EUROPEAN SYNCHROTRON RADIATION FACILITY
ESRF User Office
BP 220, F-38043 GRENOBLE CEDEX, France
Delivery address: 6 rue Jules Horowitz, 38043 GRENOBLE, France
Tel: +33 (0)4 7688 2552; fax: +33 (0)4 7688 2020; email: useroff@esrf.fr; web: http://www.esrf.fr

Experimental report

XAS study of biotransformations in the cellular environment in iron oxide-based nanoparticles for biomedical applications

The degradation of bacterial magnetosomes internalized in human stem cells was monitored by magnetic measurements, electron microscopy, and X-ray absorption spectroscopy. It evidenced major magnetosomes biotransformations over time (about a month), associated with a transition from magnetite to ferrihydrite, remarkably mirroring the reverse transition during bacterial biosynthesis of magnetosomes. These results have been summarized in the following submitted article: *Magnetosomes intracellular degradation in human stem cells results in iron state transition from magnetite to ferrihydrite*. A. Curcio, A. Van de Walle, A. Serrano, S. Preveral, C. Péchoux, D. Pignol, C. T. Lefevre, A. Espinosa, and C. Wilhelm.

Results:

X-ray absorption spectra at the near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) experiments were performed at the Fe K-edge (7112 eV) to quantitatively investigate the iron phases over the maturation of the spheroid tissue. XANES is a sensitive probe of the coordination and oxidation state of absorbing Fe ions and EXAFS gives information about their local environment, including interatomic distances and coordination numbers of surrounding shells. These two complimentary techniques can thus reveal the iron short-range geometry and identify the iron phases.[1-2] They are considered non-damaging techniques and were recently applied to study biological systems.[3]
Evolution of the XANES spectra during tissue maturation (days 0, 3, 9, and 21) was assessed and compared to the magnetite and ferrihydrite reference spectra (Figs. 1A and Fig. 1B).[4-5] The XANES spectra show a clear shift of the absorption edge over the 21 days of tissue maturation, direct evidence of iron oxidation. It coincides with a change of iron oxide structure from magnetite (composed of Fe^{2+} and Fe^{3+}) to ferrihydrite (composed of Fe^{3+} only).[6] The XANES pre-edges features are reported in Fig. 1B, whose intensity variation indicates a gradual change on the symmetry around the Fe atoms over tissue maturation and corroborating the change from magnetite (the most intense pre-edge peak) to ferrihydrite (the least intense pre-edge peak) due to their different percentage of Fe cations occupying tetrahedral sites.
Modulus of the Fourier Transform (FT) of the EXAFS signal was also performed (Fig. 1C), as detailed in the Supporting Information. The two distinct averaged distances corresponding to Fe−O and Fe−Fe shells in the FT were analyzed. For that, the amplitude reduction factor S_0^2, the interatomic distance R and the Debye-Waller (DW) factor σ^2 for the two shell distances are used as free parameters for each fitting. No trend is noted in the first coordination shell (Fe-O) with the degradation time. By contrast, the evolution of magnetosome towards ferrihydrite is evidenced at the interatomic distance of the second coordination shell finding a shrinking of values and a reduction of neighbors close to those of ferrihydrite as the degradation time increases (see Fig. 1D and Table I). It confirms the change from magnetite to ferrihydrite over time.
This evidence of a transition from magnetite to ferrihydrite after processing of the magnetosomes by the human stem cells recalls the reverse phenomenon occurring during magnetosomes (magnetite) synthesis by magnetotactic bacteria, implicating a ferrihydrite intermediate. The ferrihydrite first produced is then reduced in magnetite, the opposite process to the one evidenced here. The iron structure thus circles back to its original ferrihydrite state when stored in the ferritin.

