Stratum corneum hydration : mode of action of moisturizers on a molecular level

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Chapter 1

Skin, dry skin and moisturization

1. Function

The skin (figure 1) is the largest organ of the human body and acts as a barrier between the body and the environment. This function can be divided into three parts (Schaefer, 1996):

- defence against the penetration of chemical, biological or physical agents into the body,
- protection against water loss from the body to prevent dehydration, and
- exchange of heat between the body and its surroundings.

The defence against intrusion of biological and physical agents consist of the actual, physical barrier of the skin and the chemical defence (i.e. creation of an acidic environment by excretion of sebum). The protection against water loss and chemical agents is primarily provided by the stratum corneum (SC). This uppermost layer of the skin is very lipophilic and forms a barrier between the body and its environment, protecting it against loss of endogenous water and the penetration of (exogenous) agents.

Heat exchange between the body and its surroundings is regulated by two skin appendages, the sweat glands and the hair, as well as the vascularisation of the skin.

2. The Dermis and Epidermis

Human skin can be divided into two separate layers, the dermis and the epidermis, which are connected by a basement membrane that runs along protrusions of the dermis into the epidermis. These are called rete-ridges (Schaefer, 1996). The deepest layer, the dermis, is situated at 100 to 150 µm beneath the surface of the skin. It can be up to 3 mm in thickness and makes up the bulk of the skin. Skin appendages such as sweat glands and pilosebaceous

units are based in the subcutaneous fat layer and transverse the dermis, where they are nourished by the high degree of vascularisation. The dermis is primarily made up of connective tissue, such as collagen and elastin (Keene et al., 1997; Kielty and Shuttleworth, 1997), which give the skin its elasticity and toughness. These proteins are mostly produced by the fibroblasts, the most common cell type in the dermis, besides endothelial cells and mast cells. The mast cells play an important role in tissue regeneration and immune reactions (Sasaki, 2003).

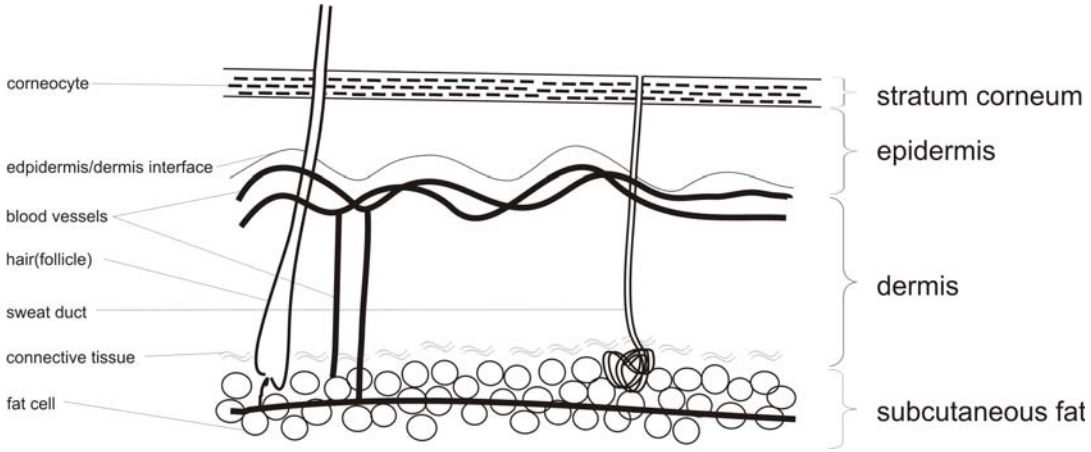

Figure 1. Schematic overview of the skin. From top to bottom four layers are shown. The deepest layer is the subcutaneous fat, consisting of fat cells. After a layer of connective tissue, the up to 1 mm thick dermis starts. This part of the skin is highly vascularized to provide nutrients to skin appendages such as sweat glands and hair follicles but also to dermal cells such as fibroblasts and the keratinocytes at the epidermis/dermis interface. The epidermis is of an undulating shape and 100 to 150 µm thick. Moving upwards in the epidermis, keratinocytes undergo differentiation into corneocytes, the cells that make up the greatest part of the uppermost layer, the 10 to 15 µm thick stratum corneum (see figure 2).

