

Moisturization and skin barrier function

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ABSTRACT: Over the past decade, great progress has been made toward elucidating the structure and function of the stratum corneum (SC), the outermost layer of the epidermis. SC cells (corneocytes) protect against desiccation and environmental challenge by regulating water flux and retention. Maintenance of an optimal level of hydration by the SC is largely dependent on several factors. First, intercellular lamellar lipids, organized predominantly in an orthorhombic gel phase, provide an effective barrier to the passage of water through the tissue. Secondly, the diffusion path length also retards water loss, since water must traverse the tortuous path created by the SC layers and corneocyte envelopes. Thirdly, and equally important, is natural moisturizing factor (NMF), a complex mixture of low-molecular-weight, water-soluble compounds first formed within the corneocytes by degradation of the histidine-rich protein known as filaggrin. Each maturation step leading to the formation of an effective moisture barrier—including corneocyte strengthening, lipid processing, and NMF generation—is influenced by the level of SC hydration. These processes, as well as the final step of corneodesmolysis that mediates exfoliation, are often disturbed upon environmental challenge, resulting in dry, flaky skin conditions. The present paper reviews our current understanding of the biology of the SC, particularly its homeostatic mechanisms of hydration.

KEYWORDS: corneocyte, corneodesmolysis, filaggrin, natural moisturizing factor, stratum corneum.

Introduction

For humans to survive in a terrestrial environment, the loss of water from the skin must be carefully regulated by the epidermis, a function dependent on the complex nature of its outer layer, the stratum corneum (SC) (1). The SC is a selectively permeable, heterogeneous, composite outer layer of the epidermis that protects against desiccation and environmental challenge, and retains sufficient water to allow it to function in arid environments. The small amount of water loss that does occur hydrates the outer layers of the SC, maintaining its flexibility and facilitating the enzymatic reactions that drive the SC's maturation (2–4). The water-retaining capacity of the SC is highly dependent upon the phenotype of

the corneocytes, their spatial arrangement, the precise composition and physical packing of extracellular lipids, and the presence of highly hygroscopic compounds found largely within the corneocytes (Fig. 1).

Under conditions of normal humidity (> 80%), a steep water gradient exists in the SC, which can be viewed by a variety of elegant techniques (Fig. 2) (5,6). The gradient is established, in part, by a discontinuity in the water-binding capacities between different corneocyte cell layers, as demonstrated via cryo-scanning electron microscopy (Fig. 3) (7). Briefly, corneocytes do not appear to be swollen at low hydration levels (18–26% w/w), suggesting that only bound water is present in the SC. However, at higher hydration levels (57–87% w/w), the corneocytes are more swollen in the central portion of the SC compared with the superficial and deeper layers. The corneocyte cell thickness is shown to increase linearly in a direction perpendicular to the skin surface with increasing hydration. Extracellular pools of water

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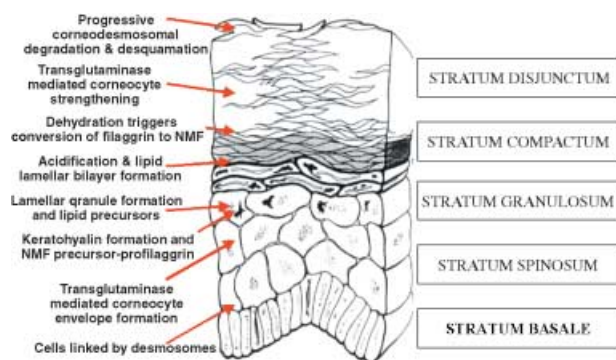


Fig. 1. Typical structure of the epidermis and critical steps in the formation of the stratum corneum. Adapted from Rawlings AV, Scott IR, Harding CR, Bowser PA. Stratum corneum moisturization at the molecular level. *J Invest Dermatol* 1994; 103: 731–740, courtesy of Blackwell Publishing, Inc. (2).

