Abstract

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Title of diploma thesis: The effect of Ca^{2+} cations and cholesteryl sulfate on the human skin barrier model

The structure and composition of the outermost layer of the *epidermis*, the *stratum corneum*, form a skin barrier to transepidermal water loss. The main components of the *stratum corneum* are corneocytes and the intercellular lipid matrix. The intercellular lipid matrix is a mixture of ceramides, cholesterol, and free fatty acids. Cholesterol esters, such as cholesteryl sulfate, are also present. This unique lipid mixture forms a multilamellar layer with short and long crystalline phases with periodicities of approximately 6 and 13 nm. A separated cholesterol phase with a periodicity of about 3,4 nm can also be found in the *stratum corneum*. The most common lateral packing of the lipid mixture is orthorhombic. Hexagonal or fluid packing is less common.

The aim of this diploma thesis was to optimize the conditions for spontaneous arrangement of the isolated human skin lipids in an aqueous medium with the addition of Ca^{2+} cations and cholesteryl sulfate. A human skin barrier model was created from the prepared lipid dispersion and used to monitor the impact of the addition of Ca^{2+} cations and cholesteryl sulfate on the lamellar phases and lateral packing of the lipids.

The lipids used for the experimental part of this diploma thesis were obtained from the isolated human *stratum corneum*. First, the *stratum corneum* was isolated from the *epidermis* and the lipids were extracted with organic solvents, followed with purification by column chromatography. The acquired lipids were then analysed by high-performance thin-layer chromatography for semiquantitative and qualitative determination. In the next step, a pilot experiment was performed to enable the formation of a dispersion of isolated lipids in an aqueous medium. Subsequently, a dispersion of isolated lipids was prepared in an aqueous medium with the addition of Ca^{2+} cations and cholesteryl sulfate. Aggregates were created by changing the pH from alkaline to acidic or by changing the temperature (cooling).

Aggregation induced by a change of pH led to insufficiently compact aggregates. The microstructure of these aggregates was studied by X-ray scattering.

X-ray scattering revealed that the presence of Ca^{2+} cations and cholesteryl sulfate allows the formation of a long phase with a periodicity of ~ 13 nm even in a non-physiological environment (pH 7,4 and 8,5). Furthermore, other phases with periodicity of ~ 5,8 nm (at acidic and neutral pH) and ~ 9 nm (at neutral and alkaline pH) were found in the presence of Ca^{2+} cations and cholesteryl sulfate. At the same time, the structure of the lateral orthorhombic packing was maintained.