The epidermis can be divided into four layers, the basal, spinous and granular layer and finally the stratum corneum (see *3 Stratum Corneum,* figure 2). In the lowest layer of the epidermis, the basal layer (*stratum basale*), the epidermal stem cells are located (Barthel and Aberdam, 2005). All keratinocytes (the most prevalent cell type in the epidermis) are derived from these cells and form column-shaped stacks in the epidermis. In the stratum basale continuous cell division takes place. Moving upward in these stacks, the keratinocytes undergo differentiation. Above the basal layer the epidermal cells appear to have spikes on their surface. These spikes, which give this epidermal layer the name spinous layer (*stratum spinosum*) are desmosomes, which tightly connect the keratinocytes to each other.

Moving towards the surface of the skin, the layer overlaying the stratum spinosum is the granular layer (*stratum granulosum*), so called because of the large abundance of keratohyalin granules and lamellar bodies present in the keratinocytes in this layer. Keratohyalin granules

(Tezuka and Freedber, 1971) are filled with a number of proteins involved in the differentiation of keratinocytes, such as profilaggrin (Harding and Scott, 1983b), loricrin (Ishidayamamoto et al., 1993), involucrin and keratins K1 and K10. Lamellar bodies are roughly spherical in shape and contain the precursors of the SC's extracellular lipid matrix, which are glucosylceramides, sphingomyelins, phospholipids, free sterols and cholesterol (CHOL) sulphate, visible as disks within them (Fartasch et al., 1993; Hamanaka et al., 2005; Holleran et al., 1993; Madison, 2003; Menon et al., 1992; Uchida et al., 2000; Vielhaber et al., 2001).

In the upper layers of the granular layer and at its junction with the SC, terminal differentiation from keratinocyte to corneocyte takes place in the time frame of approx. one day. The cell membrane is fortified with proteins (see *The Corneocyte Envelope*) and the lipids disks into the lamellar bodies are excreted in the intercellular space. The disks form the SC's extracellular matrix (see *The Extracellular Lipid Matrix*). Nucleus and other cell organelles are degraded and the keratinocyte is left as a dry, flat, polyhedral-shaped corneocyte approx. 30 µm in diameter and 1 µm in thickness, almost entirely filled with keratin.

3. The Stratum Corneum

The SC (figure 2) has a thickness of 10 to 15 µm in normal skin and comprises approx. 15 layers of corneocytes, embedded in a highly organized lipid matrix. At the interface between viable epidermis and the SC the corneocytes undergo cornification and the lipid matrix is assembled. At the surface of the skin on average one corneocyte layer is lost every day. All these processes are regulated by different enzymes. This shows that even though corneocytes are essentially dead, the SC itself is a highly active tissue.

