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CHAPTER 1

Basic Science of the Epidermis

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SUMMARY POINTS

What's Important?

1. Keratinocytes have multiple receptors that give them several important functions.
2. Every skincare product placed on the skin affects the keratinocytes in some way.
3. Stem cells in cosmeceutical products have no activity.

What's New?

1. Circadian rhythms increase TEWL at night.
2. Keratinocytes focus on cell protection in the daylight and cell repair at night.
3. Cosmeceuticals can manipulate epidermal skin cells.
4. Visible light injures mitochondria and lysosomes, and ages skin.

What's Coming?

1. New research on CBD in skincare products.
2. New treatments to remove lipofuscin and rejuvenate lysosomes.
3. New modalities to protect mitochondria.
4. More studies on the effects of light on keratinocytes.

The skin is composed of three primary layers: epidermis, dermis, and subcutaneous tissue (**Fig. 1-1**). The epidermis is the outermost superficial layer of the skin. It is very important from a cosmetic standpoint because it is this layer that gives the skin its texture and moisture, contributes to color, and affects light reflection. If the surface of the epidermis is dry, the skin feels rough and poorly reflects light. When patients complain that their skin is "dull" or "not radiant," the problem lies in the epidermis. This is the layer targeted by salespeople when they urge you to "just try" their product. The product is almost always an exfoliator that removes the uppermost layer of the epidermis providing instant smoothness and light reflection. The epidermis is the layer to target when patients want instant results or radiant skin overnight. However, the changes to the epidermis are temporary as the keratinization cycle continues to produce new cells and push away old cells to the skin's surface. The best topical formulations and skincare procedures target both the epidermis and the dermis. The epidermis is much more complex than described in this chapter, which is meant to focus on what parts of the epidermis are important to enhance the skin's beauty, appearance, and health with minimal focus on skin disease.

SKIN CELLS IN THE EPIDERMIS: THE KERATINOCYTE

Keratinocytes, also known as corneocytes, are the cells that comprise most of the epidermis. The skin cells in the epidermis are called keratinocytes because they contain keratin, a protein found in the epidermis, nails, and hair. Keratin is also found in the beaks and feathers of birds, and shells. The word "keratin" comes from the Proto-Indo-European root *ker*, which means horn. Keratin composes 30–80% of the total protein of the human epidermis.

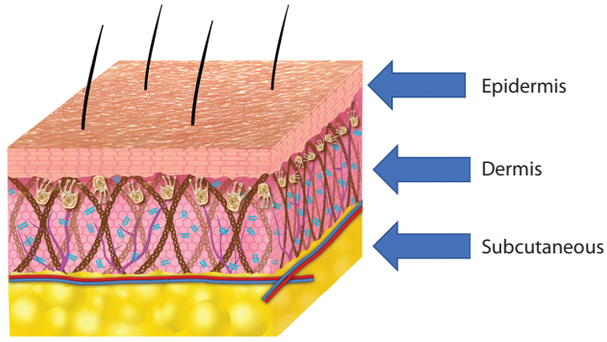

FIGURE 1-1. Human skin.

The epidermis has an abundance of the keratin filaments that make up an intermediate filament cytoskeleton. There are two classes of keratins: Type I is slightly acidic (K9–40) and Type II, more basic (K1–8 and K71–86). Both types of keratin filaments must be present for a keratin filament to develop.[1] In other words, an acidic type and a basic type form a keratin filament together. The keratin genes are found on chromosomes 12q and 17q. There are over 100 known skin, hair, and nail disorders linked to keratin genes.

Keratinocytes perform numerous functions, primarily the delivery of structural support and physical protection. Keratinocytes produce keratin filaments, which provide firmness to the skin and resilience against mechanical stress. They provide a physical barrier to prevent toxins and pathogens from entering the skin. Keratinocytes exhibit an immunomodulatory function and can secrete cytokines, activate Langerhans cells, and stimulate inflammation to protect the skin from pathogens. Keratinocytes are surrounded by bilayer lipid membranes that prevent water loss and keep hydrophilic compounds from entering the skin. They can sense peptides and function as antigen-presenting cells.

Organelles in Keratinocytes

Mitochondria

Mitochondria play a crucial role in skin health and beauty because they are responsible for energy production through a multistep process called oxidative phosphorylation or the electron transport chain. The mitochondria are the primary organelle affected by intrinsic and extrinsic aging.[2] Dysfunction of the mitochondria is a major cause of aging because energy is needed for cells to divide and differentiate, to produce the extracellular matrix (ECM) and proteins, as well as to repair themselves. Keratinocyte differentiation, for example, depends on functional mitochondria; increased Ca^{2+} causes keratinocyte differentiation based on mitochondrial calcium uptake.[2]

The mitochondria produce energy via a process called oxidative phosphorylation by converting adenosine diphosphate (ADP) to adenosine triphosphate (ATP), which occurs in the intricate inner membranes of the mitochondria. The mitochondria use oxygen as a carrier for electrons and consume oxygen in the process of oxidative phosphorylation. The flow of electrons through the electron transport chain generates energy that is stored in ATP and results in an excess number of electrons, which become reactive oxygen species. These free radicals harm the mitochondrial membranes, as well as cause damage to and mutations in the mitochondrial DNA. Mutations of mitochondrial DNA engender disorder in the energy production process and can lead to an increased number of free radicals. The cycle is perpetuated as these free radicals create more mitochondrial DNA mutations. Mutated and damaged mitochondria do not produce ATP efficiently and represent one cause of aging.[3] At this time there are no ingredients known to repair damaged mitochondria; therefore, mitochondrial damage must be prevented in order to slow aging by using sun protection, sunscreen, and antioxidants.

Cosmeceutical ingredients and mitochondria

Secreted by the pineal gland, melatonin regulates circadian rhythm. Melatonin also exerts effects on keratinocyte proliferation, skin pigmentation, inflammation, and immune response, all of which involve the mitochondria. Melatonin and the mitochondria have a symbiotic relationship. The mitochondria are the site of melatonin biosynthesis and metabolism. Melatonin directly enhances ATP production by donating electrons and provides antioxidant protection to the mitochondria.[2]

Coenzyme Q_{10} (CoQ_{10}) is a lipophilic antioxidant that plays a role in ATP production in the mitochondria. Levels of CoQ_{10}, also known as ubiquinone, are 10-fold higher in the epidermis as compared to the dermis.[2] The statin cholesterol-lowering drugs decrease levels of CoQ_{10} and have been associated with oxidative stress and mitochondrial dysfunction leading to premature aging of skin fibroblast cells in vitro.[4] Topical application of CoQ_{10} has been shown to improve the appearance of aged skin.

