The stratum corneum: a double paradox

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Summary

The stratum corneum (SC) (i.e., the outermost layer of human skin) is a complex and paradoxical tissue composed of corneocytes and a matrix of intercellular lipids playing an essential role as the skin's protective barrier. The first paradox of SC is its dual nature. It is composed of nondividing (dead) cells embedded in a metabolically active (live) environment whose function is to protect the epidermis and to maintain its integrity. In order to do so, the SC uses various strategies, including enzymatic reactions, colonization by bacterial flora, immune signaling, antimicrobial lipids and peptides, low pH, antioxidants, and natural moisturizing factor(s). The second paradox is that although its biological function is essentially that of a physicochemical barrier, cosmetologists and pharmacists are actively exploring paths for penetration through the SC to allow passage of active molecules and their penetration into the skin. Various pathways of penetration and physicochemical factors facilitating this penetration into the dermis and/or the epidermis have been defined, but the exact mechanisms of penetration of cosmetic ingredients remain elusive. For cosmetologists and pharmacists, the SC represents a major focus of interest whether for basic research or the development of novel topical approaches taking into account the fascinating properties of this complex tissue.

Keywords: antioxidants, intercellular lipids, natural moisturizing factors, skin penetration, skin protection, stratum corneum

Introduction

The stratum corneum (SC) is a target of interest for both cosmetologists and pharmacists, aiming at optimizing the delivery and efficacy of topical products in the skin. However, the question as to whether effective topical products may exert their effects via their sole action on SC, or whether they need to penetrate more deeply, remains an open question. In order to address this issue, we need to integrate current knowledge on the components of SC, clarify its functions as a live tissue composed of dead cells, and characterize its role as a barrier as well as a pathway for penetration.

The objectives of this review are, first, to present the state-of-the-art knowledge on the structure and functions of SC and, second, to highlight the ambiguity between protection by, and penetration through, the SC. The formulation of future topical products requires the understanding of the complex nature of the SC.

What is the SC?

The SC is the outermost layer of the skin. In most parts of the body, it is 10 to 20 μm thick, although this may vary from a few micrometers to millimeters. It contains 15% water[1] (whereas the whole body contains a mean of 65% and the heart 76% water) and consists of two distinct

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structural components: the corneocytes and the intercellular lipids. Elias proposed the "brick and mortar model," according to which the SC is composed of flat cells (bricks) surrounded by a lipid matrix (mortar).[2]

Corneocytes – the bricks

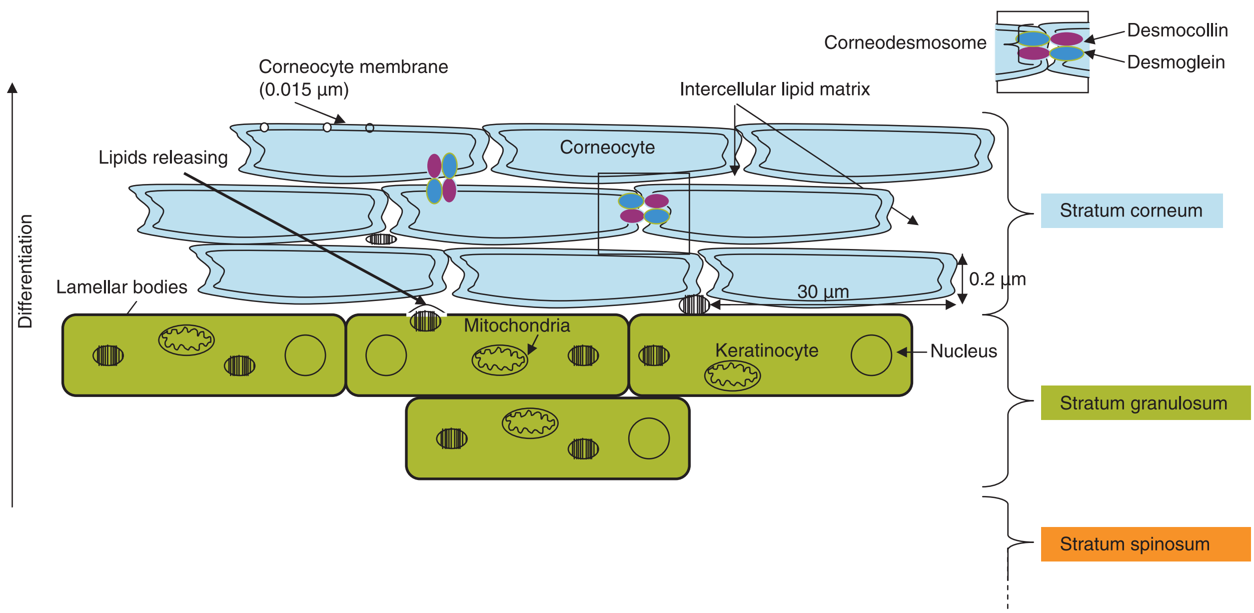
The corneocytes are keratinocyte-derived anucleated cells providing structural support for the SC. They lack key intracellular organelles such as nuclei or mitochondria that are degraded during the final differentiation of keratinocytes. The corneocytes are 0.2 to 0.3 μm thick, and their diameter is 30 to 50 μm; their membrane, 0.015 to 0.020 μm thick, consists of structural proteins such as involucrin and keratolinin. A 0.05-μm-thick structure of specialized lipids (free fatty acids, ceramides, cholesterol) is in immediate contact of the membrane and the hydrophobic lipid lamellae[3] (intercellular lipid matrix). Corneodesmosomes interconnect corneocytes as desmosomes interconnect keratinocytes in the epidermis; essential corneodesmosomal proteins include desmocollin and desmoglein (Fig. 1).

