layers. Such effects may appear over a longer time frame, for example, during the regression period that is used in some clinical protocols, or require the evaluation of endpoints other than dry appearance.

The discrepancy between visual appearance and lipid structure may be a consequence of commercial products being formulated to achieve visual improvement rather than functional change, that is, improved skin health. This is not unexpected since dry skin is readily observed by consumers and is an important signal of the need to apply moisturizer.[47] However, as this work has shown, a reduction in dry appearance does not necessarily mean that there is an improvement in the functional characteristics of the skin, only that it looks better. Thus, relying on a visual endpoint for evaluating moisturizer efficacy can yield commercially successful products that provide a marginal skin health benefit. An example are reports that some commercial moisturizing lotions may actually impede barrier recovery after experimental barrier perturbation.[45,57] A moisturizer should ideally address not only visual skin problems but also address the underlying biological causes to achieve healthy skin; there is a clear need for evaluation tools and endpoints for skin health beyond visual inspection.

We observed distinctive changes in the outer SC lipid structure with use of different treatments from soap to oil. Some of these lipid structures were sufficiently unique to provide unequivocal identification of product treatment, and to a lesser degree panelist age and skin condition. Based on TEM results, we believe that moisturizing materials enter the intercellular space of the SC and become a part of the SC, as was previously shown for petrolatum.[29,44] The mechanism by which a nonphysiological moisturizing material improves skin barrier lipids is uncertain, and multiple processes are likely involved. Given the chemical nature of the materials studied, we consider it unlikely that any of the treatments participated directly or physically in the formation of Landmann units. Of some note is the observation that petrolatum and SEFA were not as effective in reforming Landmann units when applied neat as when they were applied as reduced concentration or fully formulated products. The moisturizing body wash only contains petrolatum and polymers as moisturizing ingredients, which suggests that the quantity of petrolatum or its delivery form is important for promoting the conditions necessary for SC lipid repair. Likewise, for SEFA applied as a formulated product, the combination of humectants with some degree of occlusion may promote the internal conditions needed for the intrinsic formation of Landmann units. A semi-occluded environment is reported to accelerate TEWL barrier recovery following experimental insult[58,59]; this type

of environment might similarly favor lipid bilayer reformation in the outer SC. However, apparent lack of Landmann unit reformation following treatment with the glycerin-based moisturizer suggests that the choice of nonphysiologic ingredient or the manner in which it is formulated is also important to provide conditions that promote this reformation.[60,61] Beyond ingredient and delivery issues there remains the issue of product aesthetics and convenience; a product will benefit skin only if it is used. Of 651 dermatologist respondents in a recent survey, over 60% believed that less than half their adult female patients apply lotion as recommended.[62] Lack of convenience was cited as a factor contributing to this poor compliance by over 83% of the dermatologist respondents. The development of nontraditional product forms to deliver moisturizing benefits, such as moisturizing cleansers[54,63] and moisturizers intended for use in the shower,[62] can provide increased convenience and could improve moisturizer usage compliance.

The SC is a highly complex system and we do not claim to fully understand the lipid structure of the outer SC or its implications on the basis of this investigation. The conclusions reached are therefore predicated on certain key observations and assumptions. We observed the ideal Landmann unit lipid structure in young individuals with little or no skin dryness, the absence of this structure in individuals with a high level of dryness, and the reappearance of Landmann units with treatment by moisturizing products. We therefore assume that this Landmann unit structure is the ideal lipid structure for the outer SC, as it is throughout its lower regions. In a system undergoing desquamation that may involve lipids,[36–39] this is an important assumption. We further assume that this ideal Landmann unit structure in the outer SC is important to skin health and a parameter by which moisturizers' potential to impact skin health should be judged. Both of these hypotheses warrant further testing.

In summary our microscopy study shows that topical moisturizers enter into the SC and can affect lipid structure. The lipid structure is related to visible skin dryness but is not the primary factor determining the level of dryness. For SEFA and petrolatum, formulated products showed a greater restorative effect on ideal Landmann unit lipid structure than did the neat materials. In our experience most of the moisturizing materials and products that we investigated to date are effective at reducing visible dry skin, but far fewer materials are able to substantially reform Landmann units, particularly in individuals over age 40. Is there hope that moisturizers might restore the ideal Landmann unit lipid structure common in the healthy skin of youth? With ongoing work looking at new moisturizing agents, new delivery systems, and alternative product forms, we believe the promise is there, as shown for older individuals in Figure 28.9 and Figure 28.10.

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