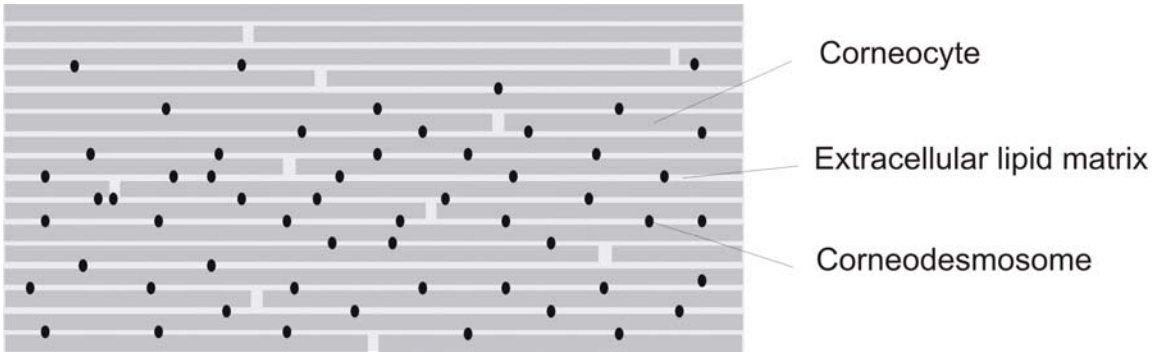

Figure 2. The stratum corneum is made up of large, flat cells called corneocytes, surrounded by a lipid matrix that comprises approx. 10% of its weight. The cells are attached to each other by modified desmosomes called corneodesmosomes, which become less abundant in the upper layers of the stratum corneum due to enzymatic degradation. This degradation facilitates desquamation, i.e. the loss of corneocytes.

The Extracellular Lipid Matrix

With its very high degree of organization, the extracellular lipid matrix provides the main barrier for water and other molecules leaving or penetrating the body. At the junction between the granular layer and the stratum corneum, the lamellar bodies contained by the corneocytes are extruded (Fartasch et al., 1993). Except for CHOL and CHOL sulphate, however, the contents of these vesicles are only the precursors of the extracellular matrix of the SC. The phospholipids are degraded into free fatty acids (FFA), while glucosylceramides and sphingomyelin are converted into ceramides (CER) (Holleran et al., 2006).

In the SC lipid matrix, the ratio's of CER, CHOL and FFA are roughly equimolar (Bouwstra et al., 1996a). Both CER and FFA are heterogeneous in nature. CER (figure 3) consist of a hydrophilic head group that can be a sphingosine (S), phytosphingosines (P) or a 6-hydroxysphingosine (H) base chemically linked to a long saturated carbon chain that can be non-hydroxylated (N) or α-hydroxylated (A) fatty acids (Ponec et al., 2003b; Robson et al., 1994; Stewart and Downing, 1999; Wertz et al., 1985). The non-hydroxy and α-hydroxy fatty acids have a chain length of mostly 24 to 26 hydrocarbon atoms, the chain length of the α-hydroxylated fatty acids can be as long as 34 carbon atoms. Three additional CER in which a linoleic acid is ester-linked to a long chain α-hydroxy fatty acid (EO) have been identified. These acylCER are EOS, EOP and EOH. The FFA vary in chain length between C14 and C28 and are mainly saturated.

The SC extracellular matrix is not only extraordinary due to its types of lipids and their heterogeneity, but also because of the high degree of organization that it displays. A three dimensional ordering with two types of organization is distinguished, the lamellar ordering parallel to the skin surface (figure 4) and the lateral packing, perpendicular to the basal plane of the lamellae (figure 5).

The lamellar ordering is characterized by multiple lipid lamellae that are stacked approx. parallel to the skin surface. Two types of lamellae with different repeat distances *d* have been found, the short periodicity phase (SPP, d = approx. 6 nm) and the long periodicity phase (LPP, d = approx. 13 nm) (Bouwstra et al., 1996a). Of these two, the LPP seems to be most important for the skin barrier function, as it is characteristic for the lipid organisation in the stratum corneum.

