Human *Stratum Corneum* homeostasis: the relevance of filaggrin and of inducers of filaggrin production

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A remarkable aspect of epidermal physiology is the repair strategy of the *stratum corneum*: instead of carrying out punctual removal of damage, the *stratum corneum* undergoes a permanent renewal. In the basal layer individual cells remove DNA damage and "chaperone" damaged proteins to the lysosomes thanks to the heat shock response. In supra-basal layers, the process of differentiation leads to a steady state regimen such that every day, the outermost layer of *stratum corneum* corneocytes sheds off and is replaced by a layer of basal cells entering the supra-basal, differentiating layer.

In the course of differentiation, the genetic material of epidermal keratinocytes is digested, the cytoskeleton undergoes structural modifications and the plasma membrane supports the synthesis of the cornified envelope on its inside face. The assembly of the cornified envelope is mediated by several transglutaminases, enzymes able to connect the amino-termini of involucrin and plakins and to generate an insoluble layer, which is then attached to the cytoskeleton. A subsequent series of steps leads to the fusion of lipid lamellar bodies with the plasma membrane, provoking the secretion of its content as well as the replacement of the membrane's lipid bilayer with ω-OH-ceramides. A final step in the differentiation of corneocytes is the synthesis of loricrin, a protein unusually rich in glycine, serine and cystein, that is the main component of the cornified envelope (about 80% of the total protein mass). Loricrin is then cross-linked with itself and with proteins of the family of the Small Proline Rich Proteins: these cross-linked oligomers are then cross-linked to the involucrin-plakin scaffold (1, 2, 3, 4).

This process can be seen as the action needed to confer resiliance to the corneocytes, making them impervious to dissolution by water or detergents, and resistant to penetration, thus protecting the inner epidermis from foreign bodies and

xenobiotics. Another essential process in the differentiation of the corneocytes is the process leading to their flat structure. This process is essentially the result of the action of filaggrin, and this is only one of the reasons of the relevance of the role of filaggrin in the *stratum corneum.*

Filaggrin was identified to be the main component (the histidine rich protein) of the keratohyalin granules of the *stratum granulosum*, the uppermost suprabasal layer, just below the *stratum corneum* (5, 6). The physiologic relevance of the components of the *stratum granulosum* was understood years before the actual identification and purification of filaggrin itself: in 1968 Vorhees and coworkers reported that the epidermis in psoriatic skin differs morphologically from the normal one, insofar as keratohyalin granules are absent in involved skin (7). The granules of keratohyalin contain profilaggrin, a high molecular mass protein (~500 kDa), which is initially highly phosphorylated and then matures to filaggrin by being dephosphorylated by ATPases and proteolytically cleaved by specific proteases, perhaps kallicrein-related peptidases, to filaggrin units with a molecular mass of about 35 kDa. Filaggrin was so named because of its capability to provoke the *in vitro* aggregation of keratin intermediate filaments (KIF) (8). Together with the tubulin of the microtubules and the actin of the microfilaments, KIFs constitute the cytoskeleton. The aggregation of intermediate filaments could provoke the intermediate filaments of the cytoskeleton to collapse and this would cause a significant change in cell shape, from an ellipsoid to a flat cell in which the keratin intermediate filaments are aligned parallel to the outer surface of the epidermis. Thus filaggrin provokes the flattening of the corneocytes and perhaps the digestion of genetic material, which is observed to still be present in the acanthotic cells typical of psoriatic *stratum corneum* where keratohyalin granules (and therefore filaggrin) are absent.

Filaggrin is a rapidly turning over molecule: its half-life is of the order of six hours and is digested to free amino acids. Interestingly enough, the composition of aminoacids of filaggrin strictly mirrors the composition of free amino acids found in the epidermis, when urocanic acid, citrulline and pyrrolidone carboxylic acid (PCA) are accounted for as derivatives of histidine, arginine and glutamic acid, respectively

(9, 10, 11). The high concentration of hydrophilic amino acids is essential for the retention of water and contributes to the flexibility of the cornified layer. Therefore, as it was very early understood by Vevy Europe's scientists in pioneering research reported in the early 80s years of the twentieth century (12), the profilaggrin-filaggrin system has at least three critical roles in epidermal differentiation: the alignment of the Keratin Intermediate Filaments, the control of the change in shape of the corneocytes, and the maintenance of epidermal texture (13).

