Structure and function of the epidermal barrier

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The skin is divided into 2 main structural layers: the epidermis and the dermis. The epidermis is generally considered to be subdivided into 5 separate strata: basal, spinous, granular, lucid, and corneum. The vital barrier function of the skin resides primarily in the top stratum of the epidermis, the stratum corneum (SC). The SC is the barrier to the passive diffusion of water out of the skin, allowing us to live in air without suffering from dehydration, and is the barrier to other molecules including irritants into the skin. The epidermis also has immunologic functions and provides some protection of the skin from ultraviolet light via the pigment system. This paper will review the structure and function of the epidermal barrier and the response to environmental challenges such as repeated handwashing. Emphasis will be placed on the SC, the front line of the skin’s defense against the insults of the outside world. (Am J Infect Control 2006;34:S98-110.)

The skin is the largest organ of the human body, accounting for approximately 16% of total body weight. Its vital role is to prevent loss of water and other components of the body to the environment and protect the body from a variety of environmental insults. The skin also has important immune and sensory functions, helps to regulate body temperature, and synthesizes vitamin D. For a review of the overall structure of the skin including the dermis and skin appendages, see Odland.1

Any discussion of the structure of skin will necessarily refer to layers. The various layers of the skin work in concert to provide strength and flexibility and perform the multiple functions of the skin. This review will focus on the barrier function residing in the top layer of the skin, the epidermis. The barrier function of the skin has been called “la raison d’etre” of the epidermis.2 The epidermal barrier serves to limit passive water loss from the body, reduce the absorption of chemicals from the environment, and prevent microbial infection. These defensive functions reside primarily in the top stratum of the epidermis, the stratum corneum (SC), at which they are integrated with SC formation and homeostasis.3,4 Thus, proper development and maintenance of the SC are keys to its remarkable ability to defend the body against both chemical and microbiologic attack as well as dehydration.

The epidermis is itself divided into several layers or strata starting with the basal layer or stratum basale just above the dermis proceeding upward through the prickle and the granular layers to the top layer, the SC. Figure 1 is a diagram representing the major strata of the epidermis. English and Latin nomenclature of the epidermal strata are given in Table 1.

The predominant cell type of the epidermis is the keratinocyte. Keratinocytes exist from the basal layer to the granular layer at which they transform into the corneocytes of the SC. Keratinocytes make keratin and many other proteins. Keratins are the major structural proteins of the SC.5 There are 2 other important cell types in the epidermis: melanocytes and Langerhans cells. These 2 cells types are shown in the micrograph in Fig 2.

Melanocytes are the pigment-producing cells of the skin and hair in all mammals. In the skin, they are found at the basal layer of the epidermis at which they make pigment granules called melanosomes containing melanin. The melanosomes are transferred from the melanocytes to the epidermal keratinocytes at which they impart some protection to the cell nucleus from ultraviolet (UV) light and give the skin its color. The process of melanin synthesis and transfer of melanosomes occurs continuously as the epidermis renews but can be speeded up in response to UV exposure to produce tanning.

Another epidermal cell shown in Fig 2 is the Langerhans cell. Langerhans cells are dendritic immune cells that are the antigen-presenting cell of the skin.5-8 They are important to the immune barrier of the epidermis and also participate in contact allergy.

Formation of the SC barrier

The granular layer or stratum granulosum (SG) is named for the granules that appear in the cells at this
point in the epidermis. Although it is only a few cells thick, it is a vital component of the epidermis because it is here that the key transformations that form the SC barrier occur. Two types of granules are formed at the SG: keratohyalin granules, which are full of protein, and lamellar bodies, which contain lipids. In the SG, the cells are transformed into the corneocytes or squames that form the SC. The nucleus is digested, the cytoplasm disappears, the lipids are released into the intercellular space, the keratin intermediate filaments aggregate to form microfibrils, and the cell membrane is replaced by a cell envelope made of cross-linked protein with lipids covalently attached to its surface. The corneocyte or squame that results from this transformation is a flat cell that tends to be in the shape of either a hexagon or pentagon approximately 25 \( \mu \text{m} \) on a side with a surface area of approximately 1000 \( \mu \text{m}^2 \) and a thickness of approximately 0.5 to 1.0 \( \mu \text{m} \). On most body sites, the SC is 12 to 16 cell layers thick, but it can vary from as little as 9 cell layers of the forehead or eyelids to as much as 25 on the dorsum of the hand and up to 50 or more on the palms or the soles of the feet. A major difference between the current and the earliest versions of the model is that we now know that the bricks are linked by desmosomes as illustrated in Fig 3 and discussed below.

