DUTCH-BELGIAN BEAMLINE
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Experiment Report Form

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

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DUBBLE	**Experiment title:**	**Experiment number**: **26-02-355**
Beamline:BM26B	**Date(s) of experiment**: From: 15-12-2006 To: 18-12-2006	**Date of report**: 07- 03 -2007
Shifts:9	**Local contact(s)**:W. Bras	
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Report: (max. 2 pages)

We performed measurements during a 3-days session in december 2006. The beam conditions (beam intensity and beam alignment) were excellent. We were able to perform all the scheduled experiments. This also includes measurements, in which the lipid phase behaviour was studied as function of temperature. Some additional studies were performed with a controlled cooling rate of the samples using a new temperature controlling device. The studies were successful performed. This provides excellent opportunities for future studies, especially focussing on the formation of particular phases.

The skin barrier for diffusion of substances is located in the horny layer, the outermost layer of the skin. The horny layer consists of dead cells embedded in lipid lamellar regions. The lipid lamellar regions are crucial for the skin barrier function. The lipid composition and organisation in the horny layer is exceptional. Mainly free fatty acids, cholesterol and ceramides (9 subclasses) are present forming two crystalline lamellar phases with repeat distances of 6 and 13 nm.
The aim of the present project was to alter lipid organisation to mimic also barrier properties of diseased skin. In order to mimic the barrier properties of diseased skin, lipid orientation and organisation should mimic that in diseased skin.
Vernix is a white biofilm covering the skin surface of the foetus during the last trimester. This layer promotes the formation of the horny layer of the foetus, most probably due to retaining an optimal hydration level in the skin, which activates the enzymes involved in the formation of the skin barrier. The structure of the biofilm is very similar to that of the horny layer: corneocytes embedded in a matrix of lipids. However, besides the barrier lipids, triglycerides, cholesterol esters and wax esters comprise the lipid matrix (STW project 6117). In the present STW project our aim is to design a synthetic biofilm with similar properties as vernix. Frequently vernix is still present on the skin surface after delivery of the baby. During birth, however, the environment of VC undergoes a substantial change from an aqueous and warm surrounding (37°C) into a gaseous, colder environment in the postnatal situation (22°C). In the present studies we mainly focused on the phase behaviour between 15 and 37°C. The final goal of these studies is to develop synthetic vernix with corneocytes and lipids. The lipids should mimic the lipid phase behaviour of the natural venix lipids.

The main goal in our research is to study the structure and orientation of lipid lamellae parallel to a porous membrane and to mimic the structure of a vernix in designing a novel biofilm.

1. Final studies were performed to examine the role sphingosine and phytospingosine based ceramides play in the formation of the 13 nm phase, the characteristic lamellar phase in the horny layer. Therefore the composition of the CER mixture was systematically varied. The studies revealed that the number of sphingosine based ceramides can be reduced, but that CER with a sphingosine moiety cannot be completely removed. The same trend is observed in another series of studies in which the number of CER with a phytosphingosine base is reduced. Both, CER based on a phytosphingosine and sphingosine moiety are thus required to form the lamellar phases present in stratum corneum. These studies will be combined with FTIR studies that are currently under investigation in our laboratory. This will provide information on whether or not the various classes of lipids participate in the orthorhombic lateral packing.
2. A second series of studies were performed with our final goal replacement of CER1- linoleate by Br-substituted CER1-linoleate. These studies aim to change the electron density profile within the 13 nm lamellar phase, which will enable us to calculate the electron density profile within the this phase. In this series of studies we observed that increasing the amount of CER1 to 30% of the total amount of ceramides promotes the formation of the 13 nm phase. The formation of the 6 nm phase, which partially obscures the reflection of the 13 nm phase almost disappeared. These compositions are excellent candidates to incorporate the Br-substituted CER1-linoleate. This is certainly a subject for the next series of experiments.
3. In an ungoing project the orientation and phase behaviour of lipids mimicking the composition of normal skin sprayed on a porous membrane were studied. The studies have been carried out very successfully. Recently we adjusted the spraying technique from the air-brush method to the linomat method, as this is a more reproducible method. Furthermore, the air-brush method is being optimized. The lipids can indeed be oriented similarly as in stratum corneum, while the formation of the 6 and 13 nm phase still occurs. However, it seems that an additional phase is also formed, which is most likely phase separated CER2. The choice of the equilibration temperature seems to be very crucial in the formation of the 13 nm phase and to avoid the formation of the additional phase. Additional studies will be performed whether the cooling rate and the equilibration period at elevated temperatures also play a role.
4. The phase behaviour of vernix and it's isolated lipid classes has been studied as function of temperature. Vernix lipids form at least two lipid domains formed by triglyceride rich and a cholesterol ester lipid mixtures at room temperature, respectively. Interestingly, the long range ordering disappeared when increasing the temperature to 37°C, the temperature of the foetus. The transitions are reversible, as observed by heating and cooling curves.
   Using synthetic ceramides, cholesterol, fatty acids, triglycerides and isolated Lanolin fractions (3 different fractions) of we attempted to mimic the lipid organisation of vernix caseosa. This was partially successful. It appeared that LAN SE fraction of lanolin together with all other lipids most closely mimics the lipid organisation of vernix caseosa. However, additional studies need to be performed.