

# Advances in Stratum Corneum Biology and Understanding of Dry Skin

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During the last decade there has been tremendous advancement in our understanding of not only the structure, composition and function of the stratum corneum, but also in its formation and the epidermal differentiation factors responsible for mediating cellular changes in keratinocytes during their journey from the basal layer to the granular layer. Moreover, the structural and biochemical changes that occur in stratum corneum quality and function have received much attention. This short paper will review some of the research conducted over the past decade on our understanding of the pathophysiology of dry skin.

## Stratum Corneum Barrier Lipid Composition and Structure

The lipid-enriched intercellular

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have contributed to the further understanding of barrier lipid structure, function and physiology over the last 10 years. For instance, it is now established that there are nine different classes of ceramides but each class is heterogeneous in its own right in terms of acyl chain length and types of fatty acids present in the ceramide structures (Figure 2).

Scientists from L'Oréal<sup>4</sup> and Beiersdorf<sup>5</sup> in particular have identified new ceramide chemical structures but the roles of the new lipid species are still not well understood.

*Structure of the lipid matrix:* Major advances in understanding the material science of the stratum corneum lipids have been made by Bouwstra's group.<sup>68</sup>

In the stratum corneum lipids, two crystalline lamellar phases are observed by X-ray diffraction with periodicities of 6.4 nm and 13.4 nm. When visualized using electron microscopy, the intercellular lipids are observed as a broadnarrow-broad sequence of bilayers. From these findings, Bouwstra<sup>7</sup> recently proposed a new "sandwich" model consisting of two broad lipid layers with a crystalline structure separated by a narrow central lipid layer with fluid domains (Figure 3).



spaces of the stratum corneum constitute the primary barrier to water loss (Figure 1).

Not only are the amount of lipids or the correct equimolar ratios of the major lipid species important (ceramide: cholesterol: fatty acids), but the precise ceramide chemical composition and physical organization of the total lipid matrix is vital for a healthy barrier.<sup>13</sup>

*Lipid composition:* Many cosmetic companies and several academic groups Cholesterol and ceramides are important for the formation of the lamellar phase whereas fatty acids play a greater role in the lateral packing of the lipids. Lipids in vivo appear to exist as a balance between a solid crystalline state (orthorhombic packing) and gel or liquid crystalline (hexagonal packing) states.

The orthorhombically packed lipids are the most tightly packed conformation and have the better barrier proper-





ties. These physical states change during migration of the corneocytes from the lower layers of the stratum corneum to the outer layers, where a greater proportion of hexagonally packed lipid conformations are observed. This is consistent with a weakening of the barrier towards the outer layers of the stratum corneum.

The importance of ceramide 1 or Cer (EOS) in facilitating the formation of the long periodicity phase (LPP) (13.4 nm) has been further elaborated by understanding the influence of the type of esterified fatty acid.<sup>8</sup>

The LPP is seen mainly with ceramide 1 linoleate, less with ceramide 1 oleate, and was absent with ceramide 1 stearate. These types of compositional changes could therefore dramatically influence the condition of the skin.

The lipid packing states will influence not only the barrier properties of the skin but also desquamation. For instance, a general decline in ceramide levels occurs with aging but is also observed more in winter months compared to summer months.<sup>9</sup> Subtle changes in the levels of ceramide 1 fatty acid types were also established where decreases in ceramide 1 linoleate levels and increases in ceramide 1 oleate levels were observed in winter and in aged skin (Figure 4).

These changes can contribute to the weakening in barrier function that is known to occur when the barrier is challenged. Interestingly, Declercq et al.<sup>10</sup> at Estée Lauder have shown similar adaptive responses in skin barrier function in subjects living in hot or humid environments. Subjects in Arizona possessed a stronger skin barrier than those living in New York and also had a greater amount of ceramides.

Further insights into differences in stratum corneum composition have also become evident on other body sites. Changes in the ceramide-to-cholesterol ratio have been described in the axilla region. Using Fourier transform infrared spectroscopy, a more ordered lipid lamellae behavior was found, suggesting that the elevated cholesterol levels might phase separate, causing a reduced barrier function.<sup>11</sup>



# Stratum Corneum Corneodesmosomes, Enzymes and Corneocyte Phenotypes

*Corneodesmosomes:* Corneodesmosomes are complexes of glycoproteins that are cross-linked into the corneocyte envelope and link cornoecytes together in the stratum corneum. The importance of the corneodesmosomes for stratum corneum integrity and the need for their degradation during desquamation has become evident over the last decade.<sup>1,2,12</sup>

*Enzymes:* A variety of enzymes are involved in the proteolytic process. Stratum corneum chymotryptic enzyme<sup>13</sup> (SCCE) and stratum corneum tryptic enzyme<sup>14</sup> (SCTE) are thought to be the main serine proteases involved in desquamation. These enzymes exist as proforms and have been immunolocalized to the intercorneocyte lipid lamellae. However, trypsin-like activity has also been demonstrated within the corneocytes themselves.<sup>15</sup> Other enzymes are also present such as the cathepsins (D, E and L). These enzymes are reported to have a more acidic pH profile compared with the serine proteases and therefore may play a greater role in the final stages of the desquamatory process. For a more detailed review see the recent paper of Rawlings.<sup>16</sup>

