	Experiment title: RESPIRATORY EFFECTS OF CARBON NANOTUBES : CHEMICAL TRANSFORMATION OF IRON CATALYST IN MACROPHAGES	**Experiment number**: SC2950
Beamline: ID21	**Date of experiment**: from: 16/06/2010 to: 21/06/2010	**Date of report**: 28/08/2010
Shifts: 15	**Local contact(s)**: Barbara Fayard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

J. Cambedouzou*, P. Launois, J. Doucet, LPS, UMR CNRS 8502, Bât. 510, Univ. Paris Sud, 91405 Orsay Cedex

S. Lanone*, J. Boczkowski, INSERM, U955 Faculté de Médecine, 8 rue Gal Sarrail, 94000 Créteil

M. Pinault*, M. Mayne-L'Hermite, CEA Saclay-Lab. F. Perrin/CEA-CNRS/URA 2453 Service des Photons Atomes et Molécules DSM-IRAMIS-SPAM Bat 522 F-91191 Gif-sur-Yvette Cedex

M. Cotte, Centre Reche. et Restau. des Musées de France C2RMF/LRMF CNRS UMR-171 Palais du

Report:

1. Context

Carbon nanotube (NT) production is constantly increasing due to extending domains of utilizations (materials, medicine, etc.). At the same time, the respiratory toxicity of NT-based products has to be carefully analyzed, as workers might be exposed to them in their workplace. It is important to disentangle biological effects of carbon nanotubes from those coming from catalyst nanoparticles, which may also induce toxic effects. Such nanoparticles can be found in two different forms: attached to the nanotubes or inside them, depending on synthesis procedures. In the present experiment, we focused on the fate of the iron-based particles attached to single-walled carbon nanotubes (SWNT) and inside multi-walled carbon nanotubes (MWNT) after cellular uptake of NT by macrophages in vitro. Their chemical state was analyzed by performing micro-XANES spectroscopy.

2. Experimental

Macrophages (RAW264.7 cell line) were exposed selectively to three types of NT samples, namely HiPCO SWNT of 'Raw' commercial grade, that we simply be noted 'SW' here, long MWNT and cut MWNT. The latter endured a 7-week long ultrasonication treatment resulting in the cutting and the shortening of the MWNT [1]. In these cut MWNT, iron nanoparticles bio-accessibility is expected to be higher. Each NT sample was dispersed in cell culture medium at a concentration of 50 µg/mL, and used to expose macrophages, already grown on an ultralene film, for 24 hours. At the end of the exposure, cells were cryofixed and further lyophilized.

Measurements were performed on the ID21 beamline at energies near the Fe Kα edge. Beam focalisation was assured by Kirkpatrick Baez (KB) mirrors allowing to reach a beam size of 0.4*0.9 µm². A silicon drift detector (Rontek) was used for fluorescence measurements.

3. Results

Reference spectra were recorded on areas of 200µm diameter (without beam focalization), on a metallic iron foil, Fe_3O_4 nanoparticles, and NT references (without any cell). The first two spectra were used as reference spectra for iron and iron oxyde, while those on NT were compared to spectra taken with the nanobeam, allowing us to conclude that *the nanobeam intensity did not induce noticeable photoreduction effects by itself*. NT spectra are fitted using linear combinations of iron, iron oxyde and Fe_3C spectra (for the latter XANES profile, see ref. [2]). It follows that i) the XANES of SW is strongly dominated by the signal of Fe_3C, ii) a majority of Fe_3C is found in long MWNT with a hint of oxide and metal Fe, and iii) a majority of iron oxide is found in cut MWNT.

We measured, using microbeam focusing, the XANES spectra of the iron nanoparticles inside long or cut MWNT, or attached to SWNT, after macrophage exposure to NT. Their detailed refinement is still in progress, but here is summarized the most interesting result (original with respect to the literature), obtained for the nanoparticles attached to SWNT. Such nanoparticles are encapsulated in graphenic coques that 'fuse' with the NT wall outside [3].

Spectra were recorded on areas were iron is co-localized with elements characteristic of cellular materials (P and K) and where the iron map is clearly contained inside the cellular contour drawn by the P and K maps (see *experimental reports MD280* and ref. [4] for NT internalization in cells). Such an area is referred to as area 'in' in the figure. Spectra were also recorded on areas where SWNT and thus iron nanoparticles are not incorporated inside macrophages. Such an area is referred as 'out' in the figure. The XANES profiles measured in the 'in' and 'out' areas are diplayed in the right part of the figure. XANES profiles measured in 'out' zones were systematically found identical to the reference XANES profile recorded on a 200µm area, showing that even using a micron-size beam, the resulting XANES is a statistical sum of all iron-based crystallites contained in the sample. This is compatible with the size of iron nanoparticles which were found to be of a few nm large in transmission electron microscopy images [1,3]. We observed that the profile of the iron absorption edge was different if spectra were recorded on 'in' areas. It shows that the chemical state of iron nanoparticles changes when SWNT are inside macrophages. A correct agreement between experimental data observed for 'in' areas and linear combinations of reference spectra is obtained considering a combination of 60% of Fe_3C, 30% of metal iron and 10% of Fe_3O_4. This indicates that a large part of the cementite is reduced in the cell while a smaller part is oxidized. It indicates that while encapsulated in graphenic sheets, in cells, iron is eventually accessible and further transformed. The main transformation turns out to be the *decarbonation of Fe_3C*. The associated biological mechanisms and toxicological effects are to be further investigated.

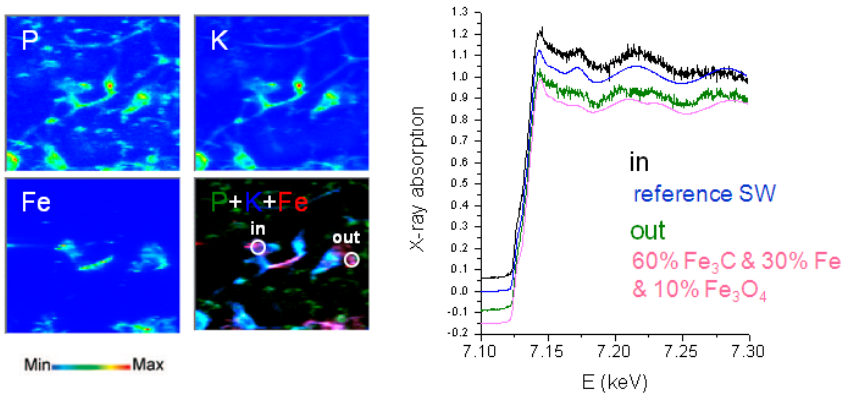

Figure 2 : *Left*: fluorescence maps of P, K and Fe of 100x100 µm² area of macrophages exposed to SWNT. *Right*: XANES spectra recorded using a submicronic beam in the indicated areas, together with those obtained for reference SWNT as well as linear combination of reference spectra of iron carbide, iron and iron oxide.

We finally underline that the *latest developments of ID21* involving KB mirrors and a silicon drift detector considerably increased the performances of the spectrometer, allowing us to detect endogeneous iron in control cells (which was not possible in our previous experiments [4]). We measured the XANES spectrum of endogeneous Fe by adding a large number of spectra on various areas containing iron in control cells. The XANES profile displays a marked oxide character.

[1] C. Bussy et al., *in preparation*
[2] N.S. Kopelev et al., Chem. Mater. **1995, 7,** 1419
[3] G. Charron et al., New J. Chem. **2009**, 33, 1211
[4] C. Bussy et al., Nano Letters **2008**, 8, 2659