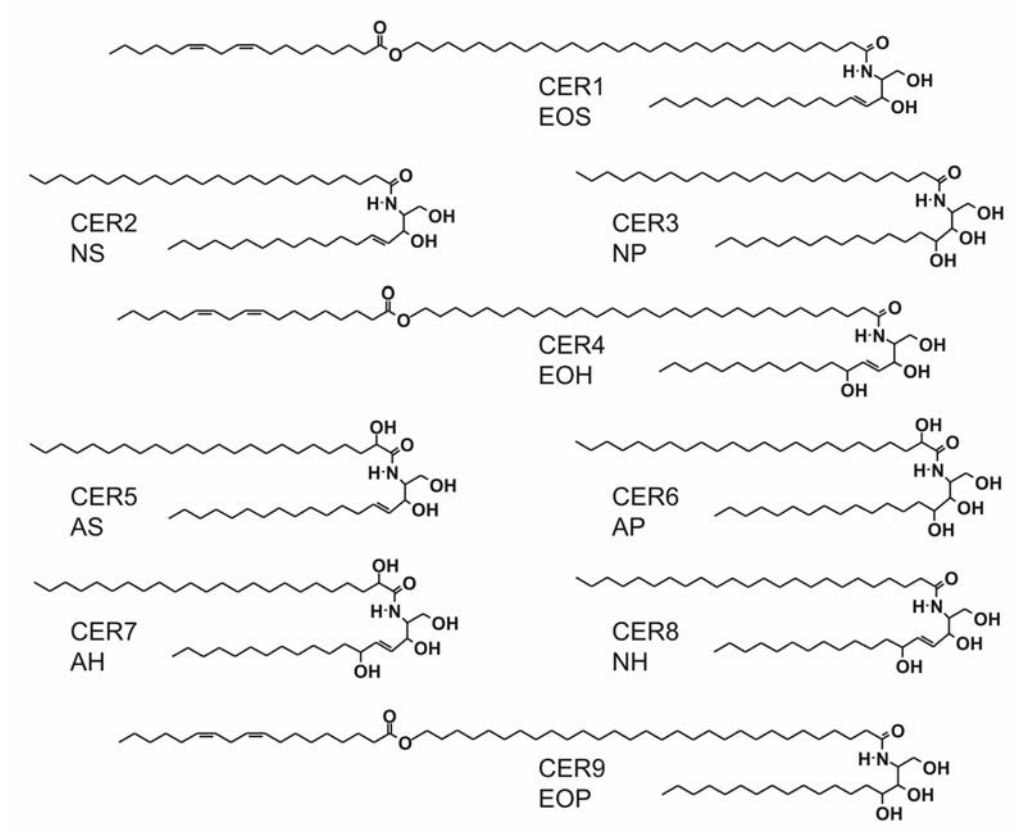

FIGURE 3. Structural formulas of human ceramides. Nine ceramides have been identified in human skin, of which three, CER EOS, EOH and EOP, are referred to as acylceramides. The numbers shown in the figure refer to the old nomenclature of ceramides, in which the compounds were numbered according to their lipophilicity, ceramide 1 being the least lipophilic.

Furthermore, it has been identified in stratum corneum of various species. Quite recently it has been reported that in the absence of the LPP the barrier function is decreased (de Jager et al., 2006). Several models have been developed to describe the lamellar ordering of the lipid lamellae, for instance the domain mosaic model (Forslind, 1994), the single gel phase model (Norlen, 2001), and Swartzendruber and Wertz's model (Swartzendruber et al., 1989), which is closely linked to the Sandwich model (Bouwstra et al., 2000b; Bouwstra et al., 2002b). The last mentioned model, the sandwich model, is a trilayer model (figure 6) in which a narrow layer with fluid domains is sandwiched by two broader, crystalline layers. The fluid domains are hypothesized to be provided by the linoleic moiety of the acylCER EOS, EOP and EOH and CHOL, while the crystalline layers are provided by CER and FFA. This structure corresponds very well with transmission electron microscopic evidence of a broad-narrow-broad segmentation of electron lucent bands in the extracellular spaces of the SC (Swartzendruber et al., 1995b), but is not in agreement with the appearance of the lipid lamellae as observed by cryo transmission electron microscopy (Al-Amoudi et al., 2005).

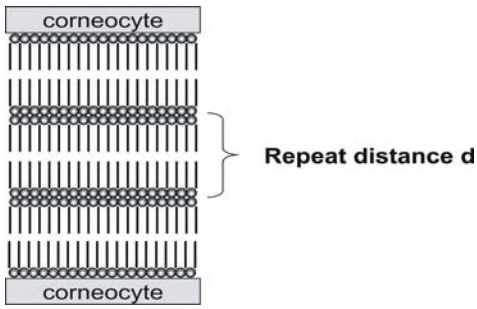

FIGURE 4. Lamellar organization of the extracellular lipid matrix in stratum corneum. In between corneocytes, the lipids are organized in bi- or trilayers, which exist in stacks of up to 12 high. The size of the bi- or trilayer is referred to as the repeat distance d.