The corneocytes act as a barrier against aggression. Their tight layering provides them with the ability to retain water while their membrane is permeable to water but impermeable to proteins. The corneodesmosomal proteins contribute to the desquamation process through their trypsine- and chymotrypsin-like serine protease activities.[4]

Intercellular lipid matrix – the mortar

The intercellular lipid matrix, located between corneocytes, constitutes about 10% to 15% of the dry weight of SC. This continuous phase of the skin barrier is organized into trilaminar units. The lipid species of the SC are composed of 50% ceramides, 10% fatty acids, 25% cholesterol, and 15% cholesterol and glucosylceramide derivatives (Fig. 1). The majority of lipids of the SC is synthesized by the keratinocytes in the upper layer of stratum spinosum and in stratum granulosum. At the stratum spinosum–stratum granulosum junction, fatty acids, sphingolipids, and other lipid precursors are extruded to reside in the intercellular lipid matrix.[5]

There are two distinct states of the intercellular lipid matrix: the nonpermeable (gel) and the permeable (liquid crystalline) matrix, the latter being permeable to water and electrons and thus the major route for skin penetration. In contrast, the covalent bonds between the ceramides of the intercellular lipid matrix and the structural proteins



The stratum corneum composed keratinocytes differenciated in corneocytes, cells which lost nuclei and mitochondria. The corneocytes connected together by corneodesmosomes consisting of desmocollin and desmoglein. Between the corneocytes there is an intercellular lipid matrix. The majority of lipids is synthesized by keratinocytes in the stratum granulosum and packaged in lamellar bodies. These organelles deliver their lipid content in the extracellular space by fusion with the plasma membrane of keratinocytes of the stratum granulosum.

Figure 1 Structure of the stratum corneum and the stratum granulosum.

of corneocytes membrane create, through esters bonds, a dense and impermeable network.[6]

The first paradox: dead cells, live tissue

The SC is a paradoxical tissue: composed of nondividing (dead) cells, it remains a metabolically active (live) tissue, able to protect itself, to communicate, and to exchange. The SC is constantly regenerated by keratinocytes' final differentiation and acts as a protective barrier thanks to key enzymatic reactions, maintains a balanced bacterial flora, releases immune signaling compounds, and preserves an acidic pH.

Enzymes

Key enzymatic reactions are taking place at the interface of the SC–stratum granulosum. A great diversity of enzymes is present in the extracellular compartment of the SC and near hair follicles, including desaturases, hydrolases, and esterases. These enzymes are responsible for the generation of intercellular lipids, cellular desquamation, and secretion of sebum and are necessary for the differentiation of the keratinocytes into corneocytes.