The vitamin A family, including retinol, influences the metabolic fitness of the mitochondria by affecting ATP production. Low levels of vitamin A are associated with minimal ATP synthesis, while increasing vitamin A results in a significantly higher energy output.[6] This may be mediated by the c-Raf and protein kinase C (PKC) families,[7] which contain high affinity retinol binding sites in their regulatory domains,[8] and play a critical role in mitochondrial function.

Cosmetic procedures and mitochondria

For the past decade, dermatologists who perform copious light and laser procedures have begun to suspect that the wavelengths of light may have effects on the skin that transcend what is presently understood. It is well known that UV light and blue light can damage mitochondria, and artificial visual light weakens mitochondrial function.[9–11] However, other studies have shown that red light and near-infrared light stimulate mitochondrial activity and ATP production by cytochrome c oxidase.[12–14] For this reason, a sunscreen that protects against visible light should be used daily. More studies are needed to understand the effects of various wavelengths of light on mitochondria.

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Lysosomes

Lysosomes are the garbage disposal of the cells. Dysfunction of the lysosome allows cellular waste products to accumulate inside the cell. Genetic defects of lysosomes lead to severe disorders called lysosomal storage diseases (e.g., Tay-Sachs disease and Gaucher's disease).

Lysosomes are intracellular organelles that contain enzymes that degrade and recycle cellular waste. The enzymes require various levels of acidity (different pHs) to work properly; therefore, the lysosome membrane contains a pump that requires ATP to propel hydrogen ions into the lysosome to regulate acidity. To rid the cell of waste, the lysosome needs (1) the correct enzymes, (2) the ideal pH for that enzyme to work, and (3) energy produced by the mitochondria in the form of ATP.

Acquired lysosome dysfunction occurs with aging. When lysosomes are unable to degrade cellular waste, lipofuscin accumulates. Lipofuscin is resistant to degradation and not subject to exocytosis; therefore, it accumulates inside cells, produces free radicals, and causes other cellular disturbances. Under a microscope and with proper staining, lipofuscin appears fluorescent and is thus easily visualized. The age of cells can be ascertained by the amount of lipofuscin seen.[15,16] It is now known that mitochondria play a role in lipofuscin accumulation.[17]

Studies have shown that oxidative stress by free radicals leads to an increase in lipofuscin. Accumulation of lipofuscin hampers the ability of lysosomes to work effectively, altering acidity and disrupting the supply of enzymes.[18] Lipofuscin in keratinocytes generates singlet oxygen free radicals and causes DNA mutations upon exposure to blue light and visible light.[19,20]

Treatments to prevent aging caused by lysosomal damage or increase of lipofuscin would have to achieve one of the following: (1) preserve lysosomal function, (2) increase breakdown of lipofuscin and cellular waste, (3) reduce free radical formation, or (4) enhance lysosomal function. Medical discoveries often occur first in areas of severe disease because these advances are most needed and may receive the most attention and funding. Gaucher's disease, which is a severe disorder caused by a lack of a lysosomal enzyme, is successfully treated with intravenous infusion of the missing enzyme.[21] Genetic treatments are being developed for Tay-Sachs disease. At this time, there are no genetic or enzyme treatments for accumulation of lipofuscin or cellular waste.

Cosmeceutical ingredients that affect lysosomes and lipofuscin

Antioxidants have been shown to slow the rate of lipofuscin accumulation. Studies have demonstrated beneficial effects from flavanols, polyphenols, catechins, and oligomeric procyanidins. Examples of ingredients that have been successful are grape seed extract, curcumin, tocopherol (vitamin E), CoQ_{10}, beta carotene, and dihydroquercetin.[22–25]

Receptors on Keratinocytes

Keratinocytes, once thought only to confer strength to the skin, play a major role in sensation, cell communication, and activation of the immune system among other functions. They contain receptors that give them several different activities. There are many more receptors on keratinocytes than discussed in this chapter, which will focus on keratinocyte receptors important in skincare.

Cannabinoid Receptors on Keratinocytes

Keratinocytes have cannabinoid receptors, both Type 1 (CB1) and Type 2 (CB2), which play a role in skin inflammation and display immunomodulatory functions (**Fig. 1-2**). Cannabidiol (CBD) regulates pathways involved in keratinocyte differentiation by inducing expression of various genes and exhibits antioxidant and anti-inflammatory properties.[26] CB1 seems to help limit topical allergic response to contact allergens by inhibiting the production of proinflammatory cytokines.[27] Activation of CB2 reduces inflammation and speeds re-epithelialization.[28] CB1 and CB2 are activated by Δ-9-tetrahydrocannabinoid (THC).[29] CB2 is also activated by eugenol, which is found in basil, cloves, and bay leaves. Although many CBD skincare products are on the market, it

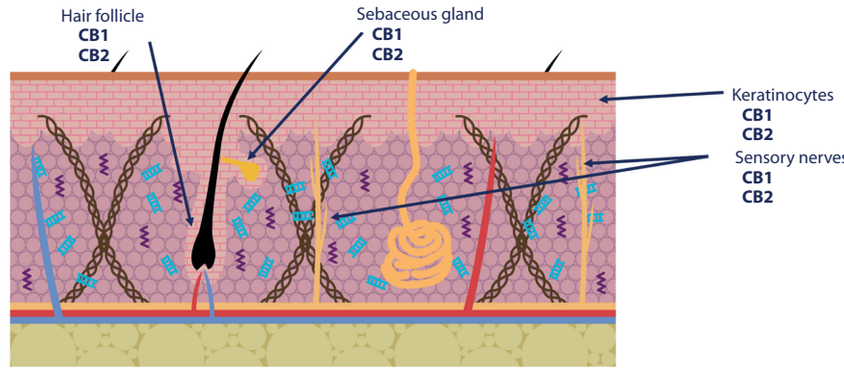

FIGURE 1-2. Cannabinoid receptors in the skin.

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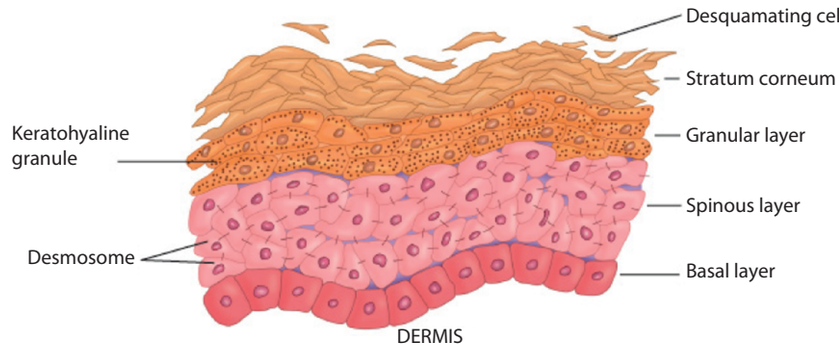

FIGURE 1-3. The layers of the epidermis.

is too early to know which are the most effective and what are the best ways to formulate them to maximize penetration.

