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| DUBBLE | **Experiment title:**<br>Lipid organization in stratum corneum sheets | **Experiment number**:<br>**26-02-504** |
| **Beamline**:BM26B | **Date(s) of experiment**:<br>From: 25-06-2010<br>To: 29-06-2010 | **Date of report**:<br>25- 09 -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 July 2010 using the microfocus setup. We combined 2 granted proposals in one session, so the same report was submitted for both proposals. 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 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. We focused on the effect of the level of CER EOS on the lipid phase behaviour

**2**. To obtain information on the lipid organization of stratum corneum of atopic dermatitis patients.

**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. We decide to use samples with 40% CER EOS: this results in the formation of only the LPP. In addition the peaks are fully separated from the crystalline CHOL peaks. The neturon diffraction will take place in December 2010.

**2.** The SC samples of atopic dermatitis patients have been measured with fantastic results. We notice a relation between reduced skin barrier function and a change in the lipid organization. This should be investigated in more details in upcoming sessions.

**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. 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. Data acquisition was excellent.