



	Experiment title: Lipid organization in stratum corneum sheets	Experiment number: 26-02-554
Beamline: BM26B	Date(s) of experiment: From: 8-06-2011 To: 11-06-2011	Date of report: 23- 09 -2011
Shifts: 9	Local contact(s): W. Bras	
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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, although in a lot of cases we had to measure samples twice with a shifted detector position in order to overcome the different intermediate gaps that separate the detector modules from each other.

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 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 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 beam time session.
- 2.** 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.
- 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. We used HSE of different culture conditions. 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 culture conditions are the cause of the change in lipid organisation.
- 4.** We finished the biopsies studies of atopic eczema patients and found that part of the samples show differences in the diffraction patterns compared to healthy volunteers. These results indicate that a subgroup of AE patients have an altered lamellar lipid organization compared to healthy subjects.
- 5.** In vitro tape-stripped native skin: Measurements from SC sheets isolated from cultured tape-stripped human skin in vitro was performed. These skin explants were also cultured at different relative humidities (R.H). Results show that only the LPP could be detected in all cultures even at various R.H. However no significant difference was observed in the LPP with change in the R.H.
- 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.