Microsc. Microanal. 8 (Suppl. 2), 2002
DOI 10.1017.S1431927602100249

New aspects of the skin barrier organisation assessed by diffraction and electron microscopic techniques

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The skin barrier for most substances is found in the upper layer of the skin, the stratum corneum (SC). The SC consists of corneocytes, flattened dead cells, which are embedded in lamellar lipid regions. The composition of the SC lipids strongly differs from that of cell membranes of living cells in which phospholipids are the major lipid components. In the SC and the major lipid classes in the SC are ceramides, cholesterol and free fatty acids. The lipid-protein matrix of the SC not only restricts the passive diffusion of lipophilic molecules, but also severely limits the transport of hydrophylic compounds across the membrane. In order to study the lipid organization of healthy and diseased skin and to understand the mode of action of formulations used to increase the drug transport, several techniques have been explored. With these techniques we intend to elucidate either the lipid organization in the SC, the penetration pathways of the active agents, or the swelling mechanism of corneocytes. The latter is of interest, especially for moisturizers.

In this presentation first a brief description of the X-ray and electron diffraction method will be given. While the X-ray diffraction technique provides integral information on the lipid organization in SC, the electron diffraction can be used to study the local lipid lateral packing and provides detailed information on the orientation of lipid crystals in the SC. Furthermore by employing a stripping technique in combination with electron diffraction, structural information can be obtained as a function of depth in vivo. This makes the electron diffraction technique extremely powerful.

Using the X-ray diffraction technique it was found that the lipids in SC form two crystalline lamellar phases with periodicities of 6.4 and 13.4 nm. Furthermore, the 13.4 nm phase consist of one narrow central lipid layer with fluid domains with on both sides a broad layer with a crystalline structure. However, using X-ray diffraction it was not possible to detect whether besides an orthorhombic phase also a hexagonal lateral packing was present. For this reason the electron diffraction technique was explored. Electron diffraction revealed that only in the superficial layers of the SC a hexagonal lateral packing was coexisting with the orthorhombic packing. When the lateral packing is compared to the lipid organization in lamellar ichthyosis skin (a skin disease), it was found that in lamellar ichthyosis skin the hexagonal lateral packing was more pronounced present. This difference in phase behavior can explain at least partly the impaired barrier function in lamellar ichthyosis skin.

Electron diffraction does not provide information on the lamellar organization in SC in vivo. For this reason we explore the freeze fracture electron microscopy and combine it with the stripping technique. In this way freeze fracture electron microscopy can be used to study the lamellar phases in the SC as function of depth in humans in vivo in a similarly as with electron diffraction. This is illustrated by studies carried with healthy and lamellar ichthyosis skin. It was found that not only the lateral packing, but also the lamellar organization in lamellar ichthyosis skin is different from that in healthy skin. Information on the relationship between lipid organization in diseased skin is of great importance to unravel the mechanism controlling the skin barrier function. This is again demonstrated in lamellar ichthyosis skin, in which an impaired barrier function parallels an altered lipid composition and organization.

However, not only changes in SC lipid organization, but also the permeation pathways of the active agents need to be studied in order to understand the mode of action of delivery systems. For this reason methods have been developed to examine the diffusion into fresh unfixed human skin. This might be the permeation pathway in the SC and the targeting of fluorescent dyes to the hair follicles.

https://doi.org/10.1017/S1431927602100249 Published online by Cambridge University Press