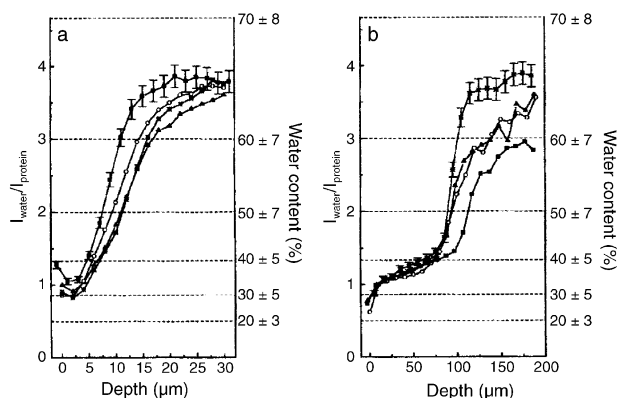


Fig. 2. *In vivo* water concentration profiles of the stratum corneum: (a) four water concentration profiles calculated from Raman measurements on the volar aspect of the forearm; and (b) four water concentration profiles based on Raman measurements on the thenar. The left-hand coordinate is the ratio between the Raman signal intensities of water and protein. The right-hand ordinate represents the absolute water content. Taken from Caspers et al. (6). Adapted from Caspers PJ, Lucassen GW, Carter EA, Bruining HA, Puppels GJ. Semiquantitative *in vivo* concentration profiles of NMF and sweat constituents in the stratum corneum of the thenar as determined by Raman spectroscopy. *J Invest Dermatol* 2001; 116: 434–442, courtesy of Blackwell Publishing, Inc.

are only observed at very high hydration levels (> 300% w/w). The explanation of these differential hydration levels is rooted in the mechanisms involved in SC moisturization.

The present review describes the most recent research that reveals the complexity and intricacy of the everyday functioning of the SC to maintain

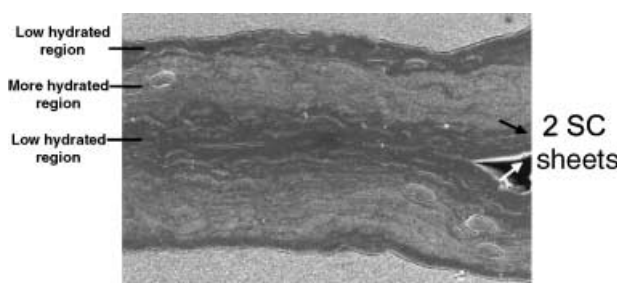


Fig. 3. High magnification cryo-scanning electron microscopy images of two sheets of stratum corneum (SC) hydrated to 90% w/w. Both sheets show an increased hydration level in their central regions, and a low hydration in the superficial and lowest part of the stratum corneum. Adapted from Bouwstra JA, de Graaff A, Gooris GS, Nijssse J, Wiechers J, van Aelst AC. Water distribution and related morphology in human stratum corneum at different hydration levels. *J Invest Dermatol* 2003; 120: 750–758, courtesy of Blackwell Publishing, Inc. (7).

hydration in relation to ever-changing environmental conditions. It shows that skin is involved in a constant battle to maintain optimal moisture barrier and protective functions. As discussed throughout this supplement, different elements of fundamental skin care exercised as a daily routine truly help the skin to achieve its healthiest state.

Stratum corneum moisturization at the molecular level

Stratum corneum

The SC consists of terminally differentiated keratinocytes (corneocytes) and the secreted contents of lamellar bodies (2,8). Each corneocyte originates from an actively proliferating keratinocyte in the epidermis beneath the SC. Corneocyte proteins are generally sequestered to the cytosol, while ceramides and other lipids are enriched in the extracellular space, where they form a continuous phase (8). The physical packing of the corneocytes creates a tortuous path for molecules to traverse, effectively increasing the diffusion length and thereby improving the SC barrier function. Extracellular lipids, tightly arranged as a covalently bound matrix in a crystalline phase called an orthorhombic packing state further reduce the rate of water flux through the tissue (9). The constitution, and thus the effectiveness, of the lipid barrier are dependent on the absolute concentrations and the relative proportions of the different

