DUBBLE	**Experiment title:** Lipid organization in skin models	**Experiment number**: **26-02-612**
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Report: (max. 2 pages)

During a 3-days session in February 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 205 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 ceramides and cholesterol with the addition of various unsaturated fatty acids to determine whether the lamellar structure changes or not.

2. To obtain information on the lipid organization of synthetic lipid mixtures of short chain ceramides.

3. Lipid organization in human skin equivalents (HSE) using a variation in culture conditions, such as inflammation and filaggrin knock down. Lipid organization in the stratum corneum cultured human skin explants.

The following results were obtained:

1. We used synthetic CER/CHOL/FFAs in an equimolar ratio where we substitute different saturated fatty acids with unsaturated ones. Most of the samples formed LPP and SPP with a certain repeat distance except where we used more than one unsaturation in the mixtures, we also noticed that the lipid organization is not well formed. So addition of more unsaturated fatty acids has an influence on the lipid lamellar organization.

2. We have also measured lipid samples prepared from synthetic CER/CHOL/FFA to form only LPP where we used different chain length of synthetic CER NS (Non hydroxy sphingosine) ranging from C16 to C24 to observe whether the repeat distance changes with increasing chain length of CER NS. Indeed the repeat distance changes with different chain length, which indicates that the chain length of CER NS has influence on the repeat distance of LPP.

3. HSE samples. We performed a series of measurements of human skin equivalents with inflammation markers in the culture medium. These studies showed that inflammation has an effect on the lipid organisation. Another study was performed with human skin explants in which the SC was removed by stripping with cyanoacrylate and thereafter the SC left to regrow in culture. This study showed that the lipid organisation in SC from stripped explants, grown in culture, differs from the native human SC. We also measured stratum corneum of HSE generated using NTERT cells (human keratinocyte cell line) .These showed a slightly different organisation. In addition, we studied the effect of filaggrin knockdown in these cultures on the lipid organisation. There is hardly any effect on the lipid organisation.