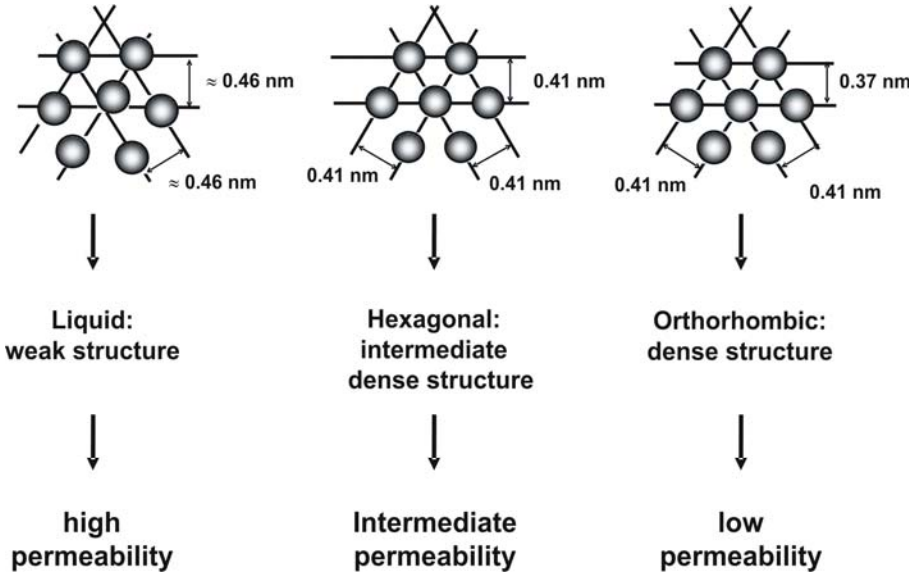

FIGURE 5. Lateral organization of the extracellular lipid matrix in stratum corneum. The lateral organization of the intercellular stratum corneum lipids is provided by the packing of the carbon tails of the lipids, here depicted as dots. The more closely the carbon tails are packed, i.e. the smaller the distance between them, the denser and therefore less permeable the lipid layer is. In normal stratum corneum, mostly the dense orthorhombic packing is present.

Additionally to the LPP, the barrier function is highly dependent on the lateral packing of the SC lipids in the lamellae. Packing density, and as a result permeability, increases in the order liquid‹hexagonal‹orthorhombic (figure 5) (Abrahamsson et al., 1978; Kitson et al., 1994; Suhonen et al., 1999b). There is evidence that all three types of lateral packing are present in SC. When focusing on human SC at physiological temperatures, the majority of the lipid carbon tails are arranged in the very dense orthorhombic lattice. At higher temperatures, this packing is converted into a less tightly packed lattice, the hexagonal lattice (Bouwstra et al., 2000a; Moore et al., 1997b; Ongpipattanakul et al., 1994).

