ESRF	**Experiment title:** Interactions between carbon nanotubes and macrophage cells: the role of iron catalyst nanoparticles	**Experiment number**: SC-2601
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Report:

Introduction:

In our previous experiment on ID21, we demonstrated the relevance and the efficiency of the X-ray fluorescence microscopy technique in order to study the biological effects of carbon nanotubes (CNT) on macrophages (see exp. report of MD-280). We managed to localize both single-walled carbon nanotubes (SWCNT) and multiwalled carbon nanotubes (MWCNT) inside or in the close vicinity of the cells through the fluorescence signal of iron nanoparticles that always remain attached to carbon nanotubes as residual catalysts. This was achieved by comparing the fluorescence maps of elements constitutive of the cells such as phosphorus (P) and potassium (K) to that of iron (Fe), which is bounded to nanotubes. A dose-response effect was detected by studying macrophages having been exposed selectively to 10 µg/mL or to 100µg/mL solutions of carbon nanotubes. Finally, an excess of calcium (Ca) concentration was observed in some of the cells exposed to 100µg/mL solutions of both unpurified MWCNT and SWCNT, compared to non exposed cells. This result has a strong biological importance since Ca is involved in many biological pathways such as the cellular inflammatory response The role of Ca in the biological response of macrophages to CNT has been further confirmed through cell viability and TNF-α assays. These results were published in a letter in 2008 (C. Bussy et al., Nano Letters 8(9), 2659 (2008)) and are the subject of an ESRF Highlight (2009).

In the present experiment, our aim was to go further in the understanding of the mechanisms of nanotube toxicity by focusing on the role of iron. We therefore planned to correlate the detection of Ca cellular excess with the oxidation state of iron nanoparticles engulfed in CNT thanks to micro-XANES. Firstly, two sorts of MWCNT were considered: *i)* MWCNT cut up using a 7-week long low-power sonication bath treatment, equivalent to the MWCNT sample we used in our previous experiment, and *ii)* a 20 µm-long uncut MWCNT sample. Beyond the difference in size of these samples, their surface state should also present major differences since the sonicated MWCNT should have much more defective walls than the uncut ones. Moreover, the sonicated ones are opened while the uncut ones are not. A Fe-rich SWCNT sample was also considered for this experiment since this sample possesses the highest Fe content (20 wt. %) of our samples. All nanotube concentrations were set at 50 µg/mL and macrophages were exposed to nanotube solutions for 24 hours.

Results and discussion

The first result of this experiment is that we observed the same trends with macrophages exposed to a 50 µg/mL solution of cut MWCNT than with macrophages exposed to a 100 µg/mL solution (previous experiment results). As a matter of facts, the iron maps are found to be inhomogeneous, and present a maximum inside the cell contour drawn by the P and K maps. This result will allow us to work in the future with 50 µg/mL solutions, which are more easily dispersed.

Macrophages exposed to uncut MWCNT were studied and figure 1 shows the absorption image together with different element maps. The shape of the cell is drawn by the P and K maps, and one can observe that the Fe map is inhomogeneous and presents a maximum of intensity in an area included inside the cell contour. Therefore it appears that 20 µm long nanotubes can also be engulfed by macrophages. Attention was also paid to the Ca contents in this cell. The Kα edge of Ca being very close to the Kβ edge of K, the detection of low Ca amounts in cells can be delicate. However, the detailed comparison between the K map and the Ca map shows small differences, which are pointed out by the red circle in figure 1. These differences allow us to be confident in the presence of Ca in this macrophage. Therefore, 20 µm long MWCNT ingestion by macrophages seems to trigger the same biological response than shorter and more defective cut MWCNT.

We finally report on the XANES study of our samples. We started by measuring some "reference" samples without cells, namely iron-based standards such Fe_3O_4 / Fe_2O_3 and metal iron, and also nanotube materials *(i.e.* cut MWCNT, 20µm uncut MWCNT and SWCNT). In order to get in a short time a good signal-to-noise ratio with these samples, the beam was unfocalised and a 200 µm pinhole was used. Then, micro-XANES spectra were recorded on cells area wherein strong iron concentrations were detected during the cell mapping. Figure 2 illustrates these XANES analyses in the case of macrophages exposed to SWCNT. The top line in figure 2 correponds to the macro XANES signal of SWCNT without cells, while the bottom line was recorded on a 1 micron² area of a macrophage showing a high Fe fluorescence signal relative to nanotubes catalyst particles. While the micro-XANES spectrum shows a lower signal-to-noise ratio than the XANES spectrum recorded on a 200µm area, the statistics of the micro-XANES is good enough to show that its profile does not fit the cell-free reference spectrum. Morever, even if the beam is only 1 micron large, its size remains much larger than that of one SWNT bundle, in which a large number of iron nanoparticles can be found. As a consequence, the XANES spectrum recorded with a 1 micron beam should result in the addition of the signal coming from all sorts of iron-based particles, and should therefore display the same profile – though with a weaker signal-to-noise ratio- than that of the reference XANES recorder on a 200 µm area. This is not the case, which opens interesting questions about the fate of iron nanoparticles once engulfed inside macrophages.

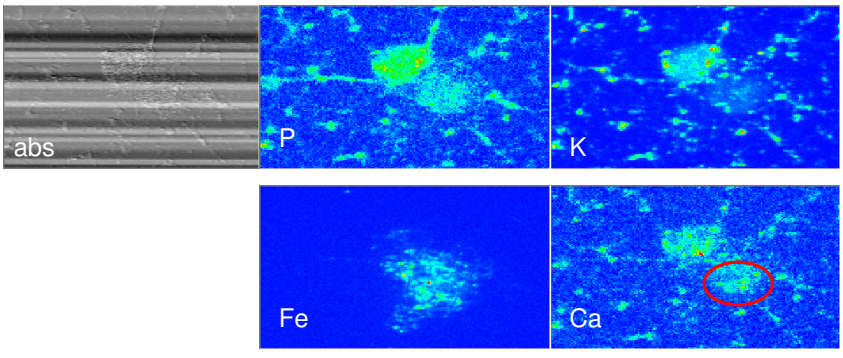

Fig.1: Absorption image (abs) and corresponding element maps (phosphorus (P), potassium (K), iron (Fe) and calcium (Ca)) of a macrophage having been exposed for 24h to 50 µg/mL of uncut MWCNT. The red circle delimitates a Ca-rich zone.

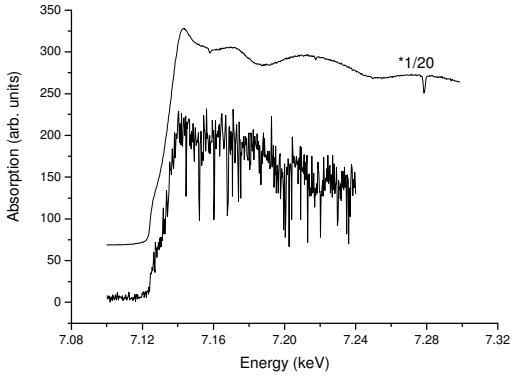

Fig.2: XANES spectrum of SWCNT alone using a pinhole of 200µm (top), and micro-XANES spectrum of a Fe-rich area in a macrophage exposed for 24h to 50 µg/mL of SWCNT (bottom).