**THE BRICKS AND MORTAR MODEL OF THE SC BARRIER**

The SC is often modeled as a brick wall (Fig 3). The SC corneocytes with their resistant cell envelopes and keratin microfibrils are considered to be the bricks, and the layers of lipids found between the cells are considered to be the mortar. The lipid “mortar” is the main barrier to water passing out through the SC. The permeability of lipid soluble molecules is modeled by considering them to wind their way around the corneocyte bricks by diffusing through the lipid mortar. Both the bricks and the mortar of the SC are produced by keratinocytes at the SG at which keratinocytes release the lipids of the mortar into the space between the cells as they are being transformed into the corneocytes “bricks.”

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<thead>
<tr>
<th>English</th>
<th>Latin</th>
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<tr>
<td>Basal cell layer</td>
<td><em>Stratum Basale</em></td>
<td><em>S Germinativium,</em> Malpighian layer</td>
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<tr>
<td>Prickle layer</td>
<td><em>S Spinosum</em></td>
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<td>Granular layer</td>
<td><em>S Granulosum</em></td>
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<td>Clear layer</td>
<td><em>S Lucidum</em></td>
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<td>Horny layer</td>
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**Fig 1. Diagram of the epidermis showing the main layers. The clear layer (not shown) is only found in the very thick epidermis of the palms and soles.**
protein. Filaggrin contains a high level of positively charge amino acids and participates in the aggregation of the keratin coiled-coils, which have an overall negative charge. After filaggrin performs this function, it is modified to come off the keratin microfibrils and is then digested by proteolytic enzymes to produce the amino acid components of the natural moisturizing factor (NMF) of the SC. NMF consists of lactate, amino acids from filaggrin breakdown, and pyrrolidone carboxylic acid (PCA) formed from the amino acid glutamine. These natural moisturizers are important to maintain proper hydration of the SC, allowing it to be flexible and to desquamate properly.

In the lower layers of the epidermis, the keratinocyte has a typical phospholipid bilayer cell membrane. Phospholipid membranes are far too permeable to water to survive exposure to air in a dry environment. At the SG, the cell membrane of the keratinocyte is transformed into the resistant cell envelope of the corneocyte. The transformation is brought about by the membrane-bound enzyme transglutaminase (T-gase). T-gase cross-links 2 proteins together by connecting a glutamine side chain of one to a lysine side chain on the other, resulting in the formation of an isopeptide bond as illustrated in Fig 4.

Several proteins participate in cross-link formation, especially some small proteins rich in the amino acid proline, known as SPRs. Eventually, the entire cell membrane is replaced by cross-linked proteins. Keratin fibers are also cross-linked to the envelope, and lipids (ceramides) are covalently attached to involucrin on the outer surface. These attached ceramides are important to the barrier function of the SC. The new structure is known as the resistant cell envelope. A schematic of the SC cell envelope based on the work of Peter Steinert is shown in Fig 5.

Recent studies indicate that the cell envelope is not always completely formed at the SG/SC interface and that some incompletely formed envelopes may persist into the SC. Harding et al have classified cell envelopes into 2 types: completely formed robust cell envelopes and incompletely formed fragile envelopes. The ratio of fragile to robust envelopes was reported to increase in dry and damaged skin.

**SC desmosomes**

Desmosomes are structures composed primarily of glycoproteins, which join cells together. The desmosomes joining corneocytes in the SC are modified from those that join epidermal keratinocytes by the addition of a protein called corneodesmosine, and SC desmosomes are sometimes referred to as corneodesmosomes. For proper desquamation of the SC to occur, the desmosomes must be digested by proteolytic enzymes. At least some of the keratin fibers are thought to connect to the desmosomes as illustrated in Fig 4. Figure 5 shows electron micrographs of desmosomes in the SC. In the lower SC, desmosomes...
are intact, but they are gradually digested by the time the skin surface is reached (Fig 6).

In SC that is desquamating at its normal rate, corneocytes persist in the SC for approximately 2 weeks, depending on body site, before being shed into the environment. On average, about one layer of corneocytes is shed each day from the surface and replaced by keratinocytes at the SG. The corneocytes that are shed each day can have a significant bacterial load and may be a source of contamination of the environment.