Sensory Receptors on Keratinocytes

Keratinocytes have numerous sensory receptors such as transient receptor potential channels (TRP), which can sense temperature, pH, touch, osmolarity, pheromones, and more. When activated, these TRP receptors release hormones, vasoactive peptides, or neurotransmitters.[29] Activation of these keratinocyte sensors can engender a cascade of effects on skin including pain, itch, activation of inflammation, or perception of heat or cooling.

Transient receptor potential vallinoid 1 (TRPV1) and TRPV4 are heat receptors and play an important role in pain and itch. TRPV1, the receptor that senses capsaicin (a component of chili peppers), gives a sensation of heat, and is activated by CBD and eugenol. TRPV3 is also triggered by eugenol. Transient receptor potential melastatin 8 (TRPM8) is a sensor of low temperatures and is activated by camphor and menthol.[30] This is why menthol, peppermint, and eucalyptus give a cooling sensation. Transient receptor potential ankyrin (TRPA) is responsible for pungent, tingling, and burning taste and feeling when bound to components of cinnamon, garlic, mustard, onion, frankincense, curcumin, and horseradish.[29]

Massage and body oils often contain these natural ingredients. They are used to deliver a sensation of cooling or heat. Knowledge of these receptors can help the practitioner choose which natural oils to include in skincare products to treat various conditions.

Keratinization

The epidermis resembles a brick wall with the bricks representing keratinocytes. It has an inner basal layer of mitotically active cells and suprabasal layers of differentiating cells. Keratinocytes are "born" at the base of the epidermis at the dermal–epidermal junction (DEJ). They are produced by stem cells, some of which reside at the base—basal layer—of the epidermis, while other stem cells are found in the hair follicle. When the stem cells divide, they create "daughter cells," which slowly migrate to the top of the epidermis.[31] This process of keratinocytes being born from stem cells, maturing, and moving to the outermost layer of the skin is called keratinization.

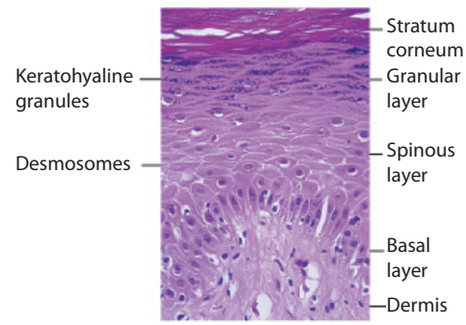

FIGURE 1-4. Histopathology of the epidermis demonstrating the four layers. (Image courtesy of George Ioannides, MD.)

After keratinization, the cells undergo apoptosis and desquamate from the skin's surface.

As keratinocyte cells migrate away from the basal layer and move outward toward the skin surface, they turn off production of proteins such as integrin and laminin and execute a terminal differentiation program. Keratinocyte cells migrate upwards in the epidermis after they loosen their attachments to each other and are pushed from below by younger keratinocytes.

As keratinocytes approach the skin's surface, they mature and develop different characteristics (known as differentiation). The layers of the epidermis are named for these characteristic traits. For example, the first (and deepest) epidermal layer is the basal layer because it is located at the base of the epidermis. The next layer is referred to as the spinous layer because the cells in this layer have prominent, spiny attachments holding the cells together. The next layer is the granular layer because these cells contain visible granules. The last, outermost layer is the stratum corneum (SC), a flattened layer of cells that have lost their nuclei and granules (**Figs. 1-3 and 1-4**). The SC is covered by a protein material called the cell envelope and bathed in lipids that protect the epidermis and help the skin remain hydrated.

As keratinocytes migrate through the layers of the epidermis, their contents and functions change according to, or depending on, the specific epidermal layer in which they are

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moving. Keratinocyte activity, such as the release of cytokines, can be affected by topical products administered to the skin.

THE LAYERS OF THE EPIDERMIS

The Basal Layer (Stratum Basale)

Cuboidal in shape, basal cells are found at the DEJ attached to the basement membrane that divides the epidermis and dermis. Basal cells produce the ECM components of the underlying basement membrane that separates the epidermis from the dermis.[32] Basal cells attach to the dermis below with hemidesmosomes and to neighboring basal cells and the overlying spinous cells via desmosomes. These basal keratinocytes contain keratins 5 and 14 that form a cytoskeleton allowing for cellular flexibility. This flexibility enables cells to proceed out of the basal layer to migrate superficially, thus undergoing the keratinization process. Mutations of the keratin 5 and 14 genes result in an inherited blistering disease called epidermolysis bullosa simplex.

Basal cells are responsible for maintaining the epidermis by constantly producing new keratinocytes. In the basal layer, 10% of keratinocytes are stem cells, 50% are amplifying cells, and 40% are postmitotic cells. Normally, stem cells are slowly dividing cells, but under certain conditions such as wounding or exposure to growth factors, retinoids, and defensins, they divide faster. Basal cells give rise to transient amplifying cells that are responsible for most of the cell division in the basal layer, producing more keratinocytes. Postmitotic cells undergo terminal differentiation and move superficially to become suprabasal cells that continue their upward migration to become granular cells and ultimately part of the SC (**Fig. 1-5**).

The Spinous Layer (Stratum Spinosum)

The stratum spinosum contains 8–10 layers of keratinocytes connected by spiny attachments called desmosomes that hold the cells together. These spiny desmosomes function as an adhesion point for the intermediate filaments, provide resistance against skin tearing, and play an important role in wound healing. Desmosomes are complex structures composed of adhesion molecules and other proteins that are important in cell adhesion and cell transport.

Lamellar granules, also called Odland bodies, first appear in the spinous layer of the epidermis. These lamellar granules contain lipids such as ceramides, cholesterol, and fatty acids that are produced by the cell. These lipids are packaged into granules, migrate to the surface, and extrude their contents into the space between keratinocytes, bathing the keratinocytes in protective lipids. The lamellar granules protect the contents until they are released at their target point. The lamellar granules also contain hydrolytic enzymes needed for desquamation such as proteases, acid phosphatase, lipases, and glycosidases. The antimicrobial peptide cathelicidin is also stored in the lamellar granules.[33]

Keratins 1 and 10 are first seen in this spinous layer of suprabasal keratinocytes. These keratins form a rigid cytoskeleton that confers mechanical strength to the cell.

