DUBBLE	**Experiment title:** Skin Lipid organization.	**Experiment number**: **26-02-796**
Beamline:BM26B	**Date(s) of experiment**: From: 9-12-2016 To: 12-12-2016	**Date of report**: 03- 01 -2017
Shifts:9	**Local contact(s)**: Daniel Hermida Merino	
Names and affiliations of applicants (* indicates experimentalists): **J.A. Bouwstra, G.S. Gooris*, R. Martins Cardoso*, R.W.J. Helder*, M.A.J. Berkers***		

Report: (max. 2 pages)

During a 3-days session in December 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 216 cm for the SAXS and the Pilatus 3k detector at a distance of 26 cm for the WAXS. 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, over a part of the diffraction circle.
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. To obtain information on the lipid organization, SC was harvested from cultured skin after a skin barrier repair lipid formulation was applied during the culturing period of 8 days. The stratum corneum was isolated, transported to Grenoble and measured.

2.) 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 of optimizing the barrier properties of this model. Several approaches are used. 1) The culture medium used to generate this model is being optimized by supplementing the culture medium with PPAR agonist/antagonist, vitamin D and SCD1 inhibitor, palmitic acid level. 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.

3.) 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 mimicking different skin diseases.

4.) We study the lipid lamellar phase of synthetic murine SC: normal and diseased (atherosclerosis models). The atherosclerotic models show changes in skin lipid composition regarding ceramides (Cer EOS a ceramide with a long chain) and free fatty acids. To investigate these changes we developed a synthetic membrane to mimic the different mouse SC compositions.

The following results were obtained:

1. Application of formulation to test skin barrier repair on cultured skin from which SC has been removed:
Formulation containing a combination of ceramide subclasses (CER EOS30 and CER NS24:CER subclasses having different molecular architecture) and one free fatty acid (fatty acid chain length of 22:0 carbon atoms) on barrier repair were studied. The X-ray diffraction profiles showed that formulations containing the ceramide subclasses form lamellar phases also present in native human skin.
2. Lipid barrier in human skin equivalents:
The isolated stratum corneum of human skin equivalents (HSE) was assessed for the lamellar phase behavior. The effect of supplements: SCD1 inhibitor, palmitic acid variation, PPAR-α agonist and antagonists on the formation of the lamellar phases were measured. In addition a study involving reducing the relative humidity was also measured. All samples showed the formation of the lamellar phases.
3. Several lipid compositions mimicking the lipid organization in diseased skin, the repeat distance of the LPP was reduced. In addition some compositions resulted in an hexagonal phase (especially when the content of Cer EOS was reduced). The hydration did not affect the repeat distance.
4. Murine SC lipid models: in the studied mixtures for both normal and diseased models we observed that the lipids were organized in two lamellar phases, LPP and SPP but with a difference in repeat distance. An excellent correlation between forming of the LPP and increased Cer EOS content was observed in the models. The lipid mixtures also formed clear orthorhombic lateral packing. Thus, we can mimic murine SC by using lipid mixtures. The changes in lipid composition can be studied by X-ray diffraction to determine the effect on the lamellar phases.