DUBBLE	**Experiment title:** Skin Lipid organization.	**Experiment number**: **26-02-772**
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Shifts:9	**Local contact(s)**: Daniel Hermida Merino	

Names and affiliations of applicants (* indicates experimentalists):

J.A. Bouwstra, G.S. Gooris*, R. Martins Cardoso*, A. Mieremet*, M.A.J. Berkers*

Report: (max. 2 pages)

During a 3-days session in June 2016, we performed measurements using the SAXS/WAXS setup. The beam conditions (beam intensity and beam alignment) were stable and excellent. We used the Pilatus 1M detector at a sample to detector distance of 207 cm for the SAXS and the Pilatus 3k detector at a distance of 27 cm for the WAXS. Although a part of the detector was damaged we could work around that. A good separation was achieved between diffraction peaks in the low q-range and weak peaks were detected.
Each sample was measured twice at two SAXS-detector positions to overcome the gap that separates the different modules in the detector. With the available software, we were able to make one image without gaps out of these 2 images. Our own software was used to merge the WAXS data and perform the integration.
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 skin and in 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. We studied the effect of formulation on the regenerated horny layer lipid organization

2.) To obtain information on the effect of a formulation on the lipid organization of regenerated skin in a clinical study, SC was harvested from volunteers after they applied a formulation for skin barrier repair during a period of 2 weeks (clinical study). The stratum corneum was isolated, transported to Grenoble and measured.

3.) In Leiden we generate an *in vitro* generated human skin used to study skin biology and to determine transport of drugs across the skin. However, currently this model does not have the same barrier properties as native human skin. One of the underlying factors is an altered lipid composition and organization. Currently we are in the process in optimizing the barrier properties of this model. Several approaches are used. 1) The culture medium used to generate this model is being optimized, such as level of glucose, insulin and isoproterenol. In addition several other supplements are added to the culture medium, such as vitamin D and antagonists/agonists of the liver-X-receptor. 2) The effect of a change in environmental factors such as temperature, hydration level (human skin equivalents are generated air exposed) on the skin barrier properties are investigated.

4.) Recently we developed a molecular model for the localization of the various lipid classes in the unit cell of the long periodicity phase (LPP). This model is based on neutron diffraction studies. Currently we study the effect of changes in the lipid composition to determine whether our molecular model can be confirmed or that we need to adjust this model to gain more insight in the importance of certain lipids to form this structure. In this session we focused on the different head groups of the ceramides.

The following results were obtained:

1. Application of formulation on the ex vivo human skin model
Application of the formulation showed slight changes in the lipid organization (lamellar phases) that were not consistent. However, it was shown that certain classes of ceramides phase separated in crystalline domains.
2. Application of formulation to test skin barrier repair on volunteers:
Formulation containing either two ceramide subclass (CER EOS30 and CER NS24:CER subclasses having different molecular architecture) and two free fatty acids (fatty acid chain length 16:0 and 18:0 carbon atoms) on barrier repair were studied. The X-ray diffraction profiles showed that after application of the formulation, the periodicity of the lipids in the SC is slightly higher compared to SC on which no formulation was applied.
3. Lipid barrier in human skin equivalents:
The isolated stratum corneum of human skin equivalents (HSE) was assessed for the lamellar phase behavior. Pilot studies were performed. The effects of agonists and antagonists on different nuclear receptors have been studied. We noticed differences in the lamellar phases, from which we conclude that the lamellar organization has been changed upon changing the medium composition.
Furthermore, the effect of the reduction of temperature during culturing of HSEs was determined.
A reduction in temperature, either severe or mild did not directly lead to an improvement of the formation of the lamellar phases. However, it provides important insight in the effects of this environmental condition and is therefore regarded as an excellent result.
4. In the mixtures with phytosphingosine ceramides (AP and NP mixtures), the lipids were organized in two lamellar phases, LPP and SPP. The other ceramide mixtures (ND, AS, NdS and NS+Nds) contained only one lamellar LPP phase. This suggests that ceramide subclasses NP and AP (especially AP which showed a much higher intensity SPP peak) may promote the formation of SPP. Repeat distances increased in case of the AP and NP mixtures.