<table>
<tr><td> </td><td>Experiment title:
Lipid organization in skin models</td><td>Experiment number:
26-02-637</td></tr>
<tr><td>Beamline:BM26B</td><td>Date(s) of experiment:
From: 28-06-2013
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24- 09 -2013</td></tr>
<tr><td>Shifts:9</td><td>Local contact(s):W. Bras</td></tr>
<tr><td colspan="3">Names and affiliations of applicants (* indicates experimentalists):
J.A. Bouwstra, G.S. Gooris*, L. Eweje*, E.H. Mojumdar*</td></tr>
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Report: (max. 2 pages)

During a 3-days session in June 2013, we performed measurements using the SAXS/WAXS setup. The beam conditions (beam intensity and beam alignment) were excellent and we used the Pilatus 1M detector at a sample to detector distance of 205 cm for the SAXS and 45 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 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 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. To gain insight in the phase behavior of lipid mixtures of synthetic CERs and CHOL with the variation of FFA chain (C16 – C28) to determine whether the lamellar structure changes or not.

2. To obtain information on the lipid organization of synthetic lipid mixtures where one of the long chain CERs known as CER EOS (esterified omega hydroxy sphingosine) was incremented from 15 mol% to 80 mol % as this CER is known to be crucial for the skin barrier.

3. We have also studied the interaction of the moisturizers ISIS (isostearyl isostearate), GMIS (glycerol monoisostearate) with the skin lipids and how they have influenced the lipid organization.

4. Lipid organization in human skin equivalents (HSE) using a variation in culture conditions, such as inflammation. We performed a series of measurements of human skin equivalents with inflammation markers

in the culture medium. These studies showed that inflammation has an effect on the lipid organization.

The following results were obtained:

1. We used synthetic CER/CHOL/FFA in an equimolar ratio where we used different saturated chain length of fatty acids ranging from C16 to C26. Most of the samples formed only LPP. But the repeat distance was increasing as the chain length of FA was increased. So the addition of long chain FFAs has an influence on increasing the repeat distance of the lipid lamellar organization.

2. The samples where we increased the CER EOS ratio were seemed to be more pronounced changed in repeat distance. As this CER is key component of the barrier, it also plays role in increasing the repeating unit of the lamellae with the increment of CER ratio. The repeat distance increased from ~12 to ~14 nm starting from 15 mol% CER EOS to 80 mol% CER EOS.

3. We did not obtained good results with the moisturizers adding to the synthetic lipid systems as what we expected. The moisturizer containing samples did not form the so called LPP and/or SPP. The reason might be due to the incorporation of large volume of moisturizers to the lipid systems. Therefore, we aimed to perform few more experiments in the next session also.

4. HSE samples. We performed a series of measurements of human skin equivalents with inflammation markers in the culture medium. These studies showed that inflammation has an effect on the lipid organisation. Another study was performed with human skin explants in which the SC was removed by stripping with cyanoacrylate and thereafter the SC left to regrow in culture. This study showed that the lipid organisation in SC from stripped explants, grown in culture, differs from the native human SC. We also measured stratum corneum of HSE generated using NTERT cells (human keratinocyte cell line) .These showed a slightly different organisation. In addition, we studied the effect of filaggrin knockdown in these cultures on the lipid organisation. There is hardly any effect on the lipid organisation.