Enzymes play important roles in structuring the lipids of the intercellular barrier. The desaturases are secreted in the sebum and convert linoleic and linolenic acids into polyunsaturated fatty acids with long chains leading to the generation of ceramides. The hydrolases, similar to lysosomal hydrolases, are essential for lipid remodelling. Furthermore, carbohydrates in the SC activate β-D-glucocerebrosidase, which catalyzes the removal of glucose from glucosylceramide to initiate the organization of the lipid barrier in the deep SC.[7] The esterases, present in the intercellular spaces, and the chymotryptic enzymes (proteases) are necessary for the desquamation, a protective mechanism contributing to the elimination of both microorganisms and cells. In turn, protease inhibitors, pH, and the ratio of cholesterol sulfate/cholesterol control the activity of these enzymes in the SC.[8]

Bacterial flora

The resident bacterial flora in the skin constitutes a complex ecosystem. These nonpathogenic microflora (predominantly *Staphylococcus* and *Micrococcus* strains and *Diphtheroid bacilli*) use the sebum as nutrient[9] and play important roles in skin defense against potentially pathogenic organisms.[10,11] Various protective mechanisms contribute to ensure the survival of nonpathogenic microorganisms and to limit skin colonization by pathogenic ones.

Immune signaling factors

The SC is a biosensor that regulates the responses of the epidermis, and a major signal sensed by SC is the level of cytokines and growth factors, which are key to the inflammatory reaction.[12] In response to cutaneous agressions, interleukin-1alpha (IL-1α) is released from SC and keratinocytes as the first step of the inflammatory cascade. IL-1α stimulates keratinocytes and fibroblasts to produce and release more IL-1α and other proinflammatory cytokines including IL-1β, IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α). This cytokine cascade leads to vasodilatation in the dermis and subsequent inflammatory cell infiltration in the epidermis and the SC. Whereas keratinocytes are essential in the initiation of inflammation, they also have the ability to limit the inflammatory processes: they produce anti-inflammatory cytokines, in particular, the IL-1 receptor antagonist that blocks the activity IL-1 by competitive binding with IL-1 receptors.[12]

pH

In the SC, pH ranges between 5 and 6. This acidic pH is crucial to the cutaneous barrier, to the integrity and cohesion of the SC, and to enzymatic activities. Endogenous and/or exogenous (originating outside the epidermis) variables cooperate to maintain the acidic pH of the SC. Endogenous mechanisms include secretion of sebum, bacterial flora, cellular degradation and metabolism, and free fatty acids generated from the hydrolysis of phospholipids that is catalyzed by phospholipase A2.[13] Exogenous factors stimulate the generation of free fatty acids by bacterial lipases, free fatty acids derived from sebum,[14] the production of microbial metabolites, and of eccrine gland secretion such as lactic acid.[15]

The SC as the ultimate protection/barrier

The SC constitutes only 10% of the entire skin but contributes to over 80% of the cutaneous barrier function. The SC functions as barrier, prevents the loss of water from the epidermis, and provides protection from the outside environment through its antioxidants.

Natural moisturizing factors

The SC is impermeable except for a small quantity of water that is delivered by epidermis into the SC, in order to hydrate its external layers, to maintain its flexibility, and to allow adequate enzymatic reactions as well as corneodesmolysis and desquamation.

The normal water flux through the SC is known as transepidermal water loss, the rate of which is influenced by the concentration of water in the epidermis, by cell integrity, the relative humidity, the diffusivity of water in the SC, and the thickness of SC. The capacity to maintain water in the skin depends on the arrangement of corneocytes, the composition and structure of intercellular lipids, and the presence of natural moisturizing factors (NMFs). In the epidermis, NMFs play an important role in the reduction of intermolecular forces between nonhelical regions of keratin and the water molecules. The specific interaction, by their glycine molecules, between NMFs and keratin fibers is key to the acquisition of the mobility of keratin fibers, which allows an increased elasticity of the SC.[16] NMFs are amino acids as serine and citrullin; organic acids as lactate; and inorganic ions as potassium, magnesium, and calcium. These NMFs influence the physical properties of SC through yet undefined mechanisms. Potassium and lactate produced in the keratinocytes migrate directly to the SC during differentiation.[17]

