DUBBLE	**Experiment title:** Lipid organization in stratum corneum and lipid models.	**Experiment number**: **26-02-670**
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Report: (max. 2 pages)

During a 3-days session in Nov/Dec 2013, we performed measurements using the SAXS/WAXS setup. The beam conditions (beam intensity and beam alignment) were excellent and we used the Pilatus 1M detector at a sample to detector distance of 212 cm for the SAXS and 45 cm for the WAXS. Because of the high resolution of the detector, a good separation was achieved between diffraction peaks in the low q-range.
Every sample was measured twice at two detector positions to overcome the gap that separates the different modules in the detector. With the software available, we were able to make one image without gaps out of these 2 images.
The skin barrier for diffusion of substances is located in the 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 and 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. To gain insight in the phase behavior of lipid mixtures of synthetic CERs and CHOL with the variation of unsaturated FFA chains (C20 – C24) to determine whether the lamellar structure changes. We performed these measurements at room and skin temperature (32C).

2. To obtain information on the lipid organization after applying a formulation on ex vivo in vitro cultured skin, we measured the formulation and cultured skin. The formulations were applied during 8 days in the incubator in Leiden. The stratum corneum was isolated and measured.

3. We have also studied the interaction of the moisturizers ISIS (isostearyl isostearate), with the stratum corneum. ISIS was incremented from 3% to 15mol% to examine whether swelling with lipophilic substances is possible.

4. To study the effect of inflammation on the Lipid organization in stratum corneum of human skin

equivalents (HSE). We performed a series of measurements of human skin equivalents with inflammation markers in the culture medium.

The following results were obtained:

1. We used synthetic CER/CHOL/FFA in an equimolar ratio. Saturated FFAs were replaced by unsaturated ones. The chain lengths ranged from C20 to C24. This change in FFA composition is of interest as in atopic eczema (inflammatory skin disease) increased levels of unsaturated fatty acids are observed. Most of the samples formed an LPP and SPP.. In most samples unsaturation of FFA did not affect the formation of the LPP. So the addition of long chain FFAs did not affect the the formation of the LPP.

2. We performed a series of measurements with ex vivo skin treated with different formulations. Results showed that the topical formulations with CERs may be beneficial for the formation of LPP. This should be considered as a pilot study and will be continued in our next series of measurements.

3. The moisturizer (ISIS) containing samples did form only the LPP. The repeat distance increased gradually with the increment of ISIS molar ratio. This indicates a small amount of swelling in the LPP system. This offers the possibility to calculate the electron density profile of the LPP system.

4. HSE samples. We performed a series of measurements with human skin equivalents generated from the NTERT-1 cell line in which filaggrin was knocked down and inflammation markers were added to the culture medium. There is hardly any effect of filaggrin knockdown on the presence of the LPP and its repeat distance. However, cytokines added to the medium (inducing inflammation) during culture changes the peak shape and positions of the LPP. Detailed interpretations are ongoing.