DUBBLE	**Experiment title:** Lipid organization of ceramide containing mixtures and horny layer sheets	**Experiment number**: **26-02-486**
Beamline:BM26B	**Date(s) of experiment**: From: 29-01-2010 To: 01-02-2010	**Date of report**: 09- 02 -2010
Shifts:9	**Local contact(s)**:W. Bras	

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Report: (max. 2 pages)

We performed measurements during a 4-days session in january 2010 using the microfocus setup. The beam conditions (beam intensity and beam alignment) were excellent and the detector condition had improved greatly as compared to our last 3 sessions. 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 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 recent studies using oriented lipid lamellae on a porous membrane the 13 nm lamellar phase (LPP) appeared to be crucial for a proper barrier function. 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 behaviour of simplified mixtures with synthetic CER:CHOL:FFA and to make a selection of the optimal samples for upcoming ILL neutron measurements, in order to unravel the molecular organisation of the LPP.

2. To obtain final results concerning mimicking the lipid phase behaviour of diseased skin in oriented lipid mixtures. These results will be related to permeability studies and FTIR studies performed in our lab.

3. Lipid organisation in a human skin equivalent (HSE): to provide detailed information on the lipid organisation of HSE that is cultured differently. Furthermore it is very important to perform these studies as function of temperature and to compare the lipid organisation in HSE to the lipid organisation in native human skin.

The results we obtained are:

1. We have measured the synthetic lipid samples with sufficient resolution to select the best samples for the upcoming neutron diffraction experiments. In future this will be a combined publication.

2. The lipid membranes mimicking the stratum corneum of diseased skin have all been measured with very good results; the long periodicity phase (LPP) (if present) could clearly be detected and separated from the short periodicity phase (SPP) due to the improved measurement conditions. These studies are now ready for publication.

3. 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. Measurements in which the position was slightly changed showed that there is a homogenous lipid organization in the stratum corneum of the different HSEs.

4. Pilot studies were performed with various lipid mixtures mimicking more closely the composition in human stratum corneum. These need to be analysed, but the data acquisition was excellent.

5. We performed some studies using mice stratum corneum. This is a collaboration with Prof Dr Holleran, UCSF, San Francisco.