Antioxidants

The SC, due to its critical location at the interface between the body and the environment, is continuously exposed to oxidants, including ultraviolet (UV) light, chemical oxidants, air pollutants, and microorganisms. α-Tocopherol is the major hydrophobic antioxidant of SC, protecting against lipid peroxidation and allowing the stabilization of lipid bilayers.[18]

The SC, similar to other living tissues of the human body, contains a number of endogenous antioxidant enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase,[19] the former two being the major antioxidants of SC.[20]

The second paradox: protection vs. penetration

Although one key role of SC is to prevent the molecules coming from outside to penetrate the skin, cosmetologists and pharmacologists are highly interested in the ways in which it could support or enhance such penetration. This is the second, not necessarily the last, of the paradoxes surrounding the SC.

The SC supports the absorption of liposoluble compounds. Furthermore, nonionized molecules penetrate more easily than ionized ones, and cationic molecules present a more efficient adhesion with the negatively charged SC. The intercellular lipids are divided into two areas: a permeable area (crystalline liquid) and a nonpermeable area (gel). The broadest gaps between the intercellular lipids measure 0.013 μm. Ninety percent of the molecules that penetrate the skin infiltrate it, thanks to the intercellular lipids, which have a lamellar organization that promotes the penetration of lipophilic molecules.

It is not certain whether all xenobiotics take the same route through the SC. There are various ways for the molecules to penetrate the SC: (i) intercellular, (ii) transcellular, (iii) intrafollicular, and/or (iv) polar.

(i) The intercellular way allows a sinuous penetration of molecules between the corneocytes through the intercellular lipids. These lipids account for 1% to 5% of the total volume of SC, but are the major way of penetration for most hydrophilic and lipophilic molecules.

(ii) The transcellular way allows penetration through the keratinized corneocytes, the way least used by xenobiotics.

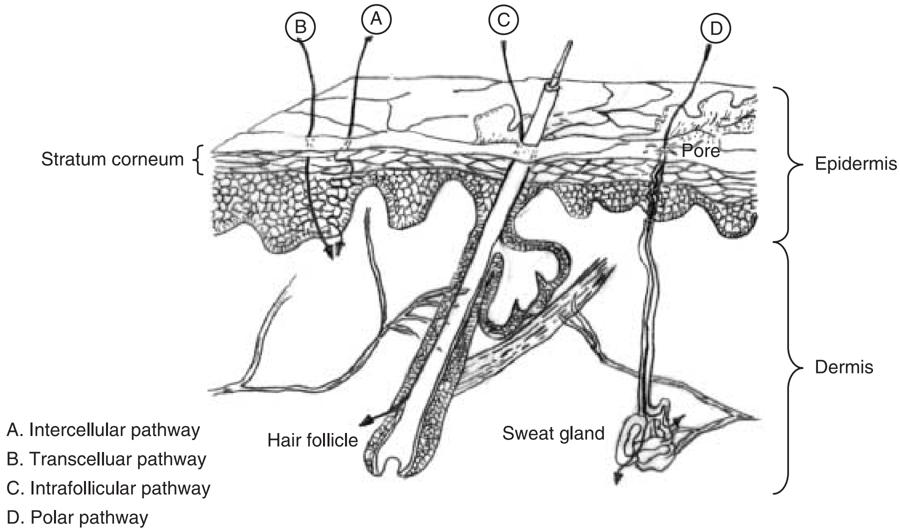
(iii) The intrafollicular way allows penetration through hair follicles (which account for 1–2% of the total skin surface), in combination with various surfactants and glycols.

(iv) The polar way, occurring through the polar pores, is hydrophilic by nature. These polar pores are present between cells and are surrounded by polar lipids, which generate discontinuities in the organization of the lipids (Fig. 2).