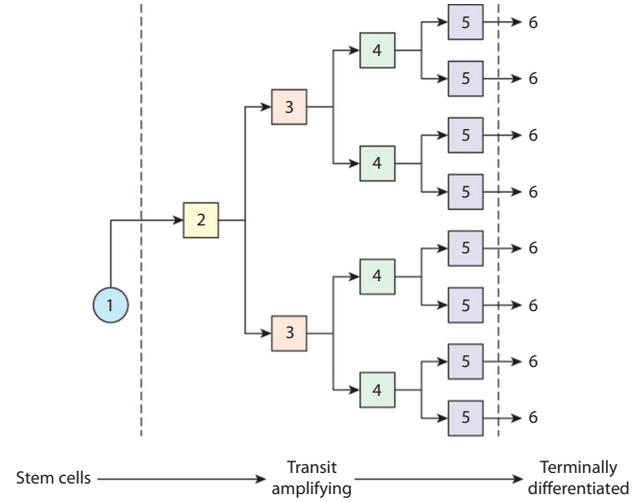

FIGURE 1-5. The stem cells divide and produce amplifying cells that greatly increase the number of keratinocytes. These in turn become the mature, terminal, and differentiated cells. The numbers indicate the cell generation.

In hyperproliferative conditions such as actinic keratosis, wound healing, and psoriasis, keratins 6 and 16 are expressed in the spinous layer. The cytoplasm of the spinous layer keratinocytes contains proteins not found in the basal layers such as involucrin, keratolinin, and loricrin. These proteins become cross-linked in the SC to impart strength to the skin.

The Granular Layer (Stratum Granulosum)

The granular layer contains 3–4 layers of keratinocytes that are characterized by granular dots seen in the keratinocytes. These keratohyalin granules contain profilaggrin, the precursor to filaggrin. Filaggrin is a **fil**ament **aggr**egating prote**in** that binds to the keratin cytoskeleton and forms a protein scaffold upon which proteins and lipids can attach. The filaggrin cross-links to keratin filaments, providing strength and structure. A genetic defect of the filaggrin gene predisposes an individual to the triad of atopic dermatitis (eczema), allergies, and asthma or may result in a dry skin disorder known as ichthyosis vulgaris.

The proteins of the cornified cell envelope (involucrin, keratolinin, the pancornulins, and loricrin) are cross-linked in this layer by the calcium-requiring enzyme transglutaminase (TGase) to form the cell envelope. There are four types of transglutaminases present in the epidermis: TGase 1 or keratinocyte TGase, TGase 2 or tissue TGase, TGase 3 or epidermal TGase, and TGase 5. Only TGases 1, 3, and 5 participate in the development of the corneocyte envelope. TGase 2 has other functions including a role in apoptosis (programmed cell death). TGase activity increases when Ca^{2+} levels increase,[34] and results in the formation of the cornified cell envelope.

Calcium is an inducer of keratinocyte differentiation,[35,36] and a suppressor of keratinocyte proliferation.[37,38] It has been shown that in the state of low Ca^{2+} levels keratinocytes are in a proliferative stage, while increases in Ca^{2+} levels lead to expression of differentiation markers such as keratins 1 and 10, TGase, and filaggrin.[37]

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BOX 1-1

1,25-Dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] stimulates the differentiation and prohibits the proliferation of keratinocytes. It exerts its effects via the nuclear hormone receptor known as vitamin D receptor (VDR). VDR operates with the aid of coactivator complexes. There are two known coactivator complexes: vitamin D interacting protein complex (DRIP) and the p160 steroid receptor coactivator family (SRC/p160). It has been proposed that the DRIP mediator complex is involved in proliferation and early differentiation, while the SRC/p160 complex is engaged in advanced differentiation.[11] The vitamin D receptors of undifferentiated keratinocytes bind to the DRIP complex, inducing early differentiation markers of K1 and K10.[12] The DRIP complex on the vitamin D receptor is then replaced by the SRC complex. The SRC complex induces gene transcription for advanced differentiation, which occurs with filaggrin and loricrin.[12] The replacement of the DRIP complex with the SRC complex on the vitamin D receptor is believed to be necessary for keratinocyte differentiation. It is important to realize that vitamin D levels are lower in older people and that this reduction may play a role in the slower wound healing characteristic in the elderly.

The form of vitamin D known as 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] plays a role in keratinocyte differentiation because it enhances the Ca^{2+} effect on the keratinocytes and increases transglutaminase activity as well as involucrin levels.[39] These combined effects induce corneocyte envelope formation.[40,41] (See **Box 1-1**.)

Granular cells exhibit anabolic properties such as synthesis of filaggrin, cornified cell envelope proteins, and high molecular weight keratins. However, they also cause catabolic events such as dissolution of the cell nucleus and organelles, which disappear prior to moving into the SC. The granular layer is the uppermost viable layer of the epidermis because the layer above it contains no organelles.

The Stratum Lucidum

This layer is 2–3 cell layers thick in most of the body and 8–10 layers thick in the palms and soles.[42] It is clear when viewed under the microscope and not easily seen in routine stains, so many skin anatomy texts contend that it does not exist other than in the palms and soles. Some consider the stratum lucidum a part of the SC. The author suggests that it is reasonable to think that most of the body has four major epidermal layers, but areas such as the palms and soles have five such layers, including the stratum lucidum.

The keratinocytes in this layer have not yet lost all their nuclei, have sparse organelles, and are filled with eleidin, which is a byproduct of keratohyalin.[42] This layer overlies the vermilion border in the lip and its lucidity allows the red blood vessels to show through, giving the vermilion border of light skin types a red appearance.

The Stratum Corneum (SC)

The most superficial layer of the epidermis is the SC or horny layer, which forms a protective layer on the skin's surface. The keratinocytes that reside in this layer are the most mature and have completed the keratinization process. These keratinocytes contain no nuclei or organelles. Although the SC plays a very important role in skin hydration and protection, it is described as the "dead layer" of the epidermis because these cells do not exhibit protein synthesis and are unresponsive to cellular signaling.[43]

The SC is approximately 15 cell layers thick but this depends upon the location on the body.[44,45] The SC has the most cell layers on the palms and the soles. If present on the lips, the SC is only about three layers thick, which is why the lips dehydrate easily and become chapped.

The SC is composed of protein-rich corneocytes embedded in a bilayer lipid lamellar matrix assembled in a "brick and mortar" fashion. The "bricks" are composed of keratinocytes and the "mortar" is made up of the contents extruded from the lamellar granules including ceramides, cholesterol, and fatty acids (**Fig. 1-6**). These lipids form bilayer lamellar membranes known as the "skin barrier." When viewed under a cross-polarizing microscope, these lamellar lipids form a Maltese cross-pattern when the skin barrier is intact (**Fig. 1-7**). One of its protective functions is to prevent transepidermal water loss (TEWL).

