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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Safe-by-Design Nanoparticles: Assessing Their Interaction with Macrophage Cells	**Experiment number**: MD-1114
Beamline: ID21	**Date of experiment**: from: 22/11/2017 to: 27/11/2017	**Date of report**: 27/02/2018
Shifts: 15	**Local contact(s)**: Ana Elena Pradas	*Received at ESRF:*

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Report:

1. Context and objectives

The experiment MD-1114 aims at determining the nature of chemical interactions of 20 nm metal oxysulfide nanoparticles of composition $Gd_{2-x}Ce_xO_2S$ ($0 \leq x \leq 1$) with macrophages. These nanoparticles are being developed in our team in a safe-by-design approach. In particular, we study their toxicity in parallel with their performance as potential photocatalysts. To evaluate their toxicity, we exposed murine macrophage to the nanoparticles. Results in metabolic activity assay showed a high dose-dependent toxicity on some samples containing cerium. In the experiment at ID21 beamline, we performed µXRF and µXAS at two absorption edges (Ce L_3-edge, Gd L_3-edge) to localize the nanoparticles in the cells and identify the oxidation state of cerium (III or IV) in this biological environment. Such an approach will provide crucial information that will help us to understand the potential toxicity of these emerging nanoparticles.

2. Experimental section

Murine macrophages (RAW 264.7 cell line) were grown on Si_3N_4 membrane windows and exposed to $Gd_{2-x}Ce_xO_2S$ nanoparticles at 10 µg/ml. After 24 h of incubation, the cells were washed with PBS and the samples were snap-frozen in isopentane cooled by liquid nitrogen. The Si_3N_4 membrane windows were then stored in 24-well plates cooled at -80 °C in a freezer or with dry ice. Non-exposed cells and free nanoparticles were also prepared as reference. Cryogenic experiments were carried out at beamline ID21 in a vacuum chamber passively cooled at *ca* -130 °C by liquid nitrogen. The Si_3N_4 membrane windows were mounted on a pre-cooled copper sample holder in liquid nitrogen and were rapidly inserted into the vacuum chamber. Measurements at Gd L_3-edge were carried out first. µXRF maps were acquired with an incoming energy of 7.4 keV with 0.5 μm^2 steps and an integration time of 100-150 ms. µXAS spectra were recorded at different points in the maps to compare with free nanoparticles. Energy of the beam was then set to 5.8 keV at Ce L_3-edge. XRF and XAS hyperspectral (fluoXAS) maps were recorded in areas presenting high

nanoparticles concentrations near the cells. Reference sample on Si_3N_4 membrane RF8-200-52454-17 purchased from AXO DRESDEN GmbH was used for quantification of elements. To calculate weight fractions, the thickness of the cells and the density of the medium were estimated at 15 µm and 1 g/ml respectively.

3. Results

We were able to visualize cryofixed macrophages exposed to the nanoparticles with potassium mapping (Figure 1a). Potassium is better distributed inside the cells than phosphorus and its XRF signal is also more intense. Therefore, potassium is a better cell marker for our experiments. Gadolinium maps of the samples show that aggregates of $Gd_{2-x}Ce_xO_2S$ nanoparticles are partly located outside the cells. Smaller aggregates were also detected on and/or in the cells (Figure 1b and 1d). The nanoparticles that contain cerium with x as low as 0.2 can be visualized with cerium mapping as well (Figure 1c). Cerium is co-located with gadolinium in the aggregates. Weight fraction of gadolinium was plotted against that of cerium (Figure 1e). The observed trend is consistent with the expected Gd/Ce ratio measured by Scanning Electron Microscopy coupled to Energy Dispersive Spectroscopy. Few points farther from the black line represent aggregates that contain slightly less Ce. They suggest a possible degradation of the nanoparticles.