These conclusions are supported by histology and molecular biology studies on ichthyosis patients, that seem to point out a causal correlation between the absence or the down-regulation of filaggrin and the impairment of epidermal homeostasis. Ichthyosis is the most common inherited disorder of keratinization. It is characterized by rough and dry skin associated with fine scaling. Light- and Electron-Microscopy studies of skin biopsies from patients with *ichthyosis vulgaris* revealed the absence or the abnormal shape of keratohyalin granules (14, 15) and immuno-staining of similar biopsies pointed out the absence of filaggrin (14). Gene sequencing results, associated to microscopy and immuno-staining studies concluded that a loss-of-function mutation within the filaggrin gene is the cause of *ichthyosis vulgaris* (15). Other pathologies were associated to decreased amounts of filaggrin: immunostaining of punch biopsies from Atopic Dermatitis patients revealed a decreased amount of filaggrin and involucrin in involved skin (16).

Aging is not a disease, *per se,* but is associated to skin lack-of-comfort statuses such as dry skin and pruritus. In old age the epidermis tends to become dry and flaky, especially in the lower leg. Immunohistochemical examination of the epidermis of the face and of the lower leg in young and old volunteers revealed a marked decrease of filaggrin and involucrin in the epidermis of the lower leg of old individuals (17). The question of the mechanisms leading to the lesser accumulation of filaggrin in old skin was tackled by Rinnerthaler and coworkers who analyzed in young, middle aged and old volunteers, the gene expression of filaggrin and loricrin, Small Proline Rich Protein (SPRR), in specimen of skin from regions non exposed to sunlight, (18). They observed a remarkable increase of SPRR mRNA (10 to 100 fold in middle-aged versus young and in old aged versus young, respectively) as well as a three-fold decrease in

the level of mRNA of filaggrin and loricrin. These authors conclude that the rise in SPRR and the decrease in loricrin could affect the mechanical properties of the *stratum corneum*. As a matter of fact, loricrin is flexible and insoluble, whereas the SPRR are rich in lysines and glutamines in the head and tail regions (the spring-like parts) and rich in prolines in the central, rigid part. And filaggrin is massively down-regulated with increasing age, and this might be the reason why the *stratum corneum* of the elderly is associated with decreased hydration and reduced water-binding capabilities.

Since old age is characterized by a down-regulation of filaggrin at the transcriptional level, one might suggest using inducers of filaggrin production to obviate the lack of comfort in the skin of older individuals. One of such inducers is Filagrinol, a mixture of pollen extract and of unsaponifiable fractions from soybean oil, olive oil and wheat germ oil (19) of proven safety (20). Upon topical application, Filagrinol stimulates the maturation of profilaggrin by activating the ATPases involved in the dephosphorylation of profilaggrin, and by inducing the incorporation of histidine in the *stratum granulosum* (21, 22). As a consequence of this, one expects topical Filagrinol to increase surface hydration and improves cutaneous sensorial conditions.

Filagrinol, stimulating filaggrin synthesis, promotes – by subsequent steps of physiological degradation of this protein – the restock of that pool of hydro soluble molecules which, playing a fundamental role in granting epidermal hydration, are called Natural Moisturizing Factor (NMF).

At the end of 80s, Vevy Europe carried out a specific experiment in which both hydration level - by means of instrumental devices - and overall condition of skin well-being - by means of a sensorial test - have been examined.

Such an experiment was performed (23) with 30 volunteers who received topically, 8% Filagrinol in a O/W emulsion or the same emulsion without Filagrinol, twice a day for 60 days. The hydration was measured with a corneometer at days 0, 10, 20, 30 40, 50 and 60 and it increased by 13%, 19%, 26%, 33%, 39% and 44% by days 10, 20, 30, 40, 50 and 60, respectively, whereas it remained practically constant in the skin treated with the O/W emulsion without Filagrinol. The cutaneous sensory conditions

were clinically assessed in a double blind experiment by trained experimenters, on a scale from 1 to 3 for the general aspect, hydration, elasticity and roughness for every volunteer, and the sum of the scores were reported, at the same days as for the hydration measurements. The scores for the Filagrinol-treated skin increased over time and were 6.83, 7.63, 8.18, 8.63, 8.98, 9.50 and 9.73 at days 0, 10, 20, 30, 40, 50 and 60 respectively, whereas the scores for control skin increased much less and reached 7.23 at day 60. As a windfall benefit, the topical administration of Filagrinol prevents in a remarkable manner the peroxidative effects of solar radiation (24). Taken together, all the results obtained via topical application of Filagrinol on human skin seem to indicate that Filagrinol is appropriate to be used in moisturizers and sunscreens prepared for aging skin.

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