Table 1. Chemical composition of natural moisturizing factor

Chemical	Composition (%)
Free amino acids	40
Pyrrolidone carboxylic acid	12
Lactate	12
Sugars	8.5
Urea	7
Chloride	6
Sodium	5
Potassium	4
Ammonia, uric acid, glucosamine, and creatine	1.5
Calcium	1.5
Magnesium	1.5
Phosphate	0.5
Citrate and formate	0.5

lipids (e.g., ceramides, cholesterol, and fatty acids). Lectins and desmosomes found in the SC also help to maintain the structural cohesiveness of the SC. The reduction of water flux and loss through the tissue is not the sole cause of the apparent discontinuity in hydration between different corneocyte layers, however. Selective retention of water is required as well, central to which is SC natural moisturizing factor (NMF).

Natural moisturizing factor

Found exclusively in the SC, NMF consists primarily of amino acids or their derivatives, such as pyrrolidone carboxylic acid (PCA) and urocanic acid, together with lactic acid, urea, citrate, and sugars (10) (Table 1). Natural moisturizing factor compounds are present in high concentrations within corneocytes and represent up to 20–30% of the dry weight of the SC (11). By absorbing atmospheric water and dissolving in their own water of hydration, hygroscopic NMF components act as very efficient humectants (2). Biologically, this humectancy allows the outermost layers of the SC to remain hydrated despite the desiccating action of the environment. Corneocytes that possess the highest concentration of NMF retain more water and appear more swollen when viewed under cryo-scanning electron microscopy (7). However, NMF is much more important than this. As our understanding of the terminal differentiation and SC maturation process has increased, it has become clear that, by maintaining free water in the SC, NMF also facilitates critical biochemical events. The most striking example of this is the regulation of several corneocyte proteases that

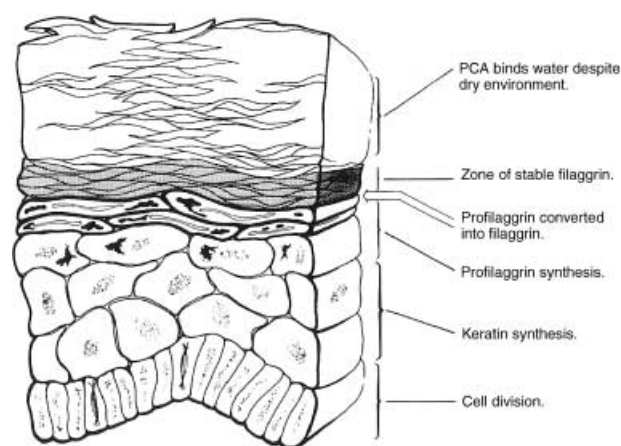


Fig. 4. Schematic of epidermis showing profilaggrin conversion to filaggrin and pyrrolidone carboxylic acid (PCA). Adapted from Rawlings AV, Scott IR, Harding CR, Bowser PA. Stratum corneum moisturization at the molecular level. *J Invest Dermatol* 1994; 103: 731–740, courtesy of Blackwell Publishing, Inc. (2).

are ultimately responsible for the very generation of NMF itself.

Origin of NMF. The components of NMF are derived from filaggrin, a 37-kDa SC protein initially synthesized in keratohyalin granules of the epidermis as profilaggrin, a large (> 500 kDa), highly basic, heavily phosphorylated, histidine-rich protein (2,12–15) (Fig. 4). First expressed in the granular layer of the SC during the final stages of epidermal differentiation, profilaggrin consists of multiple filaggrin repeats (10–12 in humans) joined by short hydrophobic linker peptides (15). Extensive phosphorylation of internal serine residues renders profilaggrin extremely insoluble. Profilaggrin is, in effect, synthesized and then precipitated within the keratohyalin granule as an insoluble, osmotically inactive precursor of NMF. During the transition of the mature granular cell into the corneocyte, profilaggrin is rapidly dephosphorylated to form filaggrin, a transient component that does not persist beyond the two to three deepest layers of the SC (16). As corneocyte maturation and progression through the upward layers of the SC proceeds, the complete proteolysis of filaggrin generates NMF. The timing of the entire process is controlled, in part, by the association of filaggrin with keratin, which is thought to shield filaggrin from premature proteolysis (15). Although the proteases catalyzing filaggrin breakdown in the SC remain to be identified, the “signal” that initializes the proteolysis at

a defined stage of SC maturation was found to be the water gradient within the SC itself (17).