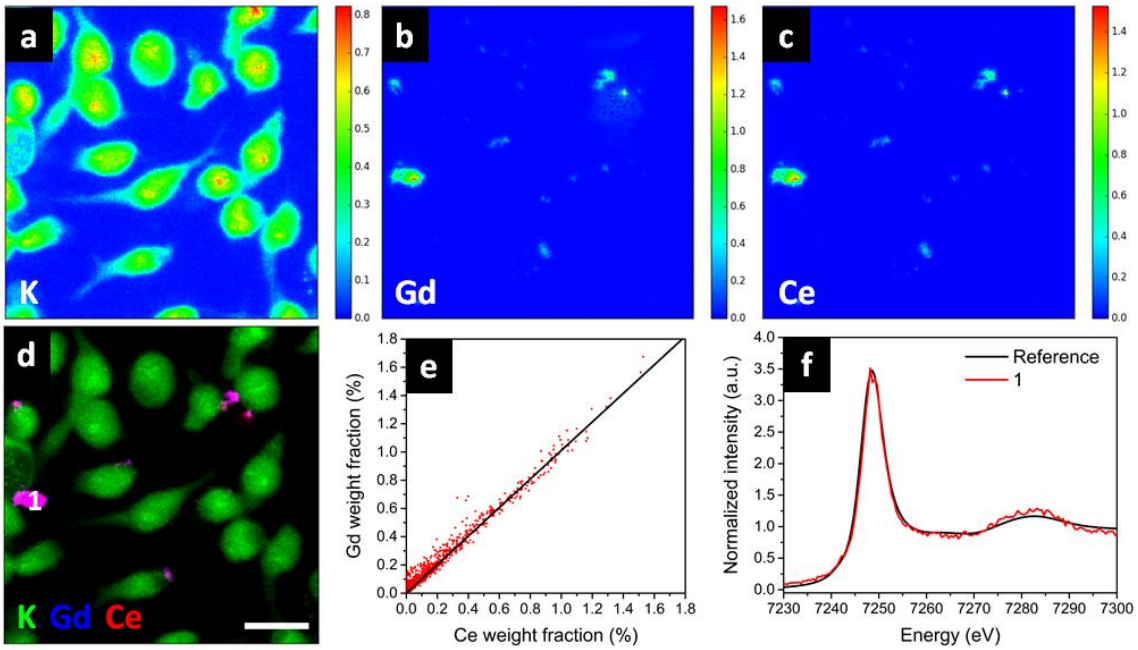

Figure 1: **a,b,c,d**: Elemental maps and RGB reconstruction of cryofixed macrophages exposed to $GdCeO_2S$ nanoparticles. The values on the color bars indicate the weight fraction of the element. The scale bar represents 20 µm. **e**: Scatter plot of weight fractions of gadolinium *vs* cerium. The expected Gd/Ce ratio measured by EDS was plotted as black line. **f**: µXANES at region 1 indicated in map **d** compared to $GdCeO_2S$ nanoparticles in water ("Reference").

We then look at the speciation of gadolinium and cerium using µXANES. Spectra at Gd L_3-edge of the aggregates show no significant modification compared to those of nanoparticles in water (Figure 1f). At Ce L_3-edge, fluoXAS maps of the samples that have high toxicity towards metabolic activity of the cells were recorded. Preliminary results indicate modification of oxidation state of cerium inside some aggregates in contact with the cells. However, this trend was not observed for all the samples.

4. Conclusions

For the first time, we employed µXRF to visualize macrophages exposed to metal oxysulfide nanoparticles of composition $Gd_{2-x}Ce_xO_2S$ ($0 \leq x \leq 1$). Aggregates of nanoparticles were detected partly outside the cells but also on and/or in the cells. Leaching of cerium seems to be present in some aggregates. Complementary elemental analyses will be done to confirm this degradation of nanoparticles in biological media. The results of fluoXAS mapping at Ce L_3-edge demonstrated that modification of oxidation state of cerium in contact with the cells can be observed even though not systematic.