EXPERIMENTAL REPORT MD904

Context:

Faced with the development of nanotechnologies, and in spite of potential benefits in numerous domains, fear is issued as for the possible human toxicity of manufactured nanoparticles (NP), particularly at the respiratory level since it is the main exposure route to nanoparticles. As of now, studies on NP toxicity have essentially focused on adults, and there are currently no data in the literature regarding the effects, on the descendants, of maternal respiratory exposure to NP during pregnancy. In the present proposal, we proposed to use X-ray micro-fluorescence (μXRF) to locate Ti, Ce and Ag elemental signals in lung and placenta tissue sections from mice pups whose mothers have been exposed, at the respiratory level, to these NP during pregnancy.

Experimental set-up:

Paraffin-embedded placenta and lung tissue sections (10 μm thick) were obtained from pups born after maternal exposure, during pregnancy, to TiO_2, CeO_2 or Ag NP. The tissue sections have been inserted between two ultralene foils. Unexposed pups and NP deposited on ultralene foils provided a control signal for comparison. The beam focalization was assured by Kirkpatrick Baez (KB) mirror, which allowed the beam focalization down to 0.4*0.9 μm^2 while fluorescence spectrum was recorded on a silicon drift diode (SDD, Bruker). Measurements were carried out on ID21 beamline, at 2 energies. The first energy chosen was 6.8 keV, which allowed investigating the Ti Kα edge as well as the Ag L_2 and L_3 edges. The detector escape peak prevented from measuring Ce L_1 edge, wherefrom the choice of a second energy, at 5.8 keV, which allowed us to measure both Ce and Ag signals. The images of the localization of the different NP in both tissues were obtained by mapping the X-ray fluorescence of S, to localize the organ of interest (placenta or lungs), together with that of Ti, Ce or Ag.

Results:

As the biological results obtained in Ag-exposed animals were the most interesting ones (i.e. decreased weight of the pups at birth, increased fetal resorption, lung histological alterations after 49 days of life as compared to unexposed animals), we mostly focus our attention on Ag-exposed animals in first intent (Figure 1).

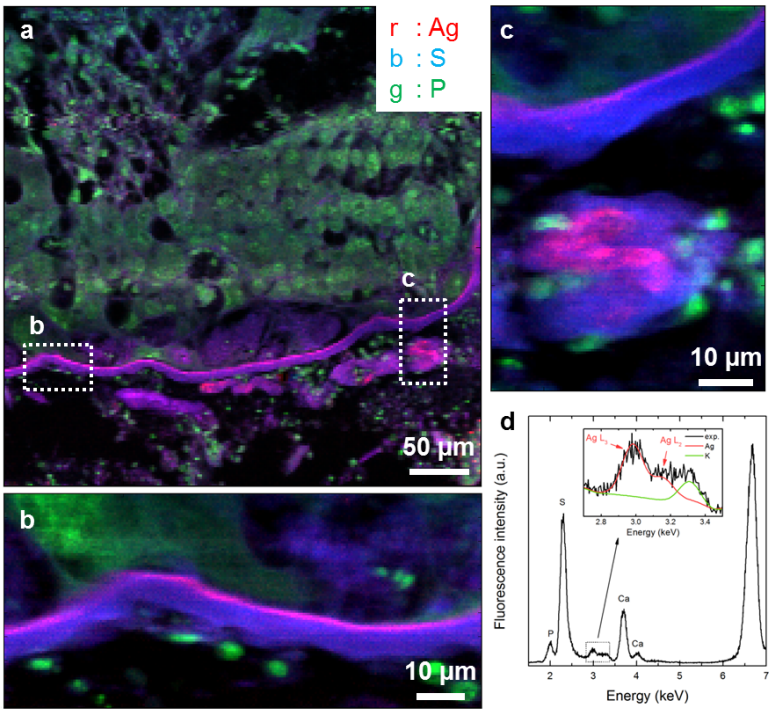

Figure 1: Fluoresence maps obtained in placenta tissue sections of an Ag-exposed animal. Signals for Ag, S and P appear in Red, Blue and Green respectively (Panels a, b and c). Typical fluorescence sepctra obtained in Ag-rich regions, is shown in Panel d.

From the different maps we obtained, we could detect a signal for Ag essentially at the level of the chorionic membrane in all 3 placenta samples that we observed (see Figure 1b). Ag signal was also detected in labyrinth and spongiotrophoblastic regions, although at a lesser extent. In lung samples, Ag signal was also detected, mainly in the airways (probably macrophages) as shown in Figure 2.

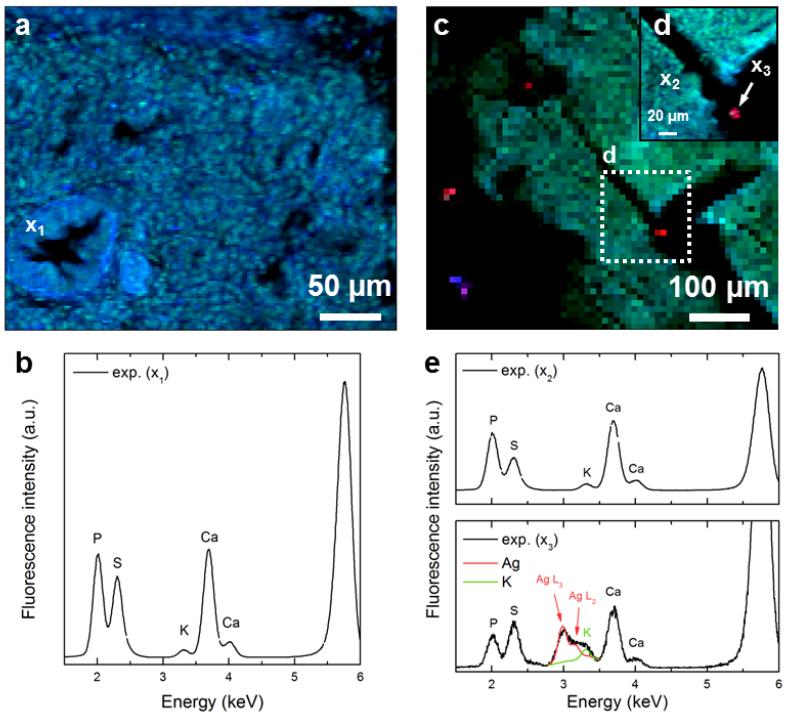

Figure 2: Fluorescence maps obtained in lung tissue sections of a Control (Panel a) or an Ag-exposed animal (Panel c and d). Signals for Ag, S and P appear in Red, Blue and Green respectively. Typical fluorescence spectra obtained in the Control animal (Panel b) and in the Ag-exposed animal (Panel e, upper spectrum, region with no Ag; Panel e, lower spectrum, Ag-rich region).

The detailed experimental results will be part of a publication currently under preparation.

It must be noted that we went through a lot of technical issues during the experiment:

computer command interface not fully operational, with frequent crashes, unstable beam. Overall, we lost almost 3 shifts.

Communication:

Exposure to manufactured nanoparticles during gestation: impact on the respiratory tract of the offspring in a mouse model. Emmanuel PAUL, Marie-Laure FRANCO-MONTOYA, Jérôme ROSE, Jorge BOCZKOWSKI, Sophie LANONE, Christophe DELACOURT, Jean-Claude PAIRON. *Journées de Recherche Respiratoire (J2R), Dijon, October 2015. Oral communication.*