Short Term Scientific Mission (STSM) Report

STSM details

Title	**Diffusion cell experiments with metal particles**
Participant	**Jolinde Kettelarij**
Host / Location	Dr. Francesca Larese-Filon, Unit of Occupational Medicine, University of Trieste, Italy
Period	1 – 14 March 2015

Objectives

The purpose of the STSM was to learn more about in vitro permeation experiments with metal particles and human skin using static diffusion Franz cells. The group of Francesca Larese-Filon has a broad experience with this type of experiments with a range of different metal particles and particle sizes. We would like to set up such experiments in our lab during my PhD, including metal particles from micron to nano size. This is why their experience was very valuable for me to learn from and bring back to our own lab.

Work description

In accordance with the objectives of the stay, the following work was carried out:

1) Theory behind skin absorption experiments

In short, skin absorption can be measured in vivo and in vitro. In vivo methods have the advantage of having kinetic and metabolic information. Advantages of in vitro methods over in vivo methods are e.g. that no animals have to be used, and human skin can be used which makes extrapolation of the results easier.

2) Set up of experiments with diffusion cells with human skin and metal particles

2.1) Particle characterization

We used citrate coated silver nanoparticles with a size of 10 nm according to the manufacturer. Particle size was confirmed with Transmission Electron Microscopy (TEM). The release of silver ions from these nanoparticles will be analysed later on in the project by filtering the solution and analysing the filtrate and the retained part with atomic absorption spectrometry (AAS).

2.2) Preparation of skin membranes

- The human skin membranes used for this study came from 3 different donors: 2 women, 1 man.
- The frozen skin membranes were put into physiological solution to thaw after which fat was removed
- A piece of skin the size of a donor chamber was cut and the thickness of the skin was measured with a caliper
- The skin pieces were dried very carefully with tissues and integrity was measured by measuring the transepidermal water loss (TEWL) with a vapometer; 3 measurements per skin

piece.

2.3) Setting up the in vitro diffusion system following the Franz method and performing experiments

- A table with 8 clean static diffusion Franz cells was prepared
- The temperature of the receptor compartment of the Franz cell was maintained at a stable 32°C by water that circulated in the jacket surrounding the compartment
- Physiological solution was prepared: 4.76 g Na2HPO4 + 0.38 g KH2PO4 + 18 g NaCl made up to volume with MQ water in a 2L volumetric flask. (NaH2PO4 • 2H2O 0.218 g).
- Each receptor compartment was filled with physiological solution
- 8 Franz cells in total:
    - 4 ml of donor phase, in this case 0.02 mg/ml citrate coated AgNP was added to five of the Franz cell donor chambers.
    - Two Franz cells were used as blanks and donor chambers were filled with 4 ml MQ water.
    - One donor chamber was filled with 160 ul PVP coated AgNP made up to a volume of 4 ml with MQ water. These are the same AgNP used in the former study on AgNP skin penetration in the same concentration as the citrate coated AgNP.
- At time intervals of 4, 8, 16, 20, 24 hours samples were taken from each receptor compartment with a syringe. At the same time, physiological solution was added to the receptor chamber to replace the sample that was taken.
- Samples were stored in the freezer (-25°C) until analysis.
- Besides the above receptor compartment samples, the following samples were collected after 24 hours and stored in the freezer (-25°C) until analysis:
    - Donor phase
    - The exposed skin area
    - Total solution from the receptor compartment

Main results

3) Analysis of the results

3.1) Skin content

Dermis and epidermis of 2 blanks and 5 samples were separated by heat shock and separately digested and mineralized. The final solution was made up to volume to 25 ml with MQ water. One skin sample is stored in the freezer until TEM analysis elsewhere (not performed during the STSM).

3.2) Measuring metal concentrations in solutions

The following samples were analysed for silver content with GF-AAS:

- Mineralized skin samples, dermis and epidermis
- Samples from receptor chamber for 20 and 24 hours

The donor chamber samples will be analysed later on with ICP-OES.

3.3) TEM analysis of skin

Unfortunately it was not possible to perform this analysis in such a short time frame, because preparation of skin samples for TEM analysis takes time.

3) How to interpret the analysed results

Not all samples could be analysed in this short time frame. For the samples that were analysed the results were inconclusive. The receptor chamber samples contained very low amounts of silver, close to the detection limit of the instrument. Further chemical analysis will be performed for these samples with a more sensitive technique, to be able to detect low levels of silver.
Analysis of the mineralized epidermis samples went well with GF-AAS, and results were in the range of what was found before for experiments performed with AgNP. However, analysis of the dermis samples revealed some strange results, because the silver content in the blanks was much

higher than in most exposed samples, and even higher than the epidermis blanks, which is strange. We went over the whole experiment and could not think of a step in which the samples could have become contaminated or where we could have switched them. Samples of unused skin of the same donors will be mineralized and analyzed for these two blanks, to see if anything is wrong with the skin itself.

Future collaboration with host institution

No clear arrangements have been made for future collaboration, although we would be pleased to work together with the group of Francesca Larese-Filon again.

Forseen articles or publications resulting from the STSM

I have drafted an introduction for a possible article about the experiments performed during the STSM. Hopefully the results of this research will result in a publication.

Other comments

During these two weeks I got to see the set-up of an entire static diffusion Franz cell experiment including sample analysis. This way I learned all kinds of details that you cannot learn from simply reading a manual, like how to handle the skin samples and finding the easiest way to take samples from the receptor compartment. This practical experience will make it much easier to set up this type of experiments in our own lab in Sweden.

In addition, I learned about the broad field of metal nanoparticles, specifically silver nanoparticles, and what difficulties you might come across when using them in experiments. For example, we discussed the function of the different particle coatings and the influence of coating and medium on dissolving nanoparticles. Furthermore, we looked at different TEM images that they had from former experiments with different types of nanoparticles, which showed the influence of coating and medium for different elements. This knowledge will be helpful when designing our own experiments and choosing the types of particles we want to use.

It will be interesting to see what the final results of the experiments with AgNP will reveal and maybe further testing is needed to get a larger set of samples. I hope to stay in contact with the group in Trieste about this.

I want to thank Francesca Larese-Filon, Marcella Mauro and Matteo Crosera for giving me the opportunity to come to Trieste, showing me around at the Occupational Medicine department in the hospital, and getting to learn their methods in the lab. I would also like to thank COST StanDerm for giving me the opportunity to go to Trieste.

Jolinde Kettelarij

27 March 2015