ESRF	**Experiment title:** RESPIRATORY EFFECTS OF CARBON NANOTUBES: THE ROLE OF INTRACELLULAR pH ON CHEMICAL TRANSFORMATION OF IRON CATALYST NANOPARTICLES INSIDE MACROPHAGES	**Experiment number**: MD589
Beamline: ID 21	**Date of experiment**: from: 01/06/2011 to: 06/06/2011	**Date of report**: 27/08/2011
Shifts: 15	**Local contact(s)**: Murielle Salome	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

E. Paineau*, P. Launois*, B. Fayard*, 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

Report:

While the use of carbon nanotubes (CNT) in composite materials is still rising, promising applications of CNT in nanomedicine have emerged in the last few years [1]. Meanwhile, the safety of CNT-based products continues to be under investigation. In particular, our interest is placed in distinguishing the respective biological roles of CNT themselves and of remaining catalyst nanoparticles present in CNT samples. Cellular components such as phagolysosomes, presenting an acidic pH when activated, are known to take in charge exogenous materials, and could therefore participate in CNT biological effects. Thus, it appeared particularly relevant to assess the role of acid pH on the chemical transformation of catalyst nanoparticles.We have recently shown the relevance of X-ray fluorescence (XRF) microscopy in localizing catalyst nanoparticles in macrophages [2]. In the present experiment, we studied the chemical state modification of CNT catalyst nanoparticles by µXANES spectroscopy on macrophages exposed to Single Wall Carbon Nanotubes (SWCNT), in presence or in absence of a proton pump inhibitor.

Experimental

Murin macrophages (RAW 264.7 cell line) were grown on ultralene films and exposed to SWCNT (50 µg/ml) in presence or in absence of a proton pump inhibitor, concanamycine (10 nM). The exposure times were of 3, 6 or 24 hours. At the end of exposure, cells were cryofixed and further lyophilized. Non-exposed cells with or without Concanamycine as well as SWCNT samples not incubated with cells were also prepared as references. Measurements were carried out on the ID21 beamline at an energy set just above the Fe Kα edge (about 7.12

keV). A Kirkpatrick Baez (KB) mirrors system allowed the beam focalisation down to 0.4*0.9 µm2 and fluorescence spectrum was recorded on a silicon drift diode (SDD). Fluorescence mapping allowed us to localize nanoparticles in macrophages [2]. To assess the chemical state of catalyst nanoparticles, µXANES spectra were performed in areas presenting high intra-cellular Fe concentrations as assessed by XRF. Calibration of the set-up was carried out by recording the spectra of a metallic iron foil.

Results

We measured on areas of 200 µm diameter (without beam focalization), as well as with beam focusing (0.4*0.9 µm²), the XANES spectra of SWCNT samples (without any cell) at different pH (4.5 and 7.2) and time exposure. The obtained spectra correspond only to Fe_3C (cementite) particles [3]. Our measurements also allowed us to ascertain that the nanoparticles are not transformed under the intense focused beam. With microbeam focusing, the recent enhancement of the beamline (larger detector surface) enables us to measure endogeneous iron on various areas in control cells. The XANES spectrum displays a good agreement with that of the ferritin, a common intracellular iron storage protein. Similar results have been obtained in control cells with concanamycine.

Focusing now on cells exposed to SWCNT, and having shown that iron areas out of cells were mainly composed of cementite, we investigated areas corresponding to iron nanoparticles inside cells, where the iron map is clearly co-localized with P and S, known as representative elements of cellular materials. In all cases, the pre-edge peak and the intensity of the white line (7.12-7.15 keV) are rather similar and compatible to those obtained for cementite. However, extended XANES spectra (up to 7.30 keV) reveal differences, suggesting modifications of the short-range local order around iron atoms (figure A). It is worth to note that multiple scattering effects occur in this energy range and prevent us to precisely describe the chemical changes of iron compounds. In this framework, we observed two main “new phases” and employed them as “references”. Using such an assumption, we were able to reproduce all measured curves (~ 90) considering a linear combination of cementite and of these biotransformed phases. Hence, we observed a marked decrease of the amount of cementite with increasing exposure time, revealing a sharp interaction of iron-based nanoparticles with macrophages (figure B).

