DUBBLE	**Experiment title:** Lipid organization in stratum corneum and lipid models.	**Experiment number**: **26-02-691**
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Report: (max. 2 pages)

During a 3-days session in June 2014, we performed measurements using the SAXS/WAXS setup. The beam conditions (beam intensity and beam alignment) were reasonable at the start but during the first hours decreased to almost zero. By opening of the slits we could partially get the beam back on the samples. We used the Pilatus 1M detector at a sample to detector distance of 250 cm for the SAXS and the Pilatus 3k detector at 27 cm for the WAXS. Because of the high resolution of the detector, a good separation was achieved between diffraction peaks in the low q-range.
Every sample was measured twice at two SAXS-detector positions to overcome the gap that separates the different modules in the detector. With the software available, we were able to make one image without gaps out of these 2 images.
The skin barrier for diffusion of substances is located in the stratum corneum (SC), also referred to as 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 healthy 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.
Besides, we use a skin model of the same lipids sprayed on a porous membrane, in which we can change the composition to get a better understanding in the forming of the lamellar systems that form the barrier of the human skin.

Our goals for the present project were:

1. Currently we develop a model for studying skin barrier repair, also relevant for patients with Atopic Eczema. Skin from which the SC is removed generates new SC when cultured in an incubator. We optimised culture conditions, and composition of culture medium and this model is now used to study the effect of formulations on the formation of the lamellar phases during generation of SC in this model. To obtain information on the lipid organization after applying a formulation on ex vivo in vitro cultured skin, we also measured the formulation and the generated SC with and without applying formulations. The formulations were applied during 8 days in the ex vivo human skin in the incubator in Leiden. The stratum corneum was isolated and measured.

2. To study the effect of Vit C and E on the culturing of skin from isolated skin cells, we performed an

experiment with 3 donors and different concentrations Vit C and E in the medium. In house we will also perform mass spectrometry to examine the lipid composition of these samples.

3.) Kinetic studies. In collaboration with Prof R. Mendelsohn (New York) we have performed kinetic studies to examine the kinetics of phase behavior: samples consisting of CERs, CHOL and FA will be increased in temperature and quenched, after which the phase behavior will be examined in time. Mendelsohn performs the FTIR studies.

4.) To gain more inside in the effect of unsaturation, chain length distribution (fatty acids) and headgroup architecture (ceramides) on the phase behaviour of complex lipid systems, we examined the lipid organization of lipid membranes mimicking the lipid composition in stratum corneum. We varied the composition of this membrane to obtain more information on the underlying factors responsible for the altered lipid organization in diseased and cultured skin.

The following results were obtained:

1. Application of formulation on cultured skin:
The stratum corneum (upper layer) of human skin was removed by a stripping method. After that, the stripped skin and non-stripped control skin were cultured for 8 days in an incubator. On the stripped skin, a formulation was applied. The composition of the formulation varied, components that were in all formulations were: cholesterol, triglycerides, supersterol esters and squalene. Formulation also contained either one ceramide class (EOS30, NS24, NP24 or NP16) or one free fatty acid (16:0, 18:0 or 22:0). The control formulation did not contain any ceramides or free fatty acids. Vaseline and supersterol esters were also used as controls.
Results show that skin that was cultured with formulations that contain one of the ceramide classes forms lamellar lipid phases. However, formulations that contain NP24, NP16 or free fatty acids only showed crystallization, but also formed the lamellar phases. However, the phases are less abundant than with the ceramide formulations. CER NS containing formulations showed the best profiles. However, the studies need to be repeated. Application of Vaseline, often used to treat diseased skin, does not result in the formation of lamellar phases; application of the formulation with only supersterol esters resulted also in strong changes in lipid organization in the generated SC.

2. The supplementation of Vit C and Vit E during the culturing process enhanced the formation of the LPP in the human skin equivalents. We also examined the lipid organization of human skin equivalents generated in two different filter systems and we noticed large differences in the quality of the lipid organization in these cultures. In the same studies we examined the effect of the type of fibroblasts (various classes fibroblasts are in the skin) on the lipid organization in the SC.

3. We were able to monitor the kinetic behavior of a ceramide containing sample under humid conditions during recrystallization upon cooling and after cooling during a period the 48 hours in time intervals of 6 hours.

4. Several mixtures were measured from which the lamellar repeat distances were determined as well as the crystalline structure (WAXS). The lipid composition was changed. We included unsaturated fatty acids in the mixtures. In addition we reduced the CHOL levels. The changes in lipid organization in the mixtures provided us important insights in the understanding the deviation in lipid organization in diseased skin.