DUTCH-BELGIAN BEAMLINE AT ESRF

EUROPEAN SYNCHROTRON RADIATION FACILITY

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:** **Lipid organization of ceramide containing mixtures and horny layer sheets**	**Experiment number**: **26-02-464**
Beamline:BM26B	**Date(s) of experiment**: From: 19-06-2009 To: 22-06-2009	**Date of report**: 19- 07 -2009
Shifts:9	**Local contact(s)**:W. Bras	
Names and affiliations of applicants (* indicates experimentalists): **J.A. Bouwstra, G.S. Gooris, D. Groen, V.S. Thakoersing**		

Report: (max. 2 pages)

Experimental conditions :

We performed measurements during a 4-days session in june 2009. The beam conditions (beam intensity and beam alignment) were excellent. The small angle X-ray detector condition was much better than in the two previous sessions. However, with respect to our measurements the peak width should be very narrow to obtain a high resolution. This was sub-optimal. We were able to perform nearly all the scheduled experiments.

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

a. elucidate in more details the lipid organisation of a lipid model for the horny layer with synthetic ceramides, cholesterol and FFA. These studies will be combined with neutron diffraction studies performed at beamline 16 at the ILL.
b. We also changed the lipid organisation to mimic the barrier properties of diseased skin. The latter are carried out with oriented lipid membranes as through these membranes we also perform diffusion studies.
c. Finally we measured isolated horny layer from human skin equivalents. This skin in grown from two cell types and mimic very closely the properties of human skin. We growth the skin using different culture conditions.

Add a. More detailed information on the lipid organization.

We performed a large number of experiments. First of all we increased the CER1 levels in our lipid mixtures. It appeared to the CER1 level can be increased to 80% of the total CERs without a measurable change in the lipid organization. This is an important observation, as high level CER1 mixtures might be an excellent tool neutron diffraction studies.

As some of the samples in the neutron studies showed a different phase behaviour as observed in the X-ray diffraction studies, we performed several studies to determine whether this was due to the replacement of protonated lipid by their deuterated counterparts, or that it was due to another support on which the lipid mixtures are prepared.

Add b. Oriented membrane studies.

1. Most of the studies will be combined with FTIR measurments to fully characterize the phase behaviour of the oriented lipid mixtures.
2. Many of the studies performed in the previous session had to be repeated, due to the poor quality of the detector in the previous session. By using oriented lipid lamellae on a porous membrane, the phase behaviour of lipid mixtures mimicking the compositions in diseased skin is being studied. We measured the affect of variation in CER composition as well as changing the CER:CHOL:FFA ratio on the lipid phase behaviour. More specifically, we selected compositions mimicking the lipid organization in dry skin, in psoriasis skin, in cultured skin and in lamellar ichthyosis skin. The studies were successfully performed and a paper will be written.
3. A simplified model with only CER1, CHOL and FFA (variation in FFA composition) was studied. These studies composition was also studied. Measurements were performed successfully. We will combine these studies with FTIR measurements and a paper is currently in preparation.
4. We measured stratum corneum isolated from various reconstructed skin models. We will continue on this subject in the next beam session. Also in this case we will combine the studies with FTIR, but also with lipid analysis using massaspectrometry.