Filaggrin's relatively short life span is dependent on the turnover time of the SC. The protease activity that results in the sudden release of a concentrated NMF pool is delayed until the corneocytes have flattened, moved far enough out into the dryer areas of the SC, and the cornified cell envelopes have strengthened. Once these conditions have been met, the SC can withstand the resulting osmotic effects of NMF generation (15). Other NMF components that may be extracellular to the corneocytes include sugars, hyaluronic acid, urea, and especially lactate (18). Since lactate is derived from eccrine sweat, its gradient is different from the amino-acid-derived NMF components (6).

Age-related decline in NMF. There is a significant age-related decline in the level of NMF. Electron microscopy studies show decreased numbers of keratohyalin granules in senile xerosis (19); this suggests that the intrinsically lower levels of NMF present in aged skin, compared with the levels in young skin, reflect a generally reduced synthesis of profilaggrin. Furthermore, it is likely that the loss of NMF is exacerbated by the age-related decline in barrier function (20). The decline in NMF production probably reflects the cumulative effects of actinic damage since it was observed in SC recovered from the back of the hand (photo-damaged), but not from the inner aspect of the biceps (photo-protected). Typical profiles of SC depth versus NMF concentration (obtained by sequential tape stripping of elderly individuals) show a decline toward the surface of the skin of elderly subjects compared to young individuals (2). This is typical of normal skin exposed to routine soap washing, during which much of the readily soluble NMF is washed out of the superficial SC (21). Indeed, recent results from *in vivo* confocal methods have demonstrated reductions in the amount of all NMF amino acids in the superficial layers of the SC (6), largely as a result of damage from bathing or ultraviolet light. Improvements in corneodesmolysis are necessary to rapidly alleviate the scaling and flaking associated with this condition.

Structural changes in corneocyte envelopes

Dry skin is further characterized by structural changes in corneocyte envelopes (CE) that are a result of reduced transglutaminase activity. This bifunctional enzyme transforms a soft or fragile corneocyte envelope (CEf) into a rigid corneocyte

envelope (CEr) by mediating the gamma-glutamyl lysine crosslinking of specialized corneocyte proteins, and in addition, the esterification of ceramides and fatty acids to the cornified envelope (22). Fragile corneocyte envelopes predominate in dry skin.

Influence of external humidity on barrier function

The processes involved in the maintenance of SC hydration appear to be under the profound influence of external humidity. Denda and colleagues (23) analyzed cutaneous barrier function, epidermal morphology, and lipid content of the SC in hairless mice maintained for 2 weeks in high relative humidity (RH > 80%) and low relative humidity (RH < 10%). Basal transepidermal water loss was reduced by 31% in animals maintained in a dry environment. Moreover, the number of lamellar bodies in stratum granulosum cells, the extent of lamellar body exocytosis, and the number of layers of SC significantly increased in animals kept in a dry environment, as did the dry weight of the SC and the thickness of the epidermis. In addition, the amount of total SC lipids increased, but lipid analysis revealed no significant differences in lipid distribution. Lastly, barrier recovery following either acetone treatment or tape stripping was accelerated after prolonged prior exposure to a dry environment, but was delayed by prior exposure to a humid environment.

Importantly, Declercq et al. (24) reported a similar adaptive response in human barrier function. Individuals living in a dry climate like that of Arizona, USA, compared with those in a humid climate like that of New York, USA, have less scaly skin, perhaps because of increased desquamatory enzyme levels and elevated ceramide levels, which improve barrier function. Similar increases in scalp lipid levels are observed between the wet and dry seasons in Thailand (25), and in the summer and winter months in the UK (26). In the latter studies, reductions in the levels of linoleate-containing ceramide with omega-hydroxy fatty acid (O), ester-linked (E) to linoleic acid and amide linked to sphingosine (S) [Cer(EOS)], and increasing levels of oleate-containing Cer(EOS) were found in the SC in winter.