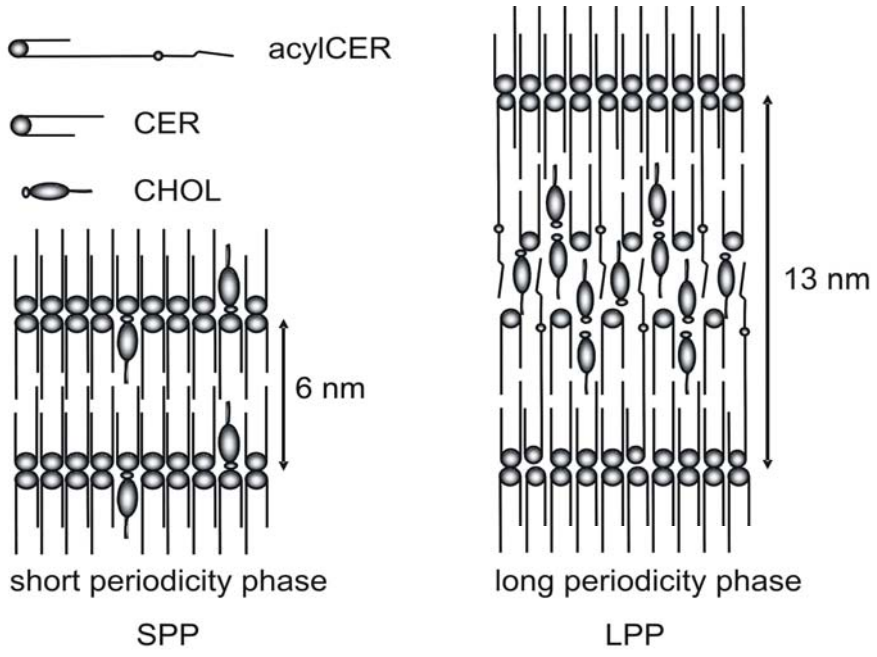

FIGURE 6. Two periodicity phases have been identified in stratum corneum, the short periodicity phase (SPP), with a repeat distance d of approx. 6 nm, and the long periodicity phase (LPP) with a repeat distance of approx. 13 nm. Of these, the LPP appears to be essential for the barrier function of stratum corneum. The LPP has been speculated combine a central fluid domain with two crystalline domains above and below it (the Sandwich Model, Bouwstra et al. *J. Lipid Res.* 1998, 2001).

The Corneocyte Envelope

The corneocyte envelope, also called cornified envelope, is a complex of several proteins surrounding the mature corneocyte, covered with covalently linked monolayer of lipids. It renders the corneocyte rigid and increases its resilience to force. The corneocyte envelope is only slightly penetrable to virtually all substances except very small hydrophilic substances such as water, making it an important part of the SC barrier (Haftek et al., 1998). Simultaneous to the cornification of the cell membrane, the cell's desmosomes are converted into corneodesmosomes (Haftek et al., 1997; Hirao, 2003; Hirao et al., 2001; Montezin et al., 1997; Serre et al., 1991). Starting in the granular layer, under the influence of calcium, envoplakin and periplakin are removed from desmosomes and linked to the keratinocyte's cell membrane. Subsequently, transglutaminases (TGase) 1 and 3 crosslink loricrin and involucrin on the keratinocyte cell membrane (Hirao, 2003; Hirao et al., 2001). Finally, lipids, mostly ω-hydroxyceramides are covalently linked to the cell envelope (Behne et al., 2000; Marekov and Steinert, 1998). Recently, it has been postulated that fatty acids are also chemically linked to this cornified layer (Lopez et al., 2007).

Desquamation

On average, one layer of corneocytes is shed per day. For this process called desquamation, the corneodesmosomes attaching the corneocytes to each other must be degraded (Serre et al., 1991). This occurs approx. halfway through the SC, as seen microscopically (Mils et al., 1992; Skerrow et al., 1989), but starts probably in much lower layers. The degradation is performed by proteases, primarily by members of the kallikrein family (Emami and Diamandis, 2007). Three important kallikreins thought to be involved in desquamation in human SC have been found, KLK5 (SC tryptic enzyme) and KLK7 (SC chymotryptic enzyme) and KLK14 (Brattsand et al., 2005; Stefansson et al., 2006). It is however suspected that several other kallikreins are also involved (Borgono et al., 2007; Komatsu et al., 2005). Additionally, the cathepsin family (Caubet et al., 2004; Egelrud, 2000) is involved. After this degradation, corneocytes are free to be lost as a result of friction or sheer stress. Interestingly, the activity of the enzymes involved in SC desquamation is dependent on SC water levels (Bouwstra et al., 2007; Watkinson et al., 2001), explaining the scaliness of dry skin.

