DUBBLE	**Experiment title:** Lipid organization of ceramide containing mixtures and horny layer sheets	**Experiment number**: **26-02-522**
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Shifts:9	**Local contact(s)**:W. Bras	

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Report: (max. 2 pages)

We performed measurements during a 4-days session in February 2011 using the microfocus setup. The beam conditions (beam intensity and beam alignment) were excellent and the detector condition was similar to our previous session. Because of the high resolution of the detector we were able to measure both SAXD and WAXD in one detector screen and due to the microfocus setup a good separation was achieved between diffraction peaks in close q-range. The microfocus cross section is less than in the previous sessions (10x20 μm)

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 organisation 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 atopic dermatitis 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 results we obtained are:

1. We have measured lipid samples prepared from pigCER/CHOL and pigCER/CHOL/FFA to observed whether they form the LPP and SPP on porous membranes. These membranes will be used in future for diffusion studies. We successfully performed the measurements and indeed both lamellar phases are formed in pigCER/CHOL as well as in pigCER/CHOL/FFA mixtures.

2. The SC samples of atopic dermatitis patients have been measured. Some of the samples of last session were measured again due to an unexpected lipid phase behavior. However, there was also a new series of SC sheets harvested from patients and from healthy subjects. These measurements confirm the results of the previous session: lipid organization in patients is different from that in healthy controls.

3. 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. However, the presence of the SPP could not be detected in these cultures, while in the native skin tissue both the LPP and SPP are present. We also measured the lamellar phases in the stratum corneum isolated from biopsy outgrow. These studies revealed that the outgrow is less reproduble. In future studies we will optimize these culture conditions and perform additional measurements.

4. We repeated pilot studies were various lipid mixtures mimicking more closely the composition in human stratum corneum of atopic eczema patients.

Data acquisition was sufficient, but less excellent than in the previous sessions. Possibly the microfocus beam was to small, which resulted in less SC tissue in the beam (not always oriented completely parallel). We will try to sort this out in the coming months.