The mortar

The lamellar bodies that appear at the SG contain lipids, which are released into the intercellular space as the SC forms. These lipids are glucosyl ceramides, cholesterol, cholesterol esters, and long-chain fatty acids. In the intercellular space, the glucosyl ceramides are converted to ceramides. The lipids spontaneously organize into multiple layers between the SC cells. These layers are illustrated in Fig 7, which shows lipid layers in the lower SC after the overlaying SC has been removed by stripping with cellophane tape.

This lipid “mortar” is critically important to the barrier function of skin, and ceramides are vital to the organization and functioning of the barrier. Ceramides are sphingolipids linked to long-chain fatty acids. There are several ceramides found in the SC, including ceramides 1 and 2 shown in Fig 8.

The SC contains no phospholipids. The phospholipids from the keratinocytes of the viable layers are broken down by phospholipases in the lower SC. This produces fatty acids, which are necessary for the development of a functional SC barrier and may play a role in producing the acid pH of the SC. The SC has a surface pH of approximately 4 to 5.5, and this acidic pH, the so-called “acid mantle” of the skin, may play a role in protecting against colonization of the skin surface by harmful bacteria.

Elias et al and Schurer and Elias have proposed that the lipids released from lamellar granules in the SG are precursor or probarrier lipids that must be processed in extracellular spaces. The necessity of this extracellular lipid processing emphasizes the dynamic nature of the SC. The SC can no longer be considered as a Saran wrap (SC Johnson, Racine, WI)-like covering of the skin. It is a dynamic tissue that is metabolically active, even if it is not a living tissue in the classic sense. For more details on the dynamic SC and the “updated” bricks and mortar model, see the excellent review by Harding.

The lamellar bodies of the SC release other important molecules into the intercorneocyte spaces in addition to the lipids that form the permeability barrier. The proteolytic enzymes involved in desmosome hydrolysis as well as inhibitors of those enzyme to control rates of desquamation are released by the lamellar bodies, as are antimicrobial peptides called defensins, which may play a role in protecting the skin from infection. Production of these peptides is increased in skin with psoriasis but not in skin with atopic dermatitis (eczema). This may account for the much higher susceptibility of eczema sufferers to skin infection, and eczematous skin may be more likely to spread nosocomial infections.
The very specialized “bricks and mortar” of the SC work together to produce a covering for the skin that is both flexible and superbly protective. When the SC is functioning properly, it defends us against dehydration, external toxins, and bacterial assault as well as protecting the more fragile keratinocytes below from mechanical disruption.

HAND HYGIENE AND EPIDERMAL BARRIER FUNCTION

Surfactants

Most handwash products rely on surfactants for their cleansing action. Surfactants are surface-active molecules. They have a hydrophobic portion referred
to as the tail and a hydrophilic portion referred to as the head group. A ball and stick representation of an ionic surfactant is shown in Fig 9.

Surfactants are classified primarily by the characteristics of the head groups. Natural soaps are the metal salts of fatty acids, so the head group is a carboxylic acid and the X is either sodium or potassium. The major classifications of surfactants are anionic, cationic, amphoteric, and nonionic, depending on whether the head group has a negative (anionic) or positive charge (cationic) or both (amphoteric) or is uncharged (non-ionic). The hydrophobic tail can vary in length and structure, but hydrocarbon chains of various lengths are the most common. The structure of one of the most frequently used anionic surfactants, sodium lauryl sulfate (SLS), is shown below. Here, the hydrophobic tail is the 12 carbon hydrocarbon chain, and the head group is the sulfate moiety.

CH₃CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂O-S-O₃⁻NA⁺

Surfactants have a wide variety of applications and are especially useful for cleansing. They can form association structures such as micelles, which can dissolve oils directly. The hydrophobic tail can associate with oils on a surface causing it to lift up from the surface, and they can help to break up other types of soil. Nearly all products for cleansing skin or hair contain surfactants.

**Surfactants effects on the bricks and mortar**

Unfortunately, the very characteristics of surfactants that make them so useful for cleaning allow them to damage both the bricks and mortar of protective barrier of the SC. Repeated washing of the skin with soaps and surfactants can negatively impact the multiple functions of the epidermal barrier. The skin problems that can be engendered by the necessity of repeated exposures in a health care environment are multiple. Skin damage from surfactants can be further exacerbated by occlusion from wearing gloves and the mechanical action of scrubbing the hands. The negative effects that soaps and surfactants can have on the epidermal barrier are illustrated in Fig 10.