The Natural Moisturizing Factor

For proper elasticity and plasticity of the SC and to ensure that the necessary enzymatic processes in SC can occur, the relative humidity must be between 40 and 80% (Scott and Harding, 1986; Watkinson et al., 2001). In normal, healthy skin, the water level is regulated not only by water loss inhibition by the extracellular lipid matrix, but also by the natural moisturizing factor (NMF) (Harding, 2000; Rawlings and Matts, 2005). NMF is a collection of small, hygroscopic molecules such as amino acids and their derivatives, the most important being pyrrolidone carboxylic acid, urocanic acid, urea and lactate. The first three of these compounds are produced by a water level dependent enzymatic process, the hydrolysis of the histidine-rich SC protein filaggrin (Barrett and Scott, 1983; Harding and Scott, 1983a; Scott, 1986) in the deepest layers of the SC, although lactate may also be derived from sweat (Nakagawa et al., 2004). Interestingly, the degradation of filaggrin and therefore the production of most of the NMF, is dependent on the SC water level, which in turn is partially dependent on the presence of NMF (Visscher, 2003). SC water and NMF therefore work in synergy: An optimal water level in SC promotes NMF production, while the presence of NMF ensures water retention in the SC. Likewise, a reduction of NMF, for instance due to excessive bathing, will inhibit NMF production (Rawlings and Matts, 2005), as is described below.

In the upper layers of the SC, where the corneocytes have lost most of their corneodesmosomes, NMF is lost from the corneocytes due to external influence (Visscher, 2003) and NMF levels are consequently low (Caspers et al., 2001).

4. Dry Skin

Dry Skin/Dry Stratum Corneum Symptoms and Causes

Dry skin and it symptoms itching, scaling and the cracking of skin, is experienced by most people at some point in their life. Mostly this is caused by climatic conditions or by excessive washing, but dry skin is also a common symptom of skin diseases such as atopic eczema or forms of ichthyosis. Whatever the source of dry skin, the underlying cause is always a water deficit in the SC caused by a lack of water-retaining NMF, either due to excessive washing or to a lack of filaggrin degradation.

The lack of water can have many consequences, as many enzymatic SC processes are dependent on SC water levels. For instance, desquamation will be inhibited, as the responsible enzymes will not function. This will result in incomplete degradation of corneodesmosomes and the consequent flaking that is so common in dry skin (Simon et al., 2000). Another consequence is incomplete corneocyte maturation (see *The Corneocyte Envelope*), i.e. the assembly of the corneocyte envelope is inhibited because transglutaminases no longer crosslink the required proteins. This will result in incomplete formation of the corneocyte envelope. Corneocytes will remain fragile and more susceptible to loss of NMF and water. Additionally, the extracellular lipid matrix will be less closely linked to the corneocyte, as fewer or no lipids will be covalently linked to the corneocyte's surface. Last but not least, low SC water levels also inhibit the degradation of filaggrin by enzymes like the kallikreins, i.e. NMF production (see *The Natural Moisturizing Factor*).

Concluding, it can be said that the decreased water levels in dry skin are not only the result of a lack of NMF, but also the cause of a lack of NMF. Dry skin has therefore only a limited capacity of healing itself and can thus be caught in a viscious dry cycle (figure 7).

