DUBBLE	**Experiment title:** **Lipid organization in stratum corneum sheets**	**Experiment number**: **26-02-576**
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Report: (max. 2 pages)

During a 3-days session in June 2011, 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 207 cm. Because of the high resolution of the detector, a good separation was achieved between diffraction peaks in close q-range. We were very happy with the automated shift in detector position, and software addition, that was arranged by the DUBBLE group. So we had an excellent series of measurements.
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 normal 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 to determine whether we can use samples with pig CERs to perform diffusion studies.

2. To obtain information on the lipid organization of stratum corneum of psoriasis patients.

3. Lipid organization in human skin equivalents (HSE) using a variation in culture conditions.

4. To obtain information on lipid composition of diseased skin lipid membranes.

The following results were obtained:

1. 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 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. Therefore, several of these samples will be measured in the next bean time session.

2. We also performed studies using less complex systems to examine whether the lipid organisation is sensitive to the various ceramides used. This is indeed the case. There is a big variation in lipid organsiation when using different ceramides.

3. We isolated ceramides from Pig skin and combined them with different amounts of cholesterol and FFA to obtain samples with either only an LPP or only an SPP. In future these samples will be used for neutron diffraction using deuterated moieties in either the fatty acids or the ceramides.

4. HSE samples. We performed measurements of SC sheets isolated from human skin equivalents. The measurements revealed that the stratum corneum of HSEs contains the LPP, regardless of the tissue culture method used. We performed the first series of measurements of human skin equivalents with inflammation markers in the culture medium. This resulted in slightly shorter repeat distances compared to controls. We also measured the lamellar phases in the stratum corneum isolated from biopsy outgrow. These studies revealed that the culture conditions are the cause of the change in lipid organisation.

5. We studied the lipid organisation in SC in psoriasis patients. In involved skin there is a big differences compared to that in healthy and uninvolved skin.

6. We examined the phase behavior of some synthetic CER mixtures with deuterated CHOL before we used the neutron diffraction. In the mean time, these neutron diffraction studies have been performed successfully in July, 2011.