DUBBLE	**Experiment title:** Lipid organization in stratum corneum and lipid models.	**Experiment number**: **26-02-702**
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Shifts:9	**Local contact(s)**:Giuseppe Portale	

Names and affiliations of applicants (* indicates experimentalists):

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

Report: (max. 2 pages)

During a 3-days session in November 2014, 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 200 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 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.) From excised human skin, skin cells are isolated: fibroblasts originate from the dermis and keratinocytes originate from the epidermis. These cells can be used to develop human skin equivalents. This is an *in vitro* generated human skin model is used to study skin biology and to determine transport of drugs. 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 organisation. Currently we are in the process in optimizing this model. Several approaches are used. 1) The culture medium used to generate this model is being optimized, such as level of glucose, insuline and vitamin D supplemented in the medium. 2) The environmental factors such as temperature, hydration level (human skin equivalents are generated air exposed) and a flow through system of the culture medium also plays a role.

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, CER NS24, CER NP24 orCER NP16: CER subclasses having slightly 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 ceramides one of the ceramide subclasses forms lamellar phases also present in native human skin. However, formulations that contain CER NP24 or CER NP16 showed crystallization on the stratum corneum surface. Skin cultured with formulations containing one of the free fatty acids also formed the same lamellar phases, but crystallisation of the fatty acid sometimes occurs.

2. We were able to monitor the kinetic behavior of 4 samples prepared from CERs, CHOL and FFA(varying in composition) during recrystallization upon cooling and after cooling during a period of 24 hours or 48 hours. A time interval of 6 hours measurements was performed. For two of the samples we could continue following the phase behavior during another 24 hours.

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 effect of vitamin C, vitamine D, microenvironment (human versus pig collagen), insuline, glucose on the formation of the lamellar phases was measured. Human versus pig collagen did not affect the formation of the lamellar phases. Vitamine D resulted in an improvement of the formation of the lamellar phases. Current results indicate that insulin, glucose and isoproterenol variation have to be fine-tuned to improve the extracellular lipid barrier.