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Experiment report

Introduction

Nanomaterials have an extensive industrial and commercial use in different fields due to their novel physical-chemical properties. However, questions have been raised concerning potential toxicological effects of these nanomaterials as a new source of exposure to humans and ecosystems. While evidences of toxicity exist in vitro, the underlying mechanisms of toxicity still remain unknown. In the present work, we used well-defined type of nanotubes: imogolite nanotubes with specific physical-chemical parameters to better understand the relationship between the aspect ratio of these tubes and the toxicity observed.

The cytotoxicity of imogolites was assessed in term of cell proliferation and viability, primary DNA lesions and chromosomic/genomic mutations. The results have shown that exposure of cells to nanoparticles of aluminogermanate with same mass per cell but with various sizes and shapes, induced different cell responses. X-ray absorption spectroscopy at the Ge K-edge was performed on the FAME beamline to study any potential changes in the structure of these nanotubes before and after interactions with biological media and cells.

Experimental details

Synthesis of the aluminogermanates imogolites-like nanotubes, and samples preparation.

AlGe imogolites were synthesized according to Levard *et al.,* (2010): Thetraethoxygermanium is added to an aluminium perchlorate with an Al/Ge ratio set to 2. The mixture is then slowly hydrolyzed by addition of a NaOH solution with the flow rate of 1.5 $mL.min^{-1}$ to reach a hydrolysis ratio [OH]/[Al] of 2. The precursor of imogolite was formed after hydrolysis step. The mixture is introduced into a thermostated reactor at 95°C for several days. At the end of incubation, each samples is dialyzed against ultrapure water by using 1000 Daltons membranes.

Three synthetic aluminogermanates were used in this experiment: protoimogolits (Precursor), i.e. a roof tile shaped structure serving as a nucleus for the subsequent growth of nanotubes, short tube and long tube. Each stock suspension was finely characterized in terms of size, shape, crystallinity, surface charge, and hydrodynamic diameter using dynamic light scattering, TEM, AFM, zeta potential measurements, and X-ray diffraction, SAXS.

Three sets of samples were analyzed on the FAME beamline:

(A) the stock suspensions of the Al-Ge synthesized nanotubes (with 2 length/diameter ratio) and their precursors.

(B) The precursors and the nanotubes (concentration 0.1 g/L) incubated within abiotic biological media *(DMEM* complemented with Bovin Serum rich in proteins) as a function of the incubation time (2h, 24h and 48h).

(C) The cells exposed during 2h to Al-Ge nanotubes or their precursorsat a concentration of 0.1g/L. At the end of the incubation time, the cells were centrifuged and separated into two phases: (C1) the cells with the internalized nanotubes and (C2) the supernatant of this centrifugation containing the nanotubes that do not interact with the cells.

Ge K-edge XAS measurements.

The samples were diluted into PVP and pressed into thin pellets. All of them were prepared at the Medical School of Marseille, and pressed into pellets at the CEREGE in Aix-en-Provence. The samples were cooled to about 10 K during spectra acquisition. This procedure improves spectrum quality by minimizing radiation damages, decreasing thermal motions of atoms. Spectra were acquired in fluorescence mode for the diluted samples and in transmission mode for the references compounds. Several scans were merged for each sample. At the end of the acquisition the signal/noise ratio of the XANES and EXAFS spectra was good. EXAFS spectra were obtained after performing standard procedures for pre-edge subtraction, normalization, polynomial removal, and wave vector conversion using the IFEFFIT software package (Ravel and Newville, 2005).

Main results

At the beginning of this experiment we wanted to be sure that the germanium present on the FAME beamline was not interfering with our samples containing low amounts of Ge. Consequently, we analyze a pellet of pure PVP with the fluorescence detector at several distances from the sample. The Ge-edge obtained with this pure PVP pellet was never intense enough to significantly interfere with our diluted samples.

Then we analyzed (i) the initial powder of nanotubes and their precursors (ii) and these particles after incubation in the abiotic culture media (complemented DMEM). In all cases, the XANES (intense edge at 11105 eV) and EXAFS spectra of the nanoparticles before and after incubations are very similar. This implies that the presence of strong complexing molecules in the complemented DMEM does not disturb the symmetry of the Ge atoms.

For the nanotubes incubated with cells, it was necessary to accumulate several scans to increase the signal/noise ratio. Once again, we do not notice drastic changes in the XANES or EXAFS oscillations of the short and long nanotubes, and their precursors.

From Levard et al. (2010), the first atomic shell is likely to be attributed to 4 oxygen atoms surrounding the Ge atoms at ~1.75 Å. This corresponds to the tetrahedral coordination of Ge previously observed in the Ge-imogolite structure. The second coordination sphere of Ge corresponds to Ge–Al atomic pairs. At this hydrolysis ratio of 2, the theoretical number of 6 Al neighbors around Ge in Ge-imogolite at ~3.27 Å is expected.