Because some of these enzymes have been immuno-localized to the intercellular space (SCCE, SCTE and cathepsin D), the physical properties of the stratum corneum lipids, together with the water activity in that microenvironment of the stratum corneum, will influence the activity of these enzymes. However, a variety of inhibitors, such as cholesterol sulfate, are present to attenuate their activities. Other protein and peptide inhibitors are present, such as antileukoproteinase, alpha-1-antitrypsin and alpha-1-antichymotrypsin, the SPINK5-derived peptides, and elfin covalently bound to the corneocyte envelope.<sup>16</sup>

Although SCCE and SCTE have been cloned and have been classified as members of the kallikrien protease family (referred to as kallikrien 7 and 5, respectively), little is understood of the molecular activation mechanisms of SCCE in the stratum corneum. Interestingly, SCCE appears to have a greater tolerance to water deprivation than other enzymes, and this may be an adaptation to maintain enzyme activity even within the water-depleted stratum corneum intercellular space.<sup>17</sup>

Again the axilla appears to be unique in that the levels of SCCE are dramatically lowered, indicating a potential for aberrant desquamation (Figure 5).<sup>18</sup> A similar adaptation response is seen in hot versus humid climates where a greater amount of SCCE is found in subjects living in Arizona.<sup>10</sup> Surprisingly, an age-related decline in SCTE, but not SCCE, exists.<sup>19</sup>

*Corneocyte phenotypes:* The more recent work on corneocyte envelope phenotype is also very interesting.



Figure 5. Comparison of SCCE activity levels in forearm (p<0.05) and axillary stratum corneum assayed by zymography



Figure 6. Double staining of corneocyte envelopes from face (A) and from forearm (B) with Nile red and anti-involucrin. Scale bars 50 µm. Green staining = involucrin Red staining = covalently bound lipid

Modified from Hirao et al, Exp Dermatol 10 35-44 (2001)

Scientists at L'Oréal originally identified that the population of corneocyte envelopes (CE) were heterogeneous, consisting of fragile (CEf) and rigid (CEr) phenotypes, and increases in the former were seen in hyperproliferative disorders such as psoriasis.<sup>20</sup>

More recently using rhodamine dyes, it has been observed that the cells undergo a maturation process from the inner (CEf) to the outer (CEr) layers of the stratum corneum being mediated by transglutaminases.<sup>21</sup>

The most beautiful work in this area has been conducted by Hirao et al.<sup>22</sup> at Shiseido who have demonstrated the changes in corneocyte hydrophobicity and expression of corneocyte proteins such as involucrin during corneocyte maturation (Figure 6). Nile red staining increases and the "apparent" expression of involucrin decreases from the CEf to CEr transition. Presumably the antibody no longer has access to the protein as involucrin becomes esterified with covalently bound lipids. Presumably these changes occur in the CEf to CEr transition. More CEr are found on exposed body sites such as the face, and further increases are observed in the winter months of the year.



Figure 7. Freeze fracture replicas show an increased number of corneodesmosomes on the surface of corneocytes from the outer SC of winter xerosis compared with those of normal skin. The morphology of intercellular spaces within the inner SC (a,c) and outer SC (b,d) from normal (a,b) and xerotic (c,d) skin strippings was analyzed on freeze fracture electron micrographs. Corneodesmosomes appear as particle aggregates (asterisks and stars). Arrowbeads indicate the direction of shadowing, Scale bar 250 nm.

From Simon et al, J Invest Dermatol 116 23-30 (2001)

#### **Understanding Dry Skin**

The reduction in ceramide levels and other lipids and the lowered corneodesmolysis that occurs in dry flaky skin conditions is now well established. SCCE levels and activity are diminished; in one experiment I found a 56% reduction in SCCE activity in dry skin versus a healthy control skin. These reductions contribute to the faulty desquamation, but barrier function is well known to be impaired.<sup>23</sup>

Desquamation results in the degradation of non-peripheral corneodes-mosomes at the stratum compactumdisjunctum interface, and the peripheral corneodesmosomes are then finally degraded in the upper layers of the stratum corneum, leading to loss of corneocytes from the surface of the skin by frictional forces. In dry flaky skin conditions the peripheral corneodesmosomes are not degraded efficiently and corneocytes accumulate on the skin's surface layer.<sup>24,25</sup> Some beautiful freeze fracture visualization of the corneodesmosomes in dry skin has been performed by Simon and Serre (Figure 7).<sup>25</sup>

Interestingly, the accumulation of the corneodesmosomal proteins desmoglein 1 (Dsg 1) and plakoglobin correlate with each other in dry skin conditions, whereas corneodesmosin does not have such an association. This

suggests different proteolytic mechanisms are occurring for the different corneodesmosomal components.25 Plakoglobin is degraded within the corneocyte; perhaps Dsg 1 is also degraded there. It is interesting that trypsin-like activity has been immunolocalized within the corneocyte matrix. The lamellar lipid matrix is also perturbed dramatically in dry skin (Figure 8). Because the main desquamatory enzymes are found within this matrix, the physical properties of this matrix will influence enzyme activity.