Surfactant molecules are well-known to bind to proteins, causing changes in their structure, and have been shown to bind to the SC. Surfactants known to be aggressive to the skin such as SLS bind more strongly than milder surfactants such as isethionates. Surfactants cause swelling of the SC in vitro, presumably by interaction with SC proteins, and the degree of swelling is well correlated with the harshness of either individual or mixtures of surfactant mixtures.

The lipid mortar is the major barrier to permeability of the SC, and surfactants are well-known to increase the ability of exogenous compounds to penetrate the skin and to increase the rate of water loss through the skin, presumably because of their effects on the lipid barrier. An obvious mechanism for the decrease in barrier function after surfactant treatment could be direct removal of SC lipids and removal of SC lipids by surfactant treatment, an effect that has been reported in some studies. Other studies on surfactant-induced irritation indicate that, rather than greatly reducing the total lipid content, the main effects are to alter lipid composition and disorder the lamellar structure of SC lipids. Figure 11 illustrates the extreme disordering of the SC lamellar lipid structure for soap-induced, winter-dry skin.

**Surfactant effects on the acid mantle**

As discussed above, the SC has a surface pH of approximately 4 to 5.5, and this acidic pH, the so-called
“acid mantle” of the SC,\textsuperscript{85,87,88,134} may play a role in protecting against colonization of the skin surface by harmful bacterial.\textsuperscript{3,4,90} Bar soaps made from natural soap are alkaline by their very nature and can raise the pH of the skin during washing.\textsuperscript{88,90,135} Trobaugh and Wickett\textsuperscript{135} reported that a single washing with typical bars soaps raised the pH from the normal range of 5.0 to 5.5 to approximately 7.5. Washing with a bar soap based on the synthetic detergent sodium cocoyl isethionate did not have this effect. The pH of soap-washed skin gradually declined toward normal over the next several hours because SC has components (fatty acids) that naturally provide buffering capacity.\textsuperscript{136,137} Trobaugh and Wickett\textsuperscript{135} further reported that washing 10 times per day with soap overcame the skin’s natural buffering capacity to some extent, and, within 3 days, the skin surface pH was consistently above 6.0, suggesting that the mechanism for maintaining the acid mantle was disrupted. In addition, cracking (fissuring) grades increased (worsened) from 1.5 to 3.0 on a 5.0-point scale. The authors interpreted the cracking as due to the harsh nature of natural soap bars and not simply to the high pH of the soap bars. Newman and Seitz\textsuperscript{138} found similar increases in skin surface pH on repeated soap washing paralleled by similar increases in cracking grade. Korting et al\textsuperscript{90}
also reported increases in skin pH following the use of natural soaps and a concomitant increase in skin colonization by coagulase-negative staphylococci.

**Surfactant effects on SC hydration and flexibility**

Since the pioneering work of Blank,\(^\text{139,140}\) we have known that water is a key factor for maintaining the pliability of the SC. Studies with isolated SC have found that well-hydrated SC is very flexible and dry SC is very stiff and brittle.\(^\text{139,141,142}\) Many in vivo studies have also found hydration to increase the elasticity of the skin.\(^\text{143-149}\) Loss of elasticity in surfactant-damaged dry skin can lead to cracking of the skin, especially around the knuckles at which stretching and flexing of the skin is required for movement.

**Effects on SC metabolism and desquamation**

In addition to the requirement of water for SC flexibility, we now understand that adequate hydration of the SC is required for the important metabolic processes occurring in the SC, such as conversion of probarrier lipids to barrier lipids\(^\text{82,91,150-152}\) to maintain barrier function and the hydrolysis of desmosomes\(^\text{67-70,153,154}\) necessary for normal desquamation. Skin in good condition maintains SC water content at approximately 30% by weight (except at the very surface), whereas very dry skin can be as low as 10% to 15% water throughout most of the SC down to nearly the level of the SG.\(^\text{155}\) The natural moisturizing factors of the SC are very important to maintaining SC hydration.\(^\text{156-160}\) NMF is easily lost from the skin, especially after the barrier is perturbed,\(^\text{157,161,162}\) and it is likely that NMF removal is a major factor in the development of dry skin following repeated hygiene procedures.