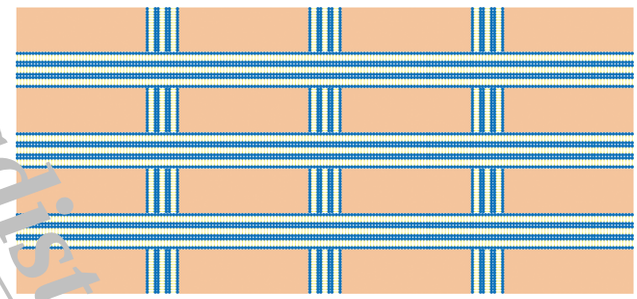

FIGURE 1-6. The keratinocytes are surrounded by multiple bilayers of lipids that form a lamellar structure. This resembles a brick and mortar structure.

FIGURE 1-7. Maltese Cross image seen under cross-polarizing microscope when bilayer lamellar membranes are intact.

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Filaggrin, the structural protein synthesized in the granular layer and packaged in keratohyalin granules, is broken down into amino acids inside the keratinocyte cells. These amino acids form a substance known as natural moisturizing factor (NMF). Genetic defects of filaggrin lead to reduced levels of NMF intracellularly in the SC.[46] Cells of the midcornified layer possess the highest amino acid content and, therefore, have the greatest capability for binding to water, while the deeper layers have less water-binding capacity.[47] Intracellularly located NMF and lipids released by the lamellar granules, located extracellularly, play an important role in skin hydration, suppleness, and flexibility (see Chapter 12, Dry Skin).

THE KERATINOCYTE CELL CYCLE

Keratinization is the process of the keratinocytes beginning in the basal layer and maturing and moving to the top of the epidermis and desquamating from the skin's surface. The keratinization process of young epidermis is about 26 to 42 days but can vary due to skin thickness, age, genetics, skincare products, and other factors.[48] Most references cite 28 days as the turnover time for the cells to transition from the basal layer to the SC.[49] However, because studies differ and it is best to manage patients' expectations about when to see changes from a new skincare regimen, this text will cite that in an average person's epidermis with a thickness of 0.1 mm, the keratinization process transpires over 4–6 weeks.[50] This is significant because changes from topical skin treatments depend on keratinization time, and treating acne or pigmentation or significantly changing the epidermis takes 4–6 weeks to see results (**Fig. 1-8**).

This series of events, known also as desquamation, normally occurs invisibly with shedding of individual cells or small clumps of cells. Disturbances of this process may result in the accumulation of partially detached keratinocytes, which cause the clinical findings of dry skin. Disease states may also alter the keratinization cycle. For example, psoriasis causes a dramatic shortening of the keratinization cycle, resulting in the formation of crusty cutaneous eruptions. Keratinization lengthens in time as humans age.[51] This means that the cells at the superficial layer of the SC are older and their function may be impaired. Results from such compromised functioning include slower wound healing and a skin appearance that is dull and lifeless.

The keratinization process is regulated by epidermal growth factor, retinoids, cytokines, and the presence of lipids such as linoleic acid and other factors.[52] Age, diet, genetics, and the use of skincare products affect keratinization duration. Using exfoliants such as scrubs, microdermabrasion, peels, and lasers can accelerate keratinization. Several cosmetic products such as retinol, growth factors, topical defensins, and alpha hydroxy acids quicken the pace of keratinization, yielding younger keratinocytes at the superficial layers of the SC, thus imparting a more youthful appearance to the skin.

Skin Stem Cells in the Epidermis

The epidermis constantly renews itself in a perpetual cycle of growth and desquamation. Epidermal stem cells (EPSCs) and hair follicles maintain this cycle and drive healing of the epidermis. EPSCs cycle between stemness, differentiation, and senescence, regulated by the p63 gene.[53] The stem cells of the epidermis are found in the hair follicle, sebaceous gland, sweat gland, and the interfollicular epidermis between the hair follicles, each with its own specialized stem cells (SCs). The interfollicular epidermal stem cells (IFE-SCs) and the sebaceous gland stem cells are constantly self-renewing, while hair follicle stem cells cycle between growth, involution, and resting stages as seen with the stages of hair growth (anagen, telogen, and catagen). IFEs are not well characterized yet. There are many stem cells in the hair follicle that are beginning to be understood (**Fig. 1-9**). LGR6+ is important in skincare science because

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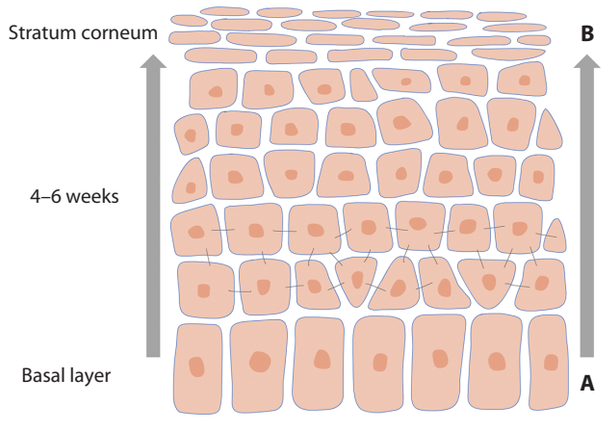

FIGURE 1-8. Movement of keratinocytes from the basal layer to the stratum corneum can take 4–6 weeks. This process is called keratinization.

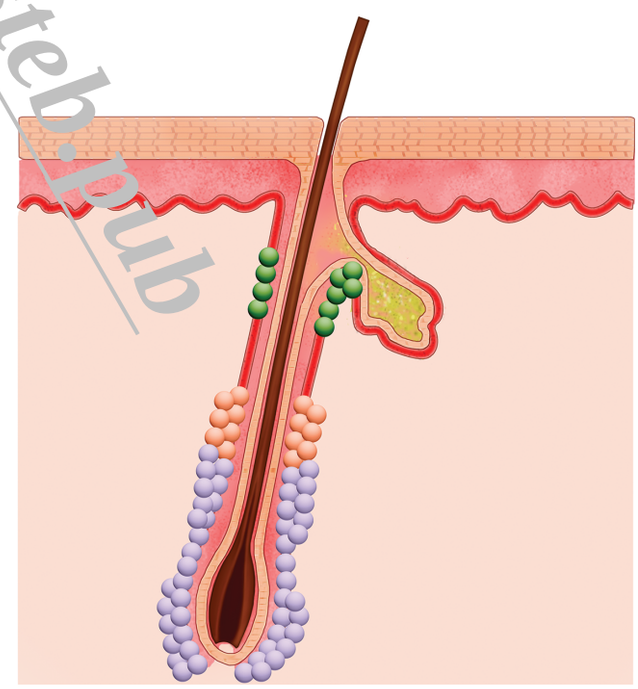

FIGURE 1-9. There are many stem cells in the hair follicle as shown in different colors along the hair follicle. The LGR6+ stem cell location is shown in green.

