Abstract

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Biophysics and Physical Chemistry

Student: Karina Suciu-Šubert

Supervisor: Mgr. Petra Pullmannová, Ph.D.

Title of diploma thesis: Preparation of the human skin barrier model by using thin lipid film deposition

The uppermost part of the human skin - the *stratum corneum*, which protects the body from the external environment, is made up of cornified cells - corneocytes, which are surrounded by the extracellular matrix of highly ordered lipids: ceramides (Cer), fatty acids and cholesterol. The protective barrier is constantly renewed with so-called keratinocytes, which differentiate to corneocytes and finally desquamate from skin to surface. The corneocyte envelope replaces plasma membrane of corneocytes. It consists of proteins (eg. involucrin) and a lipid monolayer consisting mainly of ω-hydroxyceramides which are attached to the protein segments by covalent bonds.

The task of this diploma thesis was the preparation of the human skin barrier model by using thin lipid film deposition. Two different lipid mixtures were based on Cer NS or Cer EOS and NS. The mixtures were applied on the hydrophilic or hydrophobic substrate by spraying with Linomat V. The amount of lipids and consequently the number of theoretical lipid layers on the substrate was modified in individual samples. Another thin layer was prepared by repeated deposition of monolayers of the Cer NS-based mixture using Langmuir-Blodgett technique.

Our aim was to find out how lipids organize and whether their arrangement depends on their composition, amount, number of layers or the characteristics of the substrate surface. We used XRD and light microscopy to evaluate samples created by Linomat V, and we used AFM to evaluate samples of lipid monolayers deposited by the Langmuir-Blodgett technique.

From the XRD method, we found out that 1 lamellar phase with repeat distance of ~ 5.3 nm occurred in the Cer NS-based mixture. There were 2 lamellar phases in the EOS and NS-based mixture: a long one with a repeat distance of ~12.9 nm and a short one with a repeat distance of ~5.1 nm. The relative intensity of the diffracted radiation did not change significantly in the Cer NS-based samples with reduced amount of lipids on both types of surfaces - hydrophilic and hydrophobic. The relative diffracted intensity of the Cer EOS and NS-based samples only did change on the hydrophobic surface.

Light microscopy has revealed that the thinner lipid samples did not form a continuous layer, but form mounds. Differences between hydrophilic and hydrophobic substrate were visible. We conclude that the fewer lipids in the sample, the more they are affected by the substrate surface.

By using the AFM method, we observed a Cer NS-based sample, which was created by the Langmuir-Blodgett technique. We discovered the presence of round domains with the height difference between the high and low domains up to 100 nm.

Based on the results, we concluded that the organization of lipids depends on their composition, amount, number of layers and on the surface properties of the substrate.