The physicochemical factors that influence the rate of penetration are molecular mass (a molecule of 5 kDa is the maximum mass that allows penetration in the SC), concentration, solubility, partition coefficient (lipophilic–hydrophilic coefficient), pH variations, co-solvents (used in a combination to increase the mobility of molecules), temperature (molecules penetrate better at high temperature), and penetration enhancers. The biological factors that influence the rate of penetration are age, condition, metabolism, hydration of the skin, and compound–skin interaction. The variations in the skin's structure also modulate the penetration of molecules in the skin (Table 1).

Table 1 Physical, chemical, and biological factors modulating the rate of penetration.

Physical and chemical factors	Biological factors
Molecular mass	Age
Concentration	Condition of the skin
Solubility	Metabolism of the skin
Partition coefficient	Hydration of the skin
Variations of pH	Compound–skin interactions
Co-solvents	
Temperature	
Penetration enhancers	



There are various ways for the molecules to penetrate the stratum corneum: intercellular (penetration between the corneocytes through the intercellular lipids), transcellular (penetration through the corneocytes), intrafollicular (penetration through hair follicles) and polar (penetration through the polar pores).

Figure 2 Various pathways of penetration.

The delivery of any active compound thus depends upon five major factors: structure, intrinsic activity, concentration, metabolism, and rate of clearance.

The SC: target for cosmetic ingredients

Our understanding of whether and how the ingredients of cosmetic products penetrate in and below the SC and the type of interactions between the ingredients applied and the SC are still fragmentary. Nonetheless, the SC is a prime target for cosmetologists for investigating the unique aspects of cosmetics products penetration and efficacy.

The Federal Food, Drug and Cosmetic Act defines cosmetics as articles intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance but without affecting the body's structure or functions and with no drug claims. This is a further "legislative" paradox of cosmetic products: they are intended to alter appearance, but should remain on the surface of the skin, not reach the skin's deeper layers and not be absorbed by capillaries. In contrast, products that penetrate the dermal layer are classified as pharmaceuticals and are regulated by the Food and Drug Administration. Advances in science and technology have given rise to a new class of products called "cosmeceuticals," bridging the gap between traditional cosmetics and pharmaceutical products. The Federal Food, Drug and Cosmetic Act does not recognize the term "cosmeceuticals": a product can be a drug, a cosmetic, or a combination of both, but the term "cosmeceutical" has no meaning under the law. Nevertheless, cosmeceuticals are formulated so as to penetrate the epidermis to perform definite functions, such as facilitating water retention and neutralizing free radical reactions, reducing the effects of aging, suppressing bacteria involved in acne, and calming inflammatory responses.

Numerous *in vitro* studies have been conducted to show the penetration of cosmetic ingredients and their effects on the skin cells and at the level of cellular metabolism. It is, however, not clear yet whether there is a real *in vivo* penetration of the compounds from cosmetic formulations.

An interesting, often forgotten, perspective is to suggest that the lack of penetration of the products' active ingredients would not be a negative characteristic. Indeed, these ingredients still would possess the action to protect the properties of the SC against environmental damages as we saw previously.

As mentioned above, the SC requires the contribution of antioxidants to protect itself from the environment. So many cosmetic products advocate the use of antioxidants such as vitamin E to protect the skin. α-Tocopherol is located in the lower part of the SC; that is why the use of topical products can rebalance within the SC α-tocopherol content. Other exogenous antioxidants are as important for the SC: phytoantioxidants. Plants have developed effective antioxidant strategies to protect themselves from UV because unfortunately they cannot move in the shade! These phytoantioxidants neutralize various oxidant formed by UV in the SC and also protect the SC from its toxic environment.

Conclusion

The SC, paradoxical tissue, is composed of dead cells but remains a living tissue and plays a role as a protective barrier towards exogenous agents. Different pathways for xenobiotics to penetrate through the SC have been defined. However, the penetration of cosmetic products through the outermost layer of the skin remains an enigma. If cosmetic products' ingredients do not penetrate the epidermis or dermis, this would not indicate an ineffective product, as they still serve to protect the SC and enable it to achieve its functions as the skin's very first and essential barrier. The use of antioxidant molecules in cosmetic products can help the SC to regenerate and protect itself and, thus, the underlying epidermis and dermis from the harmful effects of UV and other environmental toxins.