it is activated by defensin and can regenerate the epidermis when activated. LGR6+ is an important stem cell in wound healing.[54]

Stem cell-containing areas are divided into three niches, each with a specific microenvironment of growth factors, ECM, and various cell types. The niches are the basal layer of the epidermis, the bulge of the hair follicle, and the base of the sebaceous gland.[55] New data is emerging to reveal how stem cells interact with the ECM. For example, the stem cells interact with the ECM via integrins, and insulin-like growth factor-binding protein (IGFBP)-2 and microRNA (miR135b) have been shown to play a role in the regenerative capacity of the epidermis.[56,57] Aging, due to a multitude of reasons, results in decreases of stem cell numbers or function in these niches.[58] Stem cells are damaged from aging, exposure to UV light and free radicals, shortening of telomeres, inflammation, accumulation of senescent cells, and other factors that render them sluggish.[59–62] Reactive oxygen species, also known as free radicals, affect the proliferative ability of EPSCs and enhance their differentiation while decreasing their stemness.

EPSCs are subject to the circadian clock, which affects both proliferation and differentiation.[64,65] Circadian clock disruption leads to an increase in differentiation of EPSCs that resemble the changes seen in the aging process.[66] An increased level of senescent keratinocytes in older skin causes a reduction in stem cell population.[67]

Cosmeceuticals That Act on Epidermal Stem Cells in Skincare Products

There are several benefits to manipulating the microenvironment around EPSCs to promote renewal including improvement of the skin's appearance, strength, and texture and to speed wound healing. Using over-the-counter cosmetic products that contain stem cells is not the answer. The stem cells found in these mass-market skincare products are useless and exert no activity on the epidermis. One reason is that the stem cells in skincare products are plant derived, often from apples, and deliver no activity in human skin. Even if the stem cells were active, they would need to be cultured in stringent conditions and would not survive in a skincare product on a shelf. Companies include apple stem cells in products because consumers have heard about stem cell research and skin rejuvenation. However, the stem cells used to rejuvenate skin and treat chronic wounds are grown in controlled laboratory conditions and do not sit on a shelf in uncontrolled conditions.[68]

The stem cells that display efficacy and are used in dermatology are adult associated mesenchymal stem cells (MSCs). MSCs are divided into adipose-derived stem cells (ADSCs) that are isolated from fat and bone marrow-derived stem cells (BM-MSCs).[69] These stem cells, whether from fat or bone marrow, are extracted from the patient and injected into the target tissue. They are not formulated in products sold commercially. ADSCs are used more often because they are easily available through liposuction with minimal added procedure costs.[70] Mice receiving subcutaneous injections of ADSCs have demonstrated increased procollagen Type 1, increased collagen density, angiogenesis, and increased dermal thickness.[70,71] It is unknown exactly how the ADSCs cause these effects, but it is assumed that they activate dermal fibroblasts (which affects the dermis rather than the epidermis). Human studies using amniotic membrane stem cell-conditioned media (as compared to a saline control group) applied with microneedling have shown improvement in pore size, wrinkles, and pigmentation.[72]

Another approach is using ingredients to stimulate the stem cells or improve the microenvironment in their niche in a manner that helps them renew.[73] On a cautionary note, increasing replication of skin cancer cells is not desirable, so special care must be used when tampering with these cells to avoid causing or worsening skin cancer. Although there is no evidence that the topically applied skincare ingredients discussed in this section have ever engendered or contributed to skin cancer, it is best to first ensure that the skin is free of skin cancer before using these ingredients.

Cosmeceutical ingredients affect stem cells directly or through their microenvironment. Ascorbic acid (vitamin C) has been shown to promote formation of ECM, increase integrin expression, and augment the stemness proliferation potential of EPSCs.[67] The mushroom-derived cosmeceutical ingredient *Ganoderma lucidum*, the flowering plant *Rhodiola sachalinensis*, resveratrol from grapes and berries, and the Chinese herb *Eleutherococcus senticosus* (also known as Siberian ginseng) have been demonstrated to increase integrin expression, and enhance the stemness proliferation potential of EPSCs.[67]

Alpha and beta defensins are peptides produced by the immune system during wound healing and repair. They stimulate the usually dormant LGR6+ stem cells found in the hair follicle to form EPSCs resulting in new keratinocytes. Defensins have been found to rejuvenate skin when used topically.[74,75]

Retinol plays an important role in regulating EPSCs by maintaining self-renewal and preventing differentiation of pluripotent stem cells.[6,76] Over 500 genes are influenced by retinoic acid and many of them affect EPSCs. While retinol prevents differentiation of EPSCs, retinoic acid promotes EPSC differentiation through the expression of mRNA and microRNA, as well as by increasing DNA methylation.[77]

Cellular Senescence

Cellular senescence occurs when stem cells undergo irreversible cell cycle arrest and lose the ability to divide. Cellular senescence is the way the body naturally rids itself of dysfunctional or damaged cells. Cancer occurs when cells lose the ability to become senescent and continue dividing and reproducing even when damaged. The presence of senescent cells in the skin leads to cutaneous aging and the presence of senescent cells is characteristic of aging skin.[78] Senescent cells secrete substances that affect the microenvironment around them. They take up space, make the skin stiffer, interfere with cell-to-cell communication, damage surrounding cells, and affect the functions of EPSCs.[79] Aging, reactive oxygen species, and inflammation contribute to an increase in senescent cells.[80]

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CIRCADIAN RHYTHM IN THE EPIDERMIS

Keratinocytes have a circadian clock governed by a clock gene,[65] and evince endogenous rhythmicity.[81,82] Keratinocytes can directly sense light, which affects the circadian rhythm. Blue light specifically has been shown to directly influence keratinocyte clock gene expression when shined on cells at night.[83]

Studies have demonstrated that undifferentiated keratinocytes respond to differentiation cues between late night and early morning. Keratinocytes proliferate in daylight hours at the same time they turn on genes to help protect their DNA from UV light.[65] The repair of UV damaged cells occurs at night. In general, these rhythms allow the skin to focus on protection in the daytime and repair at night. Many circadian rhythm studies of the skin show that DNA repair occurs best at night and requires adequate sleep. Keratinocyte clock oscillations are affected by the stiffness of the surrounding ECM, the presence of inflammation, and aging.[84–86]

Circadian rhythm affects TEWL, which is higher in the evening than in the morning, and blood flow to the skin, which is highest in the late afternoon and night.[87] Skin penetration of topical products has been shown to be the highest at night with the peak at 4:00 AM.[88]

Circadian rhythms should be considered when applying skincare products. Sun protection and antioxidants are recommended in the morning to protect the skin. Apply products that do not penetrate well in the evening because TEWL is higher. Follow with a barrier repair moisturizer. DNA repair enzymes should be applied at night.

