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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:** Lipid organization of mixtures containing ceramides	**Experiment number**: **26-02-389**
Beamline:BM26B	**Date(s) of experiment**: From: 18-02-2008 To: 22-02-2008	**Date of report**: 04- 03 -2008
Shifts:9	**Local contact(s)**:W. Bras	
Names and affiliations of applicants (* indicates experimentalists): **J.A. Bouwstra, G.S. Gooris, D. Groen**		

Report: (max. 2 pages)

We performed measurements during a 4-days session in february 2008. The beam conditions (beam intensity and beam alignment) were excellent, the detector condition however was not optimal. We were not able to perform all the scheduled experiments because of repair of the beamline. This reduced our beam time to 2 days. Especially measurements in which the lipid phase behaviour was studied as function of temperature were not all performed. Some studies with a controlled heating and cooling rate of the samples were successfully performed.

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 are present forming two crystalline lamellar phases with repeat distances of 6 and 13 nm. In our synthetic mixtures 5 different CER are present mimicking the lipid organization in the horny layer.
The aim of the present project was to study the lipid organisation of only i) CER1, CHOL and FFA and to alter the 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.

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

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 removed without changing the lipid organisation. 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 have been combined with FTIR studies. Publication has been submitted to BBA.
2. 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 successfully.

Recently we adjusted the spraying technique from the air-brush method to the linomat method, as this is a more reproducible method. The lipids can indeed be oriented similarly as in the horny layer, while the formation of the 6 and 13 nm phase still occurs. The choice of the equilibration temperature seems to be crucial in the formation of the 13 nm phase. Studies have been published.

3. By using oriented lipid lamellae, the phase behaviour of lipids mixtures mimicking the compositions in diseased skin is being studied. Changes in composition are variation in CER, short chain fatty acids, addition of cholesterol sulphate. Studies were carried out successfully and additional research on the lipid organisation using FTIR will be performed.
4. The first studies using a simplified model with only CER1, CHOL and varying FA composition was examined. Measurements were performed successfully. In the future, after additional (FTIR) measurements, the molecular organisation may be determined by using cholesterol sulphate. However, currently there are not yet enough measurements performed. This is an ungoing project.