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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:** Synthetic lipids form lipid organisation mimicking the phase behaviour in the horny layer of the skin	**Experiment number**: **26-02-417**
Beamline:BM26B	**Date(s) of experiment**: From: 06-07-2008 To: 11-07-2008	**Date of report**: 30- 07 -2008
Shifts:9	**Local contact(s)**:W. Bras	
Names and affiliations of applicants (* indicates experimentalists): **J.A. Bouwstra, G.S. Gooris, D. Groen, D. de Sousa Neto**		

Report: (max. 2 pages)

Experimental conditions :

We performed measurements during a 4-days session in july 2008. The beam conditions (beam intensity and beam alignment) were excellent, and we were able to use the wide-angle detector, which gave excellent diffraction patterns. However, the small angle X-ray detector condition was far from optimal (the same situation as our previous beam time in february). An old detector is used as stand-in, while the previous detector (from before February 2008) is waiting for repair in England and the costs for a new detector are too high for DUBBLE. However, to meet the requirement of an excellent synchrotron station, For our measurements this means that the signal to noise ratio has drastically decreased when compared to measurements performed in may 2007. Although we were able to perform nearly all the scheduled experiments, due to the poor small angle X-ray detector quality a part of our measurments are not good enough for publication. So we were quite disappointed about these results.

Background experiments:

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 research the lipid organisation of a lipid model for the horny layer with synthetic ceramides, cholesterol and FFA and to alter the lipid organisation to mimic also the barrier properties of diseased skin. In order to mimic the barrier properties of diseased skin, lipid orientation and organisation of the model should mimic that in diseased skin.

The main goal in our research is to study the structure and orientation of model lipid lamellae (parallel to a porous membrane, or randomly oriented on mica).

1. 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. The phase behaviour of specialised lipid models mimicking either the 6 nm or the 13 nm phase present in the horny layer of the skin were studied. The studies have been carried out successfully. Most models were constructed in order to investigate the long periodicity phase of 13 nm. The choice of the equilibration temperature, as well as a balanced mix of CER with CHOL and FFA seem to be crucial in the formation of the 13 nm phase. Also an additional phase around 4.4 nm has been observed in some samples. It was found that both the equilibration temperature and the sample hydration are playing a role in formation of both the separate phase and the 6 nm phase.
3. By using oriented lipid lamellae on a porous membrane, the phase behaviour of lipid mixtures mimicking the compositions in diseased skin is being studied. Changes in composition are variation in CER, substitution of the CER(EOS) linoleate chain with an oleate or stearate chain, addition of short chain fatty acids, addition of cholesterol sulphate etc. Studies were carried out successfully and additional research on the lipid organisation using FTIR will be performed, as well as an assessment of the barrier function in permeation studies.
4. A simplified model with only CER(EOS), CHOL and varying FA composition was also studied. Measurements were performed successfully. In the future, after additional (FTIR) measurements, the molecular organisation of this model can possibly be revealed, helping us to understand the more complex model that mimics better the lipid composition of the horny layer.
5. We performed the first measurements concerning stratum corneum isolated from various reconstructed skin models and were able to perform the measurements. We will continue on this subject in the next beam session.