DUBBLE	**Experiment title:** Skin Lipid organization in stratum corneum of diseased skin and various models.	**Experiment number**: **26-02-838**
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Report: (max. 2 pages)

During a 3-days session in November 2017, we performed measurements using the SAXS/WAXS setup. The beam conditions (beam intensity and beam alignment) were stable and excellent, only for a few minutes the beam was not available. We used the Pilatus 1M detector at a sample to detector distance of 216 cm for the SAXS and the Pilatus 3k detector at a distance of 31 cm for the WAXS. Good separation was achieved between diffraction peaks in the low q-range and weak peaks were detected.
Each sample was measured twice at two SAXS-detector positions to overcome the gap that separates the different modules in the detector. We did temperature dependent measurements of a few samples to examine their phase behavior and some scans along one of the axis of the sample to determine homogeneity. With our own software, we were able to make one image without gaps out of these 2 images and to merge the WAXS data and perform the integration over a part of the diffraction circle. Also the plots of the spectra were done by our own scripts (Python).
The skin barrier for diffusion of substances is located in the stratum corneum (SC), also referred to as horny layer, the outermost layer of the skin. The lipid matrix in this layer is composed of ceramides (CERs), cholesterol (CHOL) and long chain free fatty acids (FFAs) forming two crystalline lamellar phases with periodicities of 6 and 13 nm. These two phases are referred to as the short periodicity phase (SPP) and long periodicity phase (LPP), respectively. In diseased skin and in human skin equivalents (HSE, cultured from isolated human skin cells) the lipid composition, lipid organization and barrier properties are different from healthy skin. Currently, we are in the process of identifying the critical parameters for a proper barrier function in order to understand the impaired barrier function in diseased skin and in human skin equivalents.
Besides, we use a skin model of the same lipids sprayed on a porous membrane, in which we can change the composition to get a better understanding in the forming of the lamellar systems that form the barrier of the human skin.

Our goals for the present project were:

1.) In Leiden we generate an *in vitro* generated human skin used to study skin biology and to determine transport of drugs across the skin. However, currently this model does not have the same barrier properties as native human skin. One of the underlying factors is an altered lipid composition and organization in the stratum corneum. Currently we are in the process of optimizing the barrier properties of this model. Several approaches are used. 1) The culture medium used to generate this model is being optimized by supplementing the culture medium with an LXR agonist, and chitosan. 2) The effect of a change in environmental factors such as temperature, hydration level (human skin equivalents are generated air exposed) on the skin barrier properties are investigated. We isolate the stratum corneum after culturing the

HSEs and the stratum corneum lipid organization was measured at the ESRF.

2.) Recently we developed a simpler two CER SC mimicking lipid molecular model, which we aim to investigate the localization of the various lipid classes in the unit cell of the long periodicity phase (LPP). This model is under investigation in combination with neutron diffraction studies, During this experiment we aim to determine the lattice parameter (LP) and lateral packing of the samples to ensure the incorporation of the deuterated components would not affect the self-assembly.

3.) In addition, we aimed to study the importance of the components of the SC lipid model system in forming the LPP by systematically altering: the concentration of CER NS (most abundant in the skin) with its dihydrosphingosine counterpart (CER NdS) which has shown LPP stabilizing effects when included with CER NS containing models.

4.) In collaboration with the group of Sparr at the Univeristy of Lund who performed the NMR-studies we performed phase behavior studies as a function of temperature.

The following results were obtained:

1. Lipid barrier in human skin equivalents:
The isolated stratum corneum of human skin equivalents (HSEs) were assessed for the lamellar phase behavior and lateral packing. The combined effects of different optimization steps were studied. The HSEs measured were cultured under reduced oxygen, supplemented with an LXR agonist, and chitosan was added to the dermal compartment. All samples showed the formation of the lamellar phases.

2. The scattering from the simpler SC lipid mimicking model, with various deuterated components (added for neutron diffraction studies), was not entirely reproducible. Although the lateral packing for all measured samples was orthorhombic, the lamellar packing varied between samples with some samples demonstrating two lamellar phases while other showing a single phase. This indicates the sample preparation still requires further development.

3. During the replacement of CER NS with CER NdS, no large difference in the LPP and packing was observed regarded of the NS/NdS ratio 66.6/33.3 to 100/0. This implies that the sample preparation is now developed that additional stabilizing lipids are not required.

4. The phase behavior from orthorhombic to hexagonal and from hexagonal to liquid was followed in SAXS and WAXS simultaneously. The heating rate was 1°C per minute in warming up as well as in cooling down. The trajectory was varied between room temperature and 85°C. Several lamellar phases could be examined. The homogeneity of the sample was checked by programmed measuring at different sample positions. Only at a few places a different phase was detected.