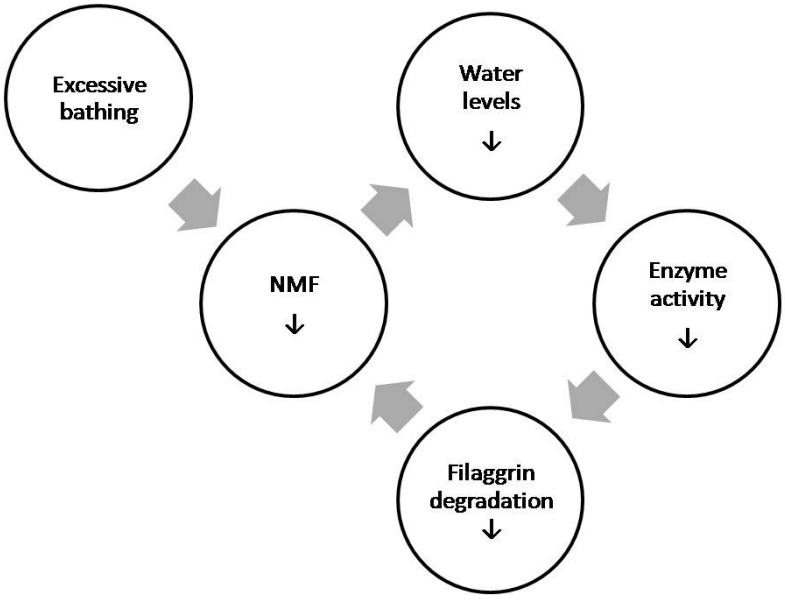

FIGURE 7. The dry skin cycle. NMF can be lost from the skin for instance by bathing. As a result, water levels in stratum corneum may drop below the minimum necessary for various enzymes to function, the enzymes involved in filaggrin degradation included. This implies that loss of NMF results in decreased NMF production.

TREATMENT OF DRY SKIN

Dry skin is generally treated by the application of moisturizers. The term moisturizer may refer to a formulation of several substances, but also to a single substance that has the ability to increase water levels in the SC. In this thesis, substances were either applied neat or dissolved in water in order to be able to exclude formulation effects as much as possible.

Moisturizer can be roughly divided into two groups, the hydrophilic and the lipophilic moisturizers. Hydrophilic moisturizers are mostly low molecular weight, hygroscopic substances. It is assumed that, because of their low molecular weight, these substances penetrate the SC, where they subsequently act as humectants (Sagiv and Marcus, 2003), mimicking the role of NMF. The best known hydrophilic moisturizers are glycerol and urea. Glycerol has been shown to increase skin moisturization (Fluhr et al., 2003) and to repair the barrier function of the skin (Fluhr et al., 1999). More recently, endogenous glycerol (glycerol transported into the epidermis by aquaporin 3 (Hara-Chikuma and Verkman, 2005) and glycerol supposedly derived from sebaceous lipids (Choi et al., 2005)) have been speculated to play an important role in natural skin moisturization. Like glycerol, urea has been used extensively in the treatment of atopic dermatitis (Abels and Proksch, 2006; Loden, 2005; Loden et al., 2001) and dry skin in general (Loden, 1996; Schoelermann et al., 2003; Swanbeck, 1992).

Besides the described small hydrophilic moisturizers, also macromolecular hydrophilic substances are used as moisturizers. The best-known member of this group is aloe vera juice or gel, the sap of the succulent plant *Aloe barbadensis Miller* that contains multiple mucopolysaccharides. Its effects are controversial, however, as some studies show no effect of aloe vera on the hydration level in SC (Dal'Belo et al., 2006; Heggie et al., 2002). As macromolecules are unlikely to penetrate the SC, their effect is most likely caused by retaining water on the surface of the skin, hereby giving the sensation of a smoother skin, rather that effecting the real increase in water levels in the SC necessary for proper enzymatic activity (Dal'Belo et al., 2006).

The second group of moisturizer are the lipophilic moisturizers. These are usually large molecules, mostly with long carbon chains, such as fatty acids, waxes or triglycerides. Due to their high molecular weight, most of them are unlikely to penetrate the skin. The most important lipophilic moisturizer is Vaseline© (petrolatum). This complex mixture of hydrocarbons is used pure or as a base for many pharmaceutical as well as cosmetic products. It has been speculated to be an occludant and to increase barrier recovery (Ghadially et al., 1992). In general, lipophilic moisturizers may have at least two mechanisms of action. Most compounds are expected to remain on the surface of the skin, thereby preventing evaporation of water from the SC by their occlusive properties. However, some lipophilic moisturizers appear to be non-occlusive (Wiechers, 1999); these may penetrate into the SC and interact with the SC lipids to provide an increased barrier to water loss.

In this thesis, several methods, discussed in Chapter 2, will be used to study the working mechanisms of hydrophilic and lipophilic moisturizer. An overview of the questions addressed is given in Chapter 3.

5. References

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