Light and the Epidermis

When light hits keratinocytes, gene expression changes depending on the type of light.[9] Blue light treatments are used for acne because the light has been shown to kill *Cutibacterium acnes.* In addition, blue light is emitted from phone screens and computer screens. Blue light up to 453 nm reduces proliferation of keratinocytes in vitro by inducing differentiation.[89] Aquaporins are water channels that play an important role in skin hydration, movement of fluids between cells, and proliferation and differentiation of keratinocytes. Blue light causes lipofuscin in aged skin cells to generate aging free radicals as discussed earlier in relation to lysosomes. Red light is often used to treat skin inflammation and its use attenuates inflammatory factors.[90]

Studies have shown that keratinocytes and fibroblasts are affected by light differently.[91,92] Fibroblasts seem to be more sensitive than keratinocytes to light, especially UV, infrared, and visible light, which may be due to a higher antioxidant capacity in keratinocytes as compared to fibroblasts.[93,94] Keratinocytes also contain higher levels of ferritin than fibroblasts, allowing them to chelate iron, reducing the levels of damaging free radicals.[95] Chapter 26, Lasers and Lights, will include a more in-depth discussion on the effects of light on the skin.

GROWTH FACTORS

Growth factors can be classified into two groups: proliferative and differentiative. Proliferative factors increase DNA synthesis and result in proliferation of cells. Differentiative factors inhibit the production of DNA and suppress growth, thereby resulting in differentiation of the keratinocytes.

Epidermal growth factor (EGF) is one of the integral chemokines in the regulation of growth in human cells. It binds to the epidermal growth factor receptor (EGFR) located on the basal and suprabasal cells in the epidermis and activates tyrosine kinase activity, which ultimately results in proliferation of the cells.[96] Keratinocyte growth factor (KGF), a member of the fibroblast growth factor family, also exerts a proliferative effect via the tyrosine kinase receptor on epidermal cells.[97] It has been shown that KGF contributes to and enhances wound healing.[98] In addition, KGF has been demonstrated to promote hyaluronan synthesis in keratinocytes.[99]

Other important growth factors include the polypeptide transforming growth factors, which consist of two types: transforming growth factor alpha (TGF-α) and transforming growth factor beta (TGF-β). They differ in both configuration and function. TGF-α is a proliferative factor, similar to EGF, and works by stimulating a tyrosine kinase response. TGF-β is a cytokine that inhibits growth of epidermal keratinocytes and stimulates growth of dermal fibroblasts. TGF-β promotes differentiation and plays an important role in controlling production of the ECM.[100] TGF-β is critical for regulating collagen synthesis. In fact, the primary cause of the decrease in Type 1 procollagen after UV exposure is inhibition of a TGF-β pathway.[101] TGF-β has also been proven to contribute to scarring, and antibodies to this factor have been shown to decrease the inflammatory response in wounds and reduce scarring.[102,103] TGF-β levels are increased by calcium, phorbol esters, as well as TGF-β itself.

Insulin-like growth factor (IGF) has been shown to yield a photoprotective effect on skin. IGF signaling regulates DNA repair.[104,105] In fact, exogenous IGF added to irradiated keratinocyte cultures has been demonstrated to rescue the irradiated cells, increase keratinocyte survival, and reduce photodamage.[106]

Growth Factors in Cosmeceuticals

Various growth factors are found in cosmeceuticals although there is a paucity of research on which growth factors are best to treat the skin.[107] Skincare products containing growth factors isolated from cells cultured in conditioned media have been on the market for decades. The efficacy and safety of these growth factors is still poorly understood; however, there have not been any proven cases of skin cancer arising from their use. The concern is that these growth factors could cause undesirable skin cells to flourish. For example, TGF-β, known as a tumor growth factor, is present in the conditioned media used in some skincare products.[108,109] Multiple cancer research studies show that TGF-β is a potent trigger of cancer-related pathways,[110,111] and prepares a favorable microenvironment for cancer cells.[112,113] These issues will be examined more completely in Chapter 37, Anti-Aging Ingredients.

ANTIMICROBIAL PEPTIDES

Antimicrobial peptides (AMPs) have recently become an area of interest because of their involvement in the innate immune

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system of human skin. AMPs exhibit broad-spectrum activity against bacteria, viruses, and fungi.[114,115] The cationic peptide of the AMPs attracts the negatively charged bacteria, becoming pervasive in the bacterial membrane in the process, and ultimately eliminates the bacteria. Cathelicidin and defensin are the two major groups of AMPs believed to have an influence in the antimicrobial defense of the skin. Cathelicidin has been identified in the keratinocytes of human skin at the area of inflammation, as well as in eccrine and salivary glands.[116–118] In addition to antimicrobial activity, cathelicidin LL-37 demonstrates a stimulatory effect on keratinocyte proliferation in the process of wound healing.[119] Pig cathelicidin PR-39 has been shown to induce proteoglycan production (specifically, syndecan-1 and -4) in the ECM in wound repair.[120] Defensin is also expressed in the human keratinocytes[121] and mucous membranes.[122,123] β-Defensin 1 seems to promote differentiation in the keratinocytes by increasing expression of keratin 10.[124] Interestingly, UVB radiation has been shown to increase the levels of human β-defensin mRNA in the keratinocytes.[125]

AMPs have been demonstrated to be involved in several dermatologic conditions including atopic dermatitis, psoriasis, and leprosy,[115] as well as wound healing, all of which are beyond the scope of our discussion. The role of AMPs in the epidermal barrier will be discussed in Chapter 12.

MOISTURIZATION OF THE STRATUM CORNEUM

The main function of the SC is to prevent TEWL and regulate the water balance in the skin. The two major components that allow the SC to perform this role are lipids and the NMF.

Natural Moisturizing Factor

Released by the lamellar granules, NMF is composed of amino acids and their metabolites, which are byproducts formed from the breakdown of filaggrin (**Box 1-2**). NMF is found exclusively inside the cells of the SC and gives the SC its humectant (water-binding) qualities (**Fig. 1-10**).

