STSM Scientific report

STSM Topic: Tape stripping and filaggrin

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Period
22/09/2014 to 01/10/2014

Reference code
COST-STSM-ECOST-STSM-TD1206-220914-049350

Background
The skin barrier has a complex structure designed to protect us from exogenous stressors in the environment including microorganisms, allergens and mechanical trauma. Filaggrin is an important protein in the upper layers of the skin (stratum corneum) and crucial for normal skin barrier functions. Together with its metabolites, they are involved in the intra- and extracellular architecture of the skin, skin hydration, UV-protection, acidification and the defense against microorganisms.

A way to estimate the amount of filaggrin protein in the skin is to do tape stripping and measure the amount of filaggrin's degradation products by High Performance Liquid Chromatography (HPLC). Tape stripping is an easy, quick, and minimally invasive method to obtain a sample of the stratum corneum. Round adhesive tape discs (Dsquame) are attached firmly to the skin, pressed for 10 seconds with a standardized force using a disc pressure applicator (CuDerm) and gently removed with tweezers.

Purpose of the visit
Sanja Kezic and her research team at the AMC and the Coronel Institute of Occupational Health are specialized in the tape stripping method and the use of HPLC to estimate the amount of filaggrin's degradation products. I have planned to use this method in several of my experimental studies during my PhD, and therefore I decided it would be of great value to learn the technique directly from Sanja and her research team. Furthermore I have limited laboratory experience and saw this as a perfect opportunity to learn some basic laboratory skills and how to behave around samples and laboratory equipment. I also

thought it would be a nice way to get to know another research team and hopefully develop a fundament for future collaborations. Additionally, I would get the opportunity to participate in two ongoing clinical studies of Sanja's PhD-student, Sjors A. Koppes. These studies involve tape stripping and measurements of skin properties such as barrier function, hydration and pH. More specific the aims for this STSM were;

1. Learn how to perform the tape stripping method
2. Learn how to analyze the tape stripping samples in the laboratory
3. Learn how to behave in a scientific laboratory and basic laboratory skills
4. Get to know a new scientific research group and help another PhD-student with his experimental studies

Work carried out during the visit

The first three days of my STSM I was together with Sjors A. Koppes at the VU Medical Centre in Amsterdam. Here we included patients and registered data for his first experimental study, which involves patients with atopic dermatitis and the effect of different topical treatments. For each patient we measured the transepidermal water loss (TEWL), skin redness, hydration and pH and performed tape stripping on two skin locations. The two last days of week 39, I spent at the Coronel Institute of Occupational Health, where I was introduced to the research team and shown around the laboratory. I had brought tape strip samples from Denmark, and I got to analyze these under the guidance of two trained laboratory analysts. I also got to see the installation of the equipment used for RAMAN spectroscopy.

The last three days of my STSM, I was at the Coronel Institute together with Sjors A. Koppes. We included patients and registered data for his second experimental study, also on patients with atopic dermatitis and the effect of different topical treatments. For each patient we measured the TEWL, skin redness, hydration and pH and performed tape stripping at four skin locations. We also did RAMAN spectroscopy at two locations.

Main results obtained

<u>Tape strip method</u>

During my time with Sjors A. Koppes, I was introduced to the proper way to do tape stripping, and I performed half of all the sampling from the participants in his study. I now feel that I have good knowledge about this technique, and that I can perform it in a proper way back in Denmark.

Figure 1. Tape stripping

Analyze of the tape strips

At the Coronel Institute I spent time with two trained laboratory analysts, and together with them I analyzed the samples that I had brought from Denmark. I took part in all the steps and now know how the analysis is done correctly and why the different parts of the analysis are necessary.

Figure 2. Preparation of the samples by adding ammonia 25%

Figure 3. Shaking of the samples

Figure 4. Evaporation of ammonium by the use of a centrifuge and vacuum at 60 ℃


Figure 4. HPLC, used to analyze the amount of NMFs in the tape strips

<u>Laboratory skills and how to behave in a scientific laboratory</u>

The laboratory analysts showed me how to use a pipette correctly and told how to handle the samples. I was explained the importance of thorough preparation and labeling of the samples, to avoid mix-ups during the analysis. They also showed me around the laboratory and told me what to be aware of to avoid exposure to ammonium that we used during the analyzing process.

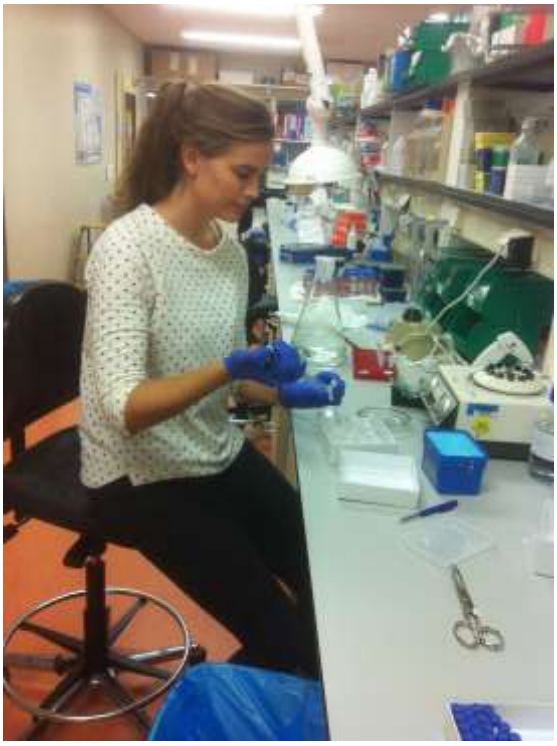

Figure 5. Working in the laboratory

Get to know a new scientific research group and help another PhD student with his experimental studies

During my stay I was introduced to the colleagues of Sanja and Sjors and they were very welcoming. I got to help Sjors with his experimental studies and I feel that a great foundation for future collaborations has been built.

Future collaboration with host institution

We will send all the tape strips from my experimental studies to Sanja and her team at the Coronel Institute for analysis of the degradation products of filaggrin, and because of this, we will have a close collaboration during the rest of my PhD period.

Projected publications/articles resulting or to result from the STSM

We will use the tape strip method in my experimental studies and at least three publications are planned from this work. Thanks to my STSM, I now know how to perform the method properly and I will have the right method description in my articles.

Other comments

I would like to greatly thank Sanja Kezic, Sjors A. Koppes and the laboratory analysts for allowing me to visit the AMC and Coronel Institute of Occupational Health, teaching me the tape stripping method and how to analyze the samples. I feel the visit was of great value and I bring back important knowledge to Denmark and my colleagues there.