DUBBLE	**Experiment title:** Skin Lipid organization.	**Experiment number**: **26-02-727**
Beamline:BM26B	**Date(s) of experiment**: From: 26-06-2015 To: 29-06-2015	**Date of report**: 16- 07 -2015
Shifts:9	**Local contact(s)**: Daniel Hermida Merino	
Names and affiliations of applicants (* indicates experimentalists): **J.A. Bouwstra, G.S. Gooris*, M.A.J. Berkers*, R. Helder*, M. Kamran***		

Report: (max. 2 pages)

During a 3-days session in June 2015, we performed measurements using the SAXS/WAXS setup. The beam conditions (beam intensity and beam alignment) were stable. 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. 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 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. To obtain information on the lipid organization after applying a formulation on ex vivo 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, transported to Grenoble and measured. We were able to detect the lamellar phases in the SC.

2.) Kinetic studies. In collaboration with Prof R. Mendelsohn (New York) we have performed kinetic studies to examine the kinetics of phase separation: samples consisting of CERs, CHOL and FFA were increased in

temperature and subsequently quenched, after which the phase behavior was examined in time. We observed very slow processes during the phase separation. Mendelsohn performed the FTIR studies using the same compositions of the lipid mixtures.

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 vitamin D supplemented in the medium. 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. 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.

5.) Effect of phospholipids on the lipid organization of the skin lipids**.**

The following results were obtained:

1. Application of formulation on ex vivo human skin barrier repair model:
Formulation containing either one ceramide subclass (CER EOS30 or CER NS24:CER subclasses having different molecular architecture) or one free fatty acid (fatty acid chain length 16:0, 18:0 or 22:0 carbon atoms) on barrier repair were studied. The X-ray diffraction profiles showed that formulations containing one of the ceramide subclasses forms lamellar phases also present in native human skin. Skin cultured with formulations containing one of the free fatty acids also formed the same lamellar phases, but crystallization of the fatty acid also occurs.

2. We were able to monitor the kinetic behavior of 2 samples prepared from CERs, CHOL and FFA(varying in composition) during recrystallization upon cooling and after cooling during a period of 30 hours measured at regular intervals. We were particularly interested in whether or not the various lipid classes crystallize in one lamellar phase or phase separate. A time interval of 6 hours measurements was performed.
3. Lipid barrier in human skin equivalents, a pilot study. We studied the effect of supplements in the medium: vitamin D, glucose, insulin, and isoproterenol on the formation of the lamellar phases were measured and there is an indication that these supplementations affect the lamellar phase behaviour. The supplements glucose, insulin, and isoproterenol have to be fine-tuned to further optimize the lipid skin barrier of the human skin equivalents. In addition a polymer has been added to the micro environment of the models. Both Vitamin D and the polymer chitosan resulted in an improvement of the formation of the lamellar phases.
4. The lipid compositional changes that were made to conform our molecular model for the localization of the lipid classes in the unit cell of the lamellar phase in SC confirmed our model. In future these results need to be confirmed and extended.
5. Adding phospholipids to skin lipids result in the formation of two different lamellar phases indicating the coexistence of phospholipid rich and a skin lipid rich phase. We have studied different molar ratios of phospholipid mixed with skin lipids, the repeat distance is effected by the amount of phospholipid in the composition.