DUBBLE	**Experiment title:** Lipids in stratum corneum model systems mimicking diseased skin.	**Experiment number**: **26-02-861**
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Report: (max. 2 pages)

During a 3-days session in July 2018, we performed measurements using the SAXS/WAXS setup. The beam conditions (beam intensity and beam alignment) were stable and excellent. We used the Pilatus 1M detector at a sample to detector distance of 198 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 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 40 degrees 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. This time the culture medium was supplemented with a PPAR isoform agonist. After isolation of the stratum corneum 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). We also aim to determine the effect lipid chain length for both the free fatty acid (FFA) and the ceramide (CER) components has in the simple model systems, investigating the lateral and lamellar packing.

3.) We altered the lipid composition in healthy human skin to mimic several changes in lipid composition reported in diseased skin. We aimed to study the lipid organization in the diseased skin and investigate how the various changes in lipid composition relate to the altered lipid organization in diseased skin

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

5) We study the lipid lamellar phase of synthetic murine SC: normal (wild-type – WT) and "diseased" (hyperlipidemic mouse model – APOE KO and SR-BI KO). The hyperlipidemic models show changes in skin lipid composition regarding free fatty acids (shorter and unsaturated chains). To investigate these changes we developed a synthetic membrane to mimic the different mouse SC compositions.

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. All conditions showed the presence of the lamellar phases.

2. The scattering showed an lipid chain length dependency on the lipid self-assembly. Regardless of the lipid type (i.e. ceramide (CER) or free fatty acid (FFA)), at shorter chain lengths, the density of the packing was reduced evident by the observed hexagonal packing. In contrast, longer chains (C22, C24) enabled the optimal packing density and lattice parameter size that is observed in natural stratum corneum. What was most interested was that the in between chain lengths (C20), and the extremely large chain lengths (C28), had the most disruptive behavior. These results imply that the lipid packing properties do not change linearly with lipid chain length.

3. The changes in the lipid composition affected the lamellar organization. Specifically, reduction in the level of the long chain acylceramide resulted in a reduction of the intensity and number of brag peaks of the LPP. Increase in the level of short chain ceramides resulted into a slight increase of the repeat distance of the LPP, while an increased level of short chain free fatty acids had no marked influence on the LPP.

4. The phase behavior from orthorhombic to hexagonal and from hexagonal to liquid was followed in SAXS and WAXS simultaneously. The temperatures at which we measured were 32°C, 55°C, 60°C and 70°C respectively. The same temperatures were used during a cooling down cycle. Several lamellar phases could be examined, in the dry state as well as in the hydrated state.

5. Murine SC lipid models: in the studied mixtures for both normal and diseased models we observed that the lipids were organized in two lamellar phases, LPP and SPP. The lipid mixtures of WT and SR-BI KO formed clear orthorhombic lateral packing while APOE KO model showed less dense packing, which is in agreements with our permeability studies. Thus, we can mimic murine SC by using lipid mixtures. The changes in lipid composition can be studied by X-ray diffraction to determine the effect on the lamellar and lateral phases.