DUBBLE	**Experiment title:** **Lipid organization in stratum corneum sheets**	**Experiment number**: **26-02-597**
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Report: (max. 2 pages)

During a 3-days session in July 2012, we performed measurements using the SAXS setup. The beam conditions (beam intensity and beam alignment) were excellent and we used the new Pilatus 1M detector at a sample to detector distance of 205 cm. Because of the high resolution of the detector, a good separation was achieved between diffraction peaks in the low q-range.
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.

Our goals for the present project were:

1. To gain insight in the phase behavior of mixtures with pig CER: CHOL: FFA at different molar ratios to determine whether we can use samples with pig CERs to perform diffusion studies.

2. To obtain information on the lipid organization of synthetic lipid mixtures of various composition.

3. Lipid organization in human skin equivalents (HSE) using a variation in culture conditions, such as inflammation and filaggrin knockdown.

4. To obtain information on lipid composition of synthetic lipid mixtures with the addition of fragrances.

The following results were obtained:

1. We used isolated CERs from Pig skin and combined them with different amounts of CHOL and FFA to obtain samples with either only an LPP or only an SPP or both .We have measured those samples and indeed they form both LPP and/or SPP depending on the mixtures. So it is possible to use them for diffusion purposes.

2. We have measured lipid samples prepared from synthetic CER/CHOL/FFA to observe whether they form the LPP and SPP on porous membranes and whether these structures are influenced by adding fragrances and moistures that are used in crèmes. Indeed both lamellar phases are formed and an influence was detected but the changes were small and changes were not always reproducible. This was a successful repeat of previous studies.

3. We also performed studies using less complex systems to examine whether the lipid organisation is sensitive to the number of ceramides used. We used single ceramides especially to use the results in simulation studies to obtain more detailed insight in the arrangement of the lipids within the lamellae. We performed measurements with only two ceramides and were successful in preparing the LPP. These samples will be used later on for neutron studies at ILL in Nov 2012.

4. HSE samples. We performed measurements of SC sheets isolated from human skin equivalents. We performed a second 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. We also measured stratum corneum of HSE generated using NTERT cells (human keratinocyte cell line). .These showed a slightly different organisation. In additions we studied the effect of filaggrin knockdown in these cultures on the lipid organisation. There is hardly any effect on the lipid organisation.

5. We examined the phase behavior of some synthetic CER mixtures with deuterated CHOL and FFA before we used the neutron diffraction. In the mean time, these neutron diffraction studies have been performed successfully in July, 2012.