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References

1 Marks R. The stratum corneum barrier: the final frontier. *J Nutr* 2004; **134**: 2017S–21S.
2 Elias PM. Epidermal lipids, barrier function, and desquamation. *J Invest Dermatol* 1983; **80**: 44S–9S.
3 Swartzendruber DC, Wertz PW, Madison KC, Downing DT. Evidence that the corneocyte has a chemically bound lipid envelope. *J Invest Dermatol* 1987; **88**: 709–13.
4 Komatsu N, Saijoh K, Sidiropoulos M *et al*. Quantification of human tissue kallikreins in the stratum corneum: dependence on age and gender. *J Invest Dermatol* 2005; **125**: 1182–9.
5 Lee SH, Jeong SK, Ahn SK. An update of the defensive barrier function of skin. *Yonsei Med J* 2006; **47**: 293–306.
6 Kalinin AE, Kajava AV, Steinert PM. Epithelial barrier function: assembly and structural features of the cornified cell envelope. *Bioessays* 2002; **24**: 789–800.
7 Rawlings AV, Matts PJ. Stratum corneum moisturization at the molecular level: an update in relation to the dry skin cycle. *J Invest Dermatol* 2005; **124**: 1099–110.
8 Elias PM, Crumrine D, Rassner U *et al*. Basis for abnormal desquamation and permeability barrier dysfunction in RXLI. *J Invest Dermatol* 2004; **122**: 314–9.
9 Katsuyama M, Ichikawa H, Ogawa S, Ikezawa Z. A novel method to control the balance of skin microflora. Part 1. Attack on biofilm of *Staphylococcus aureus* without antibiotics. *J Dermatol Sci* 2005; **38**: 197–205.
10 Roth RR, James WD. Microbial ecology of the skin. *Ann Rev Microbiol* 1988; **42**: 441–64.
11 Selwyn S, Ellis H. Skin bacteria and skin disinfection reconsidered. *Br Med J* 1972; **1**: 136–40.
12 Elias PM, Wood LC, Feingold KR. Epidermal pathogenesis of inflammatory dermatoses. *Am J Contact Dermat* 1999; **10**: 119–26.
13 De Jongh CM, Verberk MM, Withagen CET, Jacobs JJL, Rustemeyer T, Kezic S. Stratum corneum cytokines and skin irritation response to sodium lauryl sulfate. *Contact Dermatitis* 2006; **54**: 325–33.
14 Fluhr JW, Behne MJ, Brown BE *et al*. Stratum corneum acidification in neonatal skin: secretory phospholipase A2 and the sodium/hydrogen antiporter-1 acidify neonatal rat stratum corneum. *J Invest Dermatol* 2004; **122**: 320–9.
15 Bibel DJ, Miller SJ, Brown BE *et al*. Antimicrobial activity of stratum corneum lipids from normal and essential fatty acid-deficient mice. *J Invest Dermatol* 1989; **92**: 632–8.
16 Jokura Y, Ishikawa S, Tokuda H, Imokawa G. Molecular analysis of elastic properties of the stratum corneum by solid-state ^{13}C-nuclear magnetic resonance spectroscopy. *J Invest Dermatol* 1995; **104**: 806–12.
17 Nakagawa N, Sakai S, Matsumoto M *et al*. Relationship between NMF (lactate and potassium) content and the physical properties of the stratum corneum in healthy subjects. *J Invest Dermatol* 2004; **122**: 755–63.
18 Thiele JJ. Oxidative targets in the stratum corneum. A new basis for antioxidative strategies. *Skin Pharmacol Appl Skin Physiol* 2001; **14**: 87–91.
19 Thiele JJ, Schroeter C, Hsieh SN, Podda M, Packer L. The antioxidant network of the stratum corneum. *Curr Probl Dermatol* 2001; **29**: 26–42.
20 Hellemans L, Cortsjens H, Neven A, Declercq L, Maes D. Antioxidant enzyme activity in human stratum corneum shows seasonal variation with an age-dependent recovery. *J Invest Dermatol* 2003; **120**: 434–9.