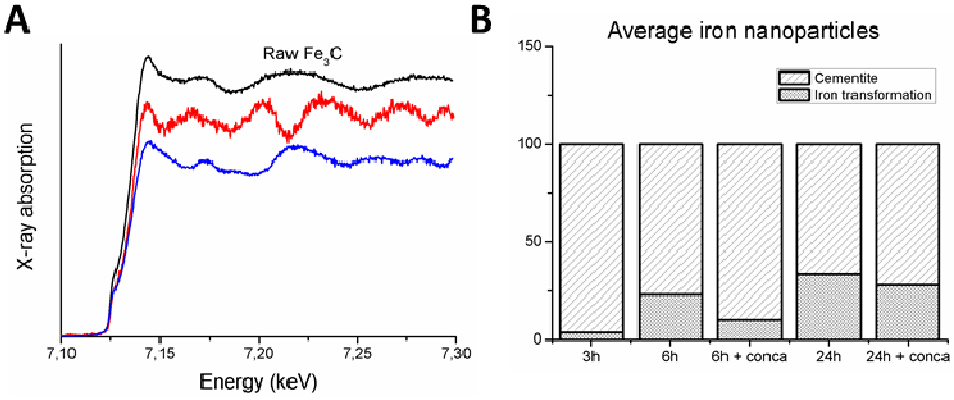

Figure: A. µXANES spectra of cementite (black) and modified iron-based nanoparticles (red and blue). B. Average amount of cementite and modified iron-based nanoparticles at different exposure time and in presence or absence of concanamycine.

The effect of concanamycine is rather limited, tending to limit the amount of iron transformation up to 6h but failing to protect cementite from chemical modifications at higher exposure time. Notwithstanding, comparing these results with those obtained from µXRF measurements, we observed a linear dependence between the ratio of biotransformed iron and the average amount of Ca in cells, confirming the role of Ca in the biological

response of macrophages to CNT. Although, the main transformation was previously suggested to be the decarbonation of Fe_3C (see experimental report SC2950), these experimental results – with much larger statistics thanks to beamline improvements – support the possibility of different atomic local ordering. The nature of these "phases" is to be further investigated by coupling XANES to others XAS techniques like µEXAFS spectroscopy.
The detailed experimental results have been reported through different communications and a publication is under preparation.

[1] K. Kostarelos et al., *Nature Nanotech.*, 2009, 4, 627
[2] C. Bussy et al., *Nano Letters*, 2008, 8, 2659
[3] N.S. Kopelev et al., *Chem. Mater.* 1995, 7, 1419

Communications:

Biotransformation of iron-based nanoparticles attached to carbon nanotubes inside cells; C. Bussy, E. Paineau, J. Cambedouzou, M. Salomé, B. Fayard, M. Pinault, N. Brun, C. Mory, J. Boczkowski, S. Lanone and P. Launois; *4th Carbon Nanomaterial Biology, Medicine & Toxicology, NT11 Satellite Symposium, Cambridge, UK, July 15-16th, 2011. (oral communication)*

Biotransformation of iron-based nanoparticles attached to carbon nanotubes inside cells; C. Bussy, E. Paineau, J. Cambedouzou, M. Salomé, B. Fayard, M. Pinault, N. Brun, C. Mory, J. Boczkowski, S. Lanone and P. Launois; *NanoteC11, International Conference on Carbon Nanoscience and Nanotechnology, Nantes, France, August 31st-September 3rd, 2011. (oral communication)*

Biotransformation of iron-based nanoparticles attached to carbon nanotubes inside cells; C. Bussy, E. Paineau, J. Cambedouzou, M. Salomé, B. Fayard, M. Pinault, N. Brun, C. Mory, J. Boczkowski, S. Lanone and P. Launois; *Nanocarbons 2011: Carbon Nanotubes and Related Materials: From Physico-Chemical Properties to Biological and Environmental Effects, Acquafredda di Maratea, Italy, September 6-11rd, 2011. (oral communication)*