The desmosomes connecting SC cells (Fig 6) are gradually digested by a cascade of proteolytic enzymes.\(^\text{67,163}\) In dry and in dry, damaged skin, these enzymes are not able to perform their function properly, thereby causing intact desmosomes to persist into the upper SC.\(^\text{59,70}\) This results in shedding of large scales or flakes rather than individual cells. It also increases the surface area of the SC available for bacterial adhesion and may be a factor in the increased presence of colonizing bacteria on damaged hands.\(^\text{90,164}\)

**Surfactant effects on cytokines-hyperproliferation**

Surfactants can induce the release of inflammatory cytokines (cell-signaling molecules). Interleukins, particularly interleukin-1α, are released upon surfactant exposure.\(^\text{165-167}\) There are 2 mechanisms that can contribute to this effect. Surfactants are clearly able to penetrate the SC and encounter keratinocytes in the viable layers of the epidermis. The interaction of surfactants with keratinocytes is known to induce cytokine release.\(^\text{166,168}\) Interleukin-1α is also released as a natural response of the epidermal barrier to disruption\(^\text{169}\) as part of the protective process intended to stimulate repair of the barrier. In surfactant-irritated skin, these effects may combine to produce overstimulation, causing the skin to hyperproliferate.\(^\text{170}\) The overstimulated skin has an increased rate of cornification that does not permit sufficient time for the complex events that must occur in the SG to form a fully functional SC. A normal SC lipid profile will not be produced,\(^\text{70,171}\) and SC cell envelopes that incompletely formed and are thus “fragile” will form in higher than normal proportion.\(^\text{61}\) Cells with fragile envelopes will lose their NMF easily and not have good barrier properties. These effects will combine to lead to SC with impaired barrier function. This resulting barrier will have weaknesses in both the bricks and the mortar. Consequently, it will be increasingly susceptible to further surfactant damage and less able to hold water to allow SC enzymes to function. The result will be a vicious cycle of skin damage.\(^\text{161}\)

**Breaking the dry skin cycle**

The challenge in the health care environment is to somehow break this dry skin cycle while meeting hygiene guidelines that require frequent cleansing of the hands. Multiple approaches are required. The first step is to use cleansers formulated with mild...
surfactants such as isethionates,\textsuperscript{172} amphodiacetates, and sulfosuccinates. Blending surfactants together in appropriate ratios can also significantly improve their mildness.\textsuperscript{110} The use of alcohol-based hand sanitizers that contain appropriately formulated emollients may also help to improve hand condition.\textsuperscript{173-175}

Although improving the surfactant cleansing system will help reduce skin problems, it is likely to be necessary to use well-formulated therapeutic lotions to help break the cycle and reestablish the formation of normal SC. Glycerin has been shown to be an effective skin-conditioning agent when incorporated into a lotion at 5\% or higher.\textsuperscript{176,177} It has several positive effects on the physical properties of the SC, including increasing hydration\textsuperscript{178-180} and improving elasticity.\textsuperscript{146,147,178} Glycerin has been reported to normalize the rate of desmosome hydrolysis in dry skin,\textsuperscript{181} and glycerin has been shown to accelerate the repair of the SC barrier after damage by SLS.\textsuperscript{182}

Petrolatum is an effective treatment for dry skin\textsuperscript{183} both as a single ingredient and as a component of creams and lotions. Petrolatum is an occlusive agent that reduces the rate of water loss through skin, increasing hydration by causing buildup of water in the upper SC. Petrolatum has been shown to penetrate deeply into damaged skin and enhance recovery of SC barrier function.\textsuperscript{184} Use of lotions and creams outside of work may have significant benefits for health care workers and should be stressed as part of the routine hand hygiene practices. Two factors must be considered while using lotions in the health care environment. One is the possibility that oils such as mineral oil and petrolatum in the lotion may compromise the integrity of latex gloves.\textsuperscript{185} The other is that lotions formulated with anionic emulsifiers may inactivate the residual activity of chlorhexidine gluconate on the skin.\textsuperscript{186,187} These issues can be addressed by providing lotions that contain lower levels of oils such as petrolatum and mineral oil and that use either nonionic or cationic emulsifiers while using latex gloves and chlorhexidine gluconate-containing cleansers.\textsuperscript{188} The consistent use of mild cleansers, alcohol rubs with emollients, and effective skin lotion both during and outside of work should go far to help maintain the protective function of the SC, keeping both the “bricks” and the “mortar” intact while meeting the hand hygiene requirements of the health care worker.

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