	Experiment title: Silver nanoparticles accumulation and fate in macrophages and gut cells	**Experiment number**: MD902
Beamline:	**Date of experiment**: from: 08/04/2015 to: 14/04/2015	**Date of report**: 01/06/2015
Shifts:	**Local contact(s)**: Giulia Veronesi	*Received at ESRF:*
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Report:

Introduction:

The objectives of the proposal were to follow Ag (NP and/or ions) translocation through the gut by µX-ray fluorescence imaging (µXRF), and to follow Ag-NP dissolution by in situ µX-ray absorption spectroscopy (µXAS) inside gut cells. We finally chose to work with lung cell models rather than intestinal tissues, choosing the A549 human lung alveolar epithelial cell line, representative of the epithelial components of the lung. This refers to inhalation exposure to nanoparticles rather than ingestion exposure, which is even more important in terms of risk to human health posed by nanoparticle exposure. This choice was driven by preliminary ICP-MS measurements of intracellular Ag accumulation in both intestinal and lung cell models, which had shown that lung cells accumulated much more Ag NPs than intestinal cells. Therefore, we thought that the level of Ag in intestinal cells would not be sufficient for proper identification and analysis of Ag distribution, and more importantly Ag speciation inside cells, while the concentration inside lung cells would be more suitable for µXRF and µXAS analysis.

Materials and methods:

The A549 alveolar epithelial cell line (ATCC CCL-185) was grown on three types of supports and exposed to Ag-NPs from two origins, i.e., 60 nm PVP-coated Ag NPs from Sigma Aldrich (#758329) and NM300K Ag-NPs provided by the NM repository at the Joint Research Center (Ispra, Italy). The exposure conditions were both acute exposure for 24 h or 48 h and repeated exposure for 4 consecutive days. Two sample preparation procedures were used, i) cell growth on Si3N4 membranes, and direct fixation and analysis on the membranes, ii) cells grown on transwell inserts, then embedded in OCT resin and cut in thin sections (10 and 20 µm) using the ID21 cryomicrotome, and iii) cells grown on ultralene films to be directly analysed. Samples were mapped at 3.42 keV, with a beam size of 0.38 x 0.83 µm² (V x H).

Results:

When analysing samples grown on ultralene films, beam damage was very intense, i.e., samples seemed to melt when the beam was applied. Therefore, we did not further analyse these samples.

Analysis of thin sections (20 µm) of cells grown on transwell inserts showed some cells that could be analysed and contained high levels of Ag. XAS analysis on Ag-rich regions showed diverse Ag speciation, depending on the intracellular location. Some Ag-rich regions contained Ag in the Ag(I)-S chemical configuration,

representative of Ag NPs that had dissolved intracellularly and recombined with S-rich cellular molecules (either glutathione or S-containing proteins), while other regions rather showed reduced Ag (Ag°), representative of intact Ag NPs. In some regions, it was not posible to acquire XAS spectra due to beam shift.

The best sample preparation was cells grown and exposed on Si3N4 membranes, where the most stable samples and the highest cell density was obtained. In this condition, we acquired images of control (unexposed) cells, then cells exposed for 24 h or 48 h to NM300K and for 48 h to Ag-PVP. Figure 1 shows µXRF maps of A549 cells exposed to NM300 k for 48 h, showing intense intracellular accumulation of Ag, which located around the cell nucleus, representative of distribution of Ag-NPs in late endosomes and/or lysosomes. Unfortunately, we were not able to acquire XAS spectra on these samples, as no Ag was detected when the beam was set for XAS analysis. Our hypothesis is that the beam location shifted out of the Ag-rich areas when changing the settings from the imaging configuration to the XAS analysis configuration.

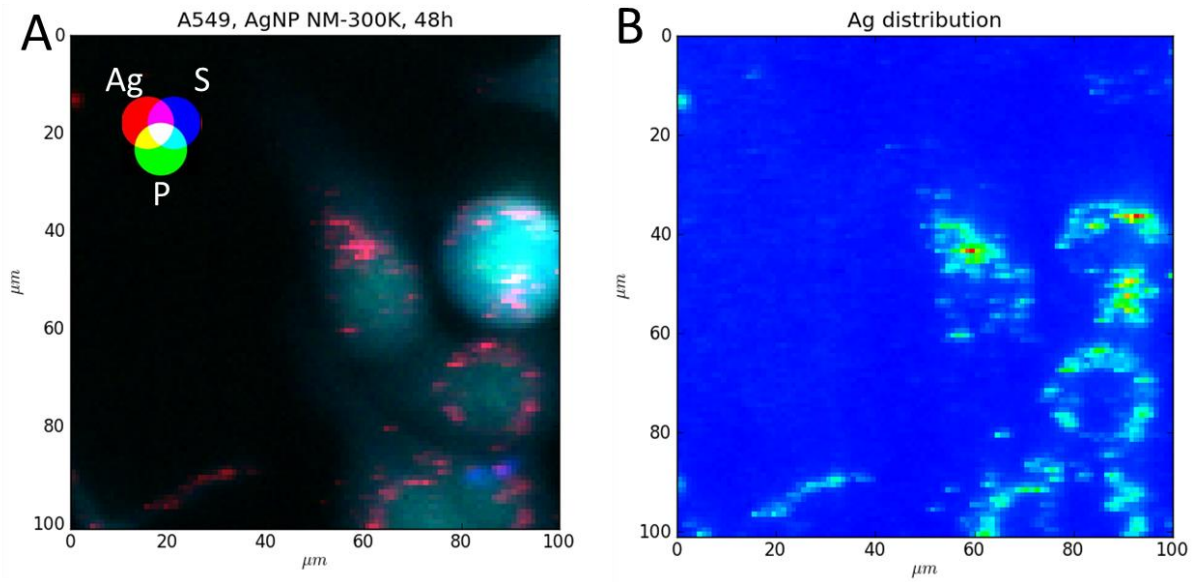

Figure 1. µXRF image of A549 cells exposed to NM300K Ag nanoparticles for 48 h, showing intracellular accumulation arounf the cell nucleus.

Conclusions:

This experiment enabled to choose the best cell sample preparation procedure, which was Si3N4 cell culture and exposure, followed by cryofixation and direct analysis on the cryostage. Our results showed that Ag-NP accumulated inside A549 human alveolar epithelial cells and localised around the cell nucleus, suggesting their distribution in late endosomes and/or lysosomes. XAS analysis showed that Ag NPs were either intact inside cells, or had dissolved in the intracellular compartments, leading to Ag ion recombination with intracellular S-rich biomolecules that could be either small molecules such as glutathione, or proteins like metallothioneins. Still, since µXRF imaging and µXAS analysis were not performed on the same cell preparation, thiese results could not be valorised and would necessitate a follow-up experiment.