## Summarizing the Lipid Changes in Dry Skin

The long periodicity phase has been reported to be lacking in subjects with dry skin and has been attributed to reduced levels of Cer (EOS) and Cer (EOH). Pilgram et al<sup>26</sup> also have reported on the changes in the lateral packing of lipids in dry skin conditions leading to a greater amount of hexagonal phase. Cer (EOS) is reduced in these dry skin conditions, again contributing to the phase changes in the barrier lipids, as well as increasing the barrier lipid's viscosity and thereby presumably reducing enzyme diffusivity.<sup>27</sup>

However, other changes occur to the biochemistry of the lipids. It has been well established in hyperproliferative disorders such as dry skin that there is a change in the stratum corneum lipid composition. In particular the ceramide subtypes change and a predominance of sphingosine-containing ceramides at the expense of the phytosphingosine-containing ceramides can be observed.28 There also appears to be a hydrophobic chain length deficiency in the ceramides and fatty acids in subjects with hyperproliferative skin disorders such as atopic dermatitis.<sup>29</sup> In a similar finding, scientists at L'Oréal28 have shown



Figure 8. Organization of stratum corneum lipids and ultrastructural changes in lipid organization towards the surface of the stratum corneum, as seen in transmission electron micrographs of tape strippings from individuals with clinically normal skin (left) and severe xerosis (right). A. First strip; absence of bilayers and presence of amorphous lipidic material B. Second strip; disruption of lipid lamellae C. Third strip; normal lipid lamellae (x200,000) D. First strip; disorganized lipid lamellae E. Second strip; disorganized lipid lamellae F. Third strip; normal lipid lamellae (x200,000) Modified from Rawlings et al, J Soc Cosmet Chem 45 203-220 (1994)

that the chain length of the ceramide species gets shorter in subjects with dry skin.

These changes in ceramide composition will obviously influence the packing of the lipids and the barrier properties.

From this understanding, four aspects of ceramide chemistry need to be corrected in dry skin:

- The lowered levels of ceramides;
- The lowered levels of covalently bound ceramides;
- The phytosphingosine-containing ceramide insufficiency;
- The precise chain length of the ceramides and fatty acids; and
- The ceramide 1 linoleate (Cer (EOS)) insufficiency.

Overall, however, the lipid lamellar architecture in the outer layers of the stratum corneum needs to be normalized in dry flaky skin conditions.

Dry skin also results in changes in the CEf and CEr levels, where CEf predominates. This appears to be related to the reduction in the level of the enzyme, transglutaminase, which normally cross links the corneocyte envelope proteins and attaches lipids to the corneocyte envelope.<sup>30</sup>

*Itch:* Tremendous advances in the understanding of the textural symptoms associated with dry skin have been made but itch is still a poorly treated aspect of the dry skin condition. Epidermal SCCE is reported to be increased in psoriasis and atopic dermatitis and probably contributes to the disease pathology.<sup>31</sup>

Very interestingly, scientists at Arexis have developed an SCCE transgenic mouse model that over expresses SCCE. This phenotype spontaneously itches and has a weakened barrier function. The histochemical changes seen in the mouse model are similar to those observed in the inflammatory scaling skin diseases.<sup>32</sup> Other inflammatory enzymes, such as plasmin and urokinase, are also increased in dry skin.<sup>33</sup>

#### Conclusions

The structure and function of the stratum corneum has been the subject of intense research over the past few decades. Recent developments in understanding lipid composition have led to a new ceramide nomenclature system, a new proposal for a molecular model of the interactions between the lipid species and the demonstration of the presence of predominantly crystalline orthorhombic lipid phases in the stratum corneum. Linoleate-containing ceramide 1, now known as Cer (EOS), has been shown to be essential for the formation of the 13 nm long periodicity phase.

The role of the corneocyte envelope and its transglutaminase-mediated maturation processes have been shown to be essential for good skin condition. Several proteases – particularly serine and cathepsin-like enzymes – may have a role in corneodesmolysis.

Disruption to lipid packing states, reduction in the level of lipids – particularly the phytosphingosine-containing ceramides – and loss of the LPP largely account for the perturbations in lipid structure that occur in dry skin. The reduced corneodesmolysis that occurs in this xerotic skin disorder is due to reductions in the levels and activities of stratum corneum proteases together with elevated levels of corneodesmosomes in the superficial layers of the stratum corneum. Additionally, increased levels of fragile corneocytes are associated with reduced transglutaminase activity. However, the somatosensory problems that occur in dry skin are poorly understood and the itching associated with dry skin is still an under-researched area.

Technologies to treat the surface textural skin problems, to enhance the differentiation process – particularly lipid biosynthesis – and to control the somatosensory problems in dry skin have received much attention in the last decade and will be reviewed in the next column in this two-part series.

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