Further work has to be done regarding Al atoms in the second coordination sphere in our samples. Simulations using FEFF and the ifeffit package are in progress to precisely recalculate the Ge-Al coordination number. This will provide interesting information of the biopersitance of these AlGe nanotubes in contact with cells.

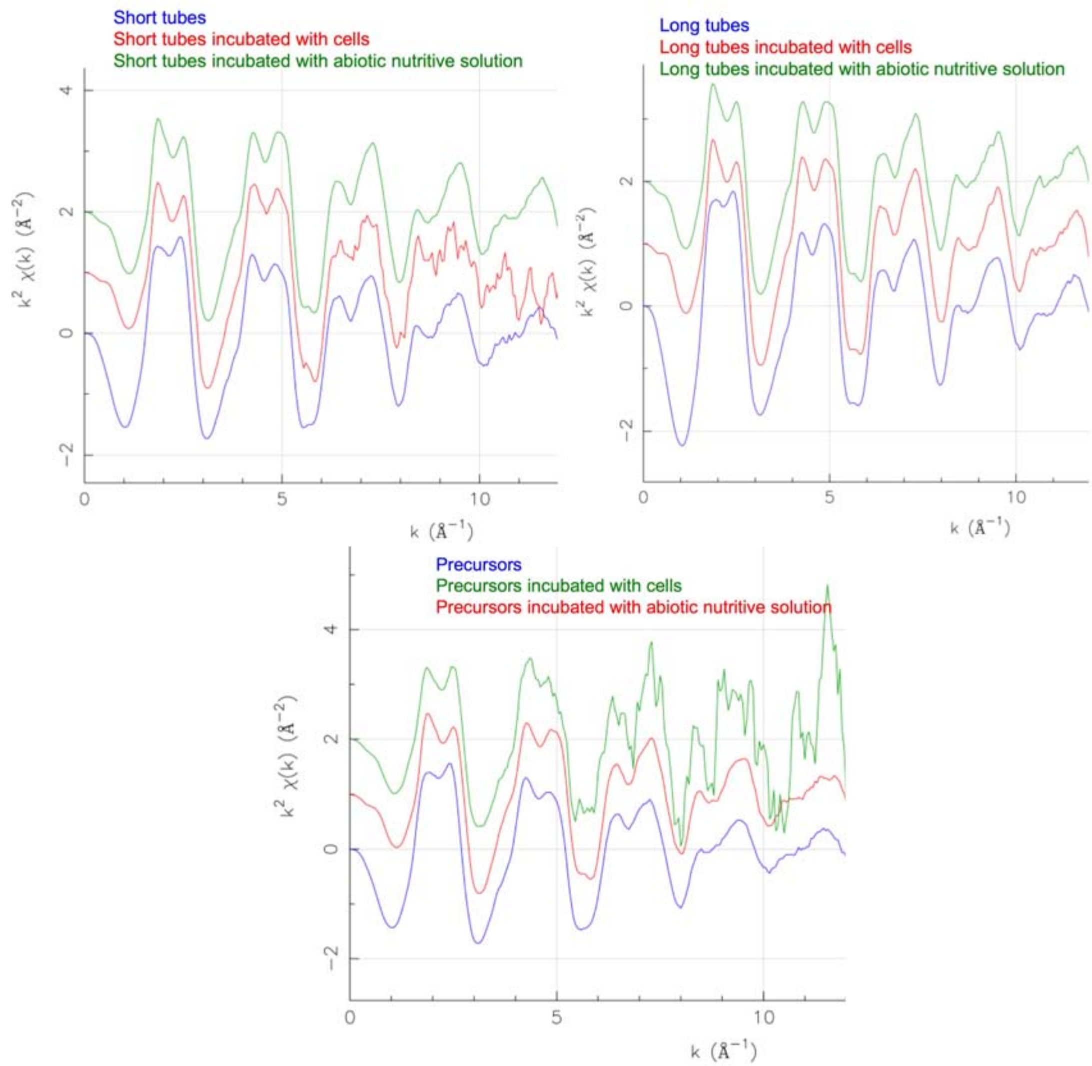

Figure 2, EXAFS at the Ge K-edge of the precursors, short and long tubes after incubation in the abiotic cell culture medium, and with cells.

References

Levard C, Rose J, Thill A, Masion A, Doelsch E, Maillet P, Spalla O, Olivi L, Cognigni A, Ziarelli F, Bottero JY (2010) Formation and Growth Mechanisms of Imogolite-Like Aluminogermanate Nanotubes. Chemistry of Materials 22 (8):2466-2473.

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