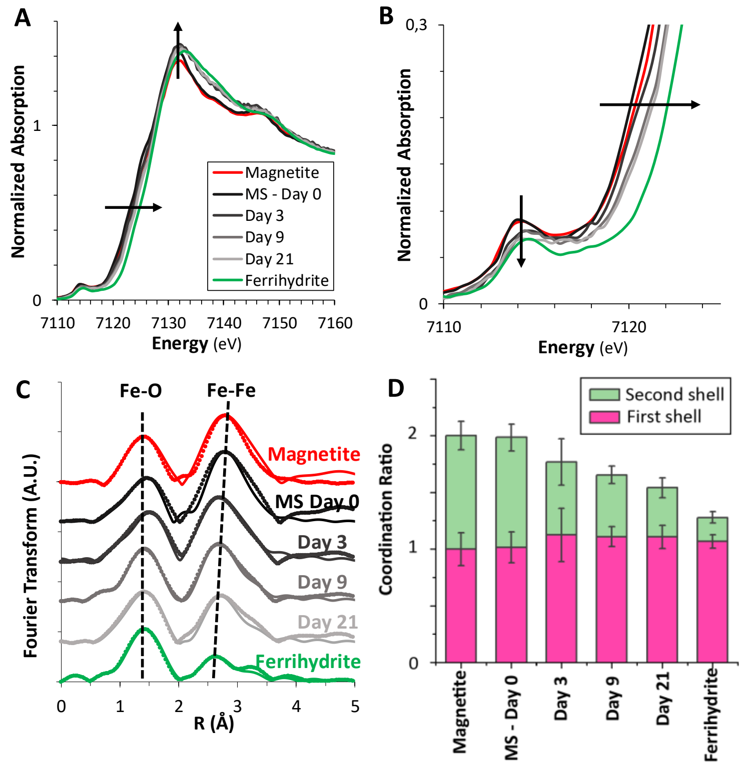

Figure 1: Transition of iron oxide structure from magnetite to ferrihydrite over tissue maturation. The iron phases are followed using XANES and EXAFS analyses (at the ESRF synchrotron facilities, CRG beamline BM25-SpLine). (A-B) XANES absorption spectra at the Fe K-edge display a progressive shift of absorption edge in iron phases that coincide with a progressive transition from the magnetite to the ferrihydrite structure. (C) Modulus of the FT of the EXAFS signal and fitting simulations (continous lines) show similar results, with an iron structure that is progressively shifted from magnetite to ferrihydrite. (D) Coordination ratio of the first and second shell of samples and references obtained from experimental EXAFS fitting measurements at room temperature.

References

1. Piquer, C.; Laguna-Marco, M.; Roca, A. G.; Boada, R.; Guglieri, C.; Chaboy, J., Fe K-edge X-ray absorption spectroscopy study of nanosized nominal magnetite. *The Journal of Physical Chemistry C* **2014,** *118* (2), 1332-1346.
2. Sánchez-Marcos, J.; Laguna-Marco, M.; Martínez-Morillas, R.; Céspedes, E.; Menéndez, N.; Jiménez-Villacorta, F.; Prieto, C., X-ray absorption and Mössbauer spectroscopies characterization of iron nanoclusters prepared by the gas aggregation technique. *Journal of nanoscience and nanotechnology* **2012,** *12* (11), 8619-8623.
3. Marcano, L.; Munoz, D.; Martín-Rodríguez, R.; Orue, I.; Alonso, J.; García-Prieto, A.; Serrano, A.; Valencia, S.; Abrudan, R.; Fernández Barquín, L., Magnetic Study of Co-Doped Magnetosome Chains. *The Journal of Physical Chemistry C* **2018,** *122* (13), 7541-7550.
4. Fdez-Gubieda, M. L.; Muela, A.; Alonso, J.; García-Prieto, A.; Olivi, L.; Fernández-Pacheco, R.; Barandiarán, J. M., Magnetite biomineralization in Magnetospirillum gryphiswaldense: time-resolved magnetic and structural studies. *ACS nano* **2013,** *7* (4), 3297-3305.
5. Malferrari, D.; Castellini, E.; Bernini, F.; Rubio, A. S.; Castro, G. R.; Sainz-Díaz, C. I.; Caleffi, M.; Brigatti, M. F.; Borsari, M., Chemical trapping of gaseous H 2 S at high and low partial pressures by an iron complex immobilized inside the montmorillonite interlayer. *Microporous and Mesoporous Materials* **2018,** *265*, 8-17.
6. Carta, D.; Casula, M. F.; Corrias, A.; Falqui, A.; Navarra, G.; Pinna, G., Structural and magnetic characterization of synthetic ferrihydrite nanoparticles. *Materials Chemistry and Physics* **2009,** *113* (1), 349-355.