NMF is composed of highly water-soluble chemicals; therefore, it can absorb large amounts of water, even when humidity levels are low. This allows the SC to retain substantial water content even in a dry environment. The NMF also provides an important aqueous environment for enzymes that require such conditions to function. The importance of NMF is clear when one notes that ichthyosis vulgaris patients, who have been shown to lack NMF, manifest severe dryness, and scaling of the skin.[126] It has been demonstrated that normal skin exposed to normal soap washing has significantly lower levels of NMF when compared to normal skin not washed with surfactants.[127] NMF levels have also been reported to decline with age, which may contribute to the increased incidence of dry skin in the elderly population (see Chapter 12).

BOX 1-2

Filaggrin, named for *filament aggr*egating prote*in*, derived its name from the fact that it binds keratin filaments to form a structural matrix in the SC. Genetic defects in the filaggrin gene are known to play a role in a subset of ichthyosis vulgaris cases.[38] Interestingly, filaggrin is not present in the superficial layers of the SC. Studies have shown that it is completely degraded into amino acids within 2 to 3 days of profilaggrin formation and its constituents are further metabolized to form the NMF.[40] This is nature's way of keeping its water-binding capabilities in the top layer of the SC where they are needed while preventing the lower layers of the SC from being disrupted by having too much water present. In addition, the level of NMF is regulated by the water activity present in the SC.

Lipids

In order of abundance, the composition of skin surface lipids includes triglycerides, fatty acids, squalene, wax esters, diglycerides, cholesterol esters, and cholesterol.[128] These lipids are an integral part of the epidermis and are involved in preventing TEWL and the entry of harmful bacteria. They also help prevent the skin from absorbing water-soluble agents. For decades it has been known that the absence of lipids in the diet leads to unhealthy skin (see Chapter 12). More recently, it has been shown that inherited defects in lipid metabolism, such as the deficiency of steroid sulfatase seen in X-linked ichthyosis, will lead to abnormal skin keratinization and hydration.[129] It is now known that SC lipids are affected by age, genetics, seasonal variation, and diet. Deficiency of these lipids predisposes the individual to dry skin. This has been demonstrated in mice with essential fatty acid deficiency (EFAD): when fed a diet deficient in linoleic acid these mice developed increased TEWL.[130] Interestingly, the administration of hypocholesterolemic drugs has also been associated with dry skin changes.[131]

Skin lipids are produced in and extruded from the lamellar granules as described earlier or are synthesized in the sebaceous glands and then excreted to the skin's surface through the hair follicle. The excretion of sebum by sebaceous glands is hormonally controlled (see Chapter 11, Oily Skin). Lipids help keep the NMF inside the cells where it is needed to maintain hydration and aqueous enzyme functioning. Although this is less well characterized, lipids can themselves influence enzyme function.

Role of lipids in TEWL

The major lipids found in the SC that contribute to the water permeability barrier are ceramides, cholesterol, and fatty acids.

Since the 1940s, when the SC was first identified as the primary barrier to water loss, many hypotheses have been developed as to exactly which lipids are important in the SC. The research with the EFAD mice described earlier led to a focus on phospholipids because they contain linoleic acid. However, it was later found that phospholipids are almost completely absent from the SC.[132] In 1982, ceramide 1 was discovered. This linoleic acid-rich compound is believed to play a major role in structuring SC lipids essential for barrier function.[133] Later, five more distinct types of ceramides were discovered and

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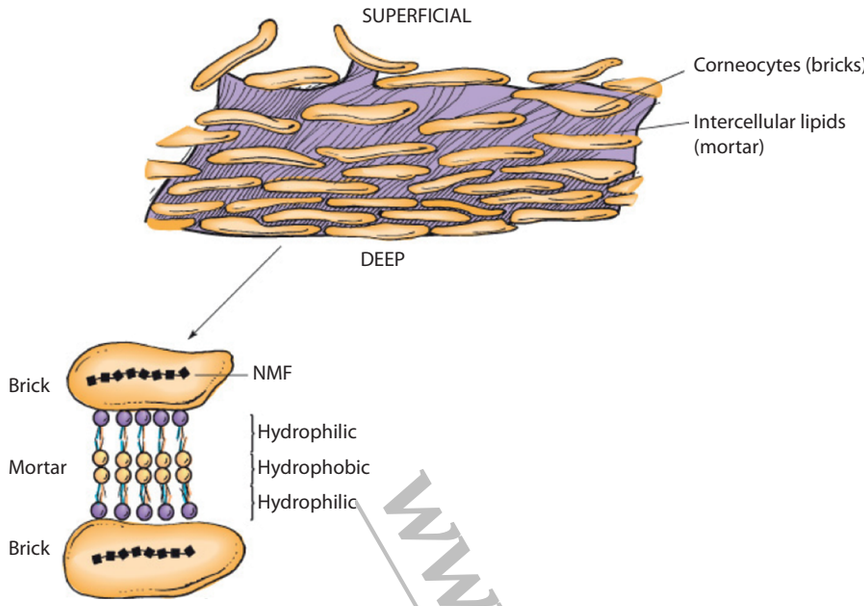

FIGURE 1-10. The keratinocytes are embedded in a lipid matrix that resembles bricks and mortar. Natural moisturizing factor (NMF) is present within the keratinocytes. NMF and the lipid bilayer prevent dehydration of the epidermis.

named according to the polarity of the molecul. Ceramide 1 is the most nonpolar and ceramide 6 is the most p.lar.

Although the ceramides were once thought to be th. key to skin moisturization, studies now suggest that no .rtic-ular lipid is more important than the others. It appea. that the proportion of fatty acids, ceramides, and choleste.ol is the most significant parameter. This was demonstrated in a study in which after altering the water barrier with acetone the application of a combination of ceramides, fatty acids, and cholesterol resulted in normal barrier recovery.[134] Application of each of the separate entities alone resulted in delayed barrier recovery. Manufacturers now include ceramides or a mixture of ceramides, cholesterol, and fatty acids in several available products based on these findings. However, the use of these mixtures to treat atopic dermatitis and other ichthyotic disorders has been disappointing.

CONCLUSION

The epidermis is implicated in many of the skin complaints of cosmetic patients. It is the state of the epidermis that causes the skin to feel rough and appear dull. A flexible, well-hydrated epidermis is more supple and radiant than a dehydrated epidermis. The popularity of buff puffs, exfoliating scrubs, masks, moisturizers, chemical peels, and microdermabrasion attests to the obsession that cosmetic patients have with the condition of their epidermis. It is important to understand the properties of the epidermis in order to understand which cosmetic products and procedures can truly benefit patients as opposed to those that are based on myths or hype.

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