DUBBLE	**Experiment title:** Lipids in stratum corneum model systems mimicking diseased skin.	**Experiment number**: **26-02-881**
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Report: (max. 2 pages)

During a 3-days session in October 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 205 cm for the SAXS and the Pilatus 3k detector at a distance of 29 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. With our own software (Python scripts), 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.
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.) Investigate the combination of 2 ceramides with different headgroup architecture, this in collaboration with a molecular dynamics simulating group in the U.S, Vanderbilt University in Nashville.

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 Lafleur at the University of Montreal, Canada who performed AFM-IR-studies, we performed phase behavior studies as a function of hydration in a 4 component system.

5.) To use lipid models as substitute for native skin in studies, it is important that the models exhibit the lipid organization similar to that of the native skin. We studied the lamellar organization as a function of equilibration temperature, sample heating up and cooling rate as well as duration of equilibration in both simple and complex lipid models.

6.) We investigated the importance of CER headgroups interactions for the formation of the LPP, by their subsequence reduction.

7.) We altered the chain length of the import ceramide EOS chain length, to determine if there is a linear relationship with the length of the long periodicity phase (LPP).

The following results were obtained:

1. When CER EOS (an exceptional ceramide (CER) that is only present in the stratum corneum) was part of the composition a single LPP phase was formed, without CER EOS, phase separation occurred.
2. The X-ray diffraction patterns showed a lipid chain length dependency on the lipid self-assembly. Regardless of the lipid type (i.e. 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. We didn't detect any phase separation, nor in the soaked buffer condition nor in the fully hydrated state.
5.) Temperature of annealing is important for the LPP formation. When equilibrated below the melting temperature of the lipid mixture, the characteristic lamellar phases were not formed while very high equilibration temperature resulted in a reduced fraction of lipids forming the LPP.
6.) The role of the ceramide headgroup that provides both the functional groups for hydrogen bonding as well adhering the two carbon chains together showed a significant change in the lipid packing after 30 mol% of the ceramide was exchanged with free fatty acids, hence removing the headgroup. The packing continued to deteriorate to multiple shorter phases as more ceramide headgroups were removed.
7.) The change in the carbon chain length of ceramide EOS did not show to have a clear relationship to the LPP. The lipids remained in an LPP, packed in the orthorhombic phase regardless of the chain length.