Influence of external humidity on filaggrin hydrolysis, desquamation, and cutaneous disorders

Scott and Harding (13–15,17) found that filaggrin hydrolysis during development of rat SC is

controlled by environmental humidity as well. Filaggrin distribution in the rat SC, as shown by immunofluorescence, changed dramatically during the first hours of postnatal life. During late fetal development, filaggrin accumulated through the entire thickness of the SC, suggesting an inhibition of its proteolytic degradation to free amino acids. Immediately after birth, filaggrin proteolysis occurred in the outer part of the SC and followed the normal adult pattern. Thus, activation of filaggrin proteolysis was dependent on the drop in external water activity caused by the transition from an aqueous environment *in utero* to a dryer environment after birth. Filaggrin proteolysis could be blocked by maintaining a 100% humidity atmosphere around the newborn rat after birth. Interestingly, profilaggrin levels vary inversely with humidity, indicating yet another homeostatic regulatory mechanism that balances the SC hydration state (23).

To clarify the effects of environmental humidity on skin pathology, Katagiri and coworkers (27) studied skin surface conductance (a measure of surface hydration using a skin surface hygrometer; Skicon 200 I.B.S. Co., Ltd., Japan), free amino acid content, and the immunoreactivity of filaggrin in the epidermis of hairless mice subjected to varying levels of humidity. In this study, the skin surface conductance in the SC of mice was significantly lower 3–7 days after transfer from a humid environment (RH > 80%) to a dry environment (RH < 10%), as compared to mice transferred from a normal environment (RH = 40–70%) to a dry environment. Furthermore, the free amino acid content in the SC, while reduced 24 h after the mice had been transferred from a normal to a dry condition, recovered to the original level within 3 days. By contrast, mice transferred from a humid to a dry condition showed a significantly lower amino acid content even 7 days after the transfer. The immunoreactivity of filaggrin became faint in the epidermis of the mice transferred from a humid or normal to a dry environment. Taken together, the results suggest that a drastic decrease in the environmental humidity reduces total free amino acid generation (and thus the level of NMF and the capacity of the SC to maintain hydration), and consequently, induces skin surface dryness in the SC.

In addition to modulating the profilaggrin-filaggrin system, the water content of the SC also regulates desquamation (28). The action of specific hydrolases in the SC that degrade the glycoprotein complexes of corneodesmosomes facilitates cell loss from the surface of the skin (8).

As demonstrated by Rawlings and colleagues (29), desmosomal degradation is significantly reduced at low relative humidity (RH = 10%). The sub-optimal water level in the SC may inhibit SC hydrolase activity and, thereby, suppress desquamation (28).

Given the role that environmental humidity plays in influencing epidermal structure and function, low humidity is widely thought to induce or exacerbate various cutaneous disorders. For example, xerosis and other common dry skin conditions are characterized, in part, by an accumulation of corneocytes on the surface layers of the skin, a result of flawed corneodesmosomal degradation, changes in ceramide biochemistry and structure, and reduced NMF levels.

Surface pH

When the skin is fully occluded and exposed to humid climates, the SC skin surface pH rises. Whereas barrier repair after acute perturbations proceeds normally at an acidic skin pH, recovery is delayed at a neutral pH, which impedes the post-secretory processing of newly secreted polar lipids (glucosylceramides) into mature lamellar bilayers (ceramides). Furthermore, increased proteolytic activities have been observed under high pH conditions, leading to increased corneodesmolysis and aberrations in corneocyte cohesion (30).

Summary

The SC possesses a biosensor function able to respond to variable environmental conditions, especially humidity, so that it remains optimally moisturized. Dry skin conditions may result from SC dysfunction, as determined by several factors, including advanced age, abnormal hydration levels, and pH. Profilaggrin synthesis and dephosphorylation, filaggrin hydrolysis and the resulting NMF generation, corneocyte maturation, and desquamation are all essential to SC hydration and maintenance. Fundamental skin care—the ally of skin in optimizing SC structure and function—is based, in part, on our understanding of these physiological mechanisms.

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