EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** The assessment of iron oxide nanoparticles uptake, distribution and interaction within 3D liver spheroids for nanotoxicology studies.	**Experiment number**: LS-2442
Beamline: ID16B-NA	**Date of experiment**: from: 10/10/2015 to: 14/10/2015	**Date of report**: 15/10/2015
Shifts: 12	**Local contact(s)**: Sylvain Bohic	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Preliminary report being submitted in order to support proposals LS-2459 and LS-2501.

Experiment LS-2442 successfully determined the distribution of internalised Fe_2O_3 nanoparticles within HepG2 cellular spheroids. Modifications had to be done to the sample preparation method as various cracks within the tissue sections were being formed during the freeze drying process. A 4% paraformaldehyde fix for 30 minutes at room temperature step was done in between harvesting and snap freezing. This resulted in spheroid sections with significantly less cracks after freeze drying. Flux intensity had to be adjusted as the beam was causing the scarring of the sections. This was adjusted by adding a 500 µm silicon window pre-set at the beamline.

For the 24 hour exposure period, spheroid sections contained sufficient internalised nanoparticles to give adequate iron fluorescent intensities with exposure times of 200ms and 300ms for exposed and control samples respectively. This allowed the acquisition time for a 100X100 µm section to be acquired in about 34-50 minutes for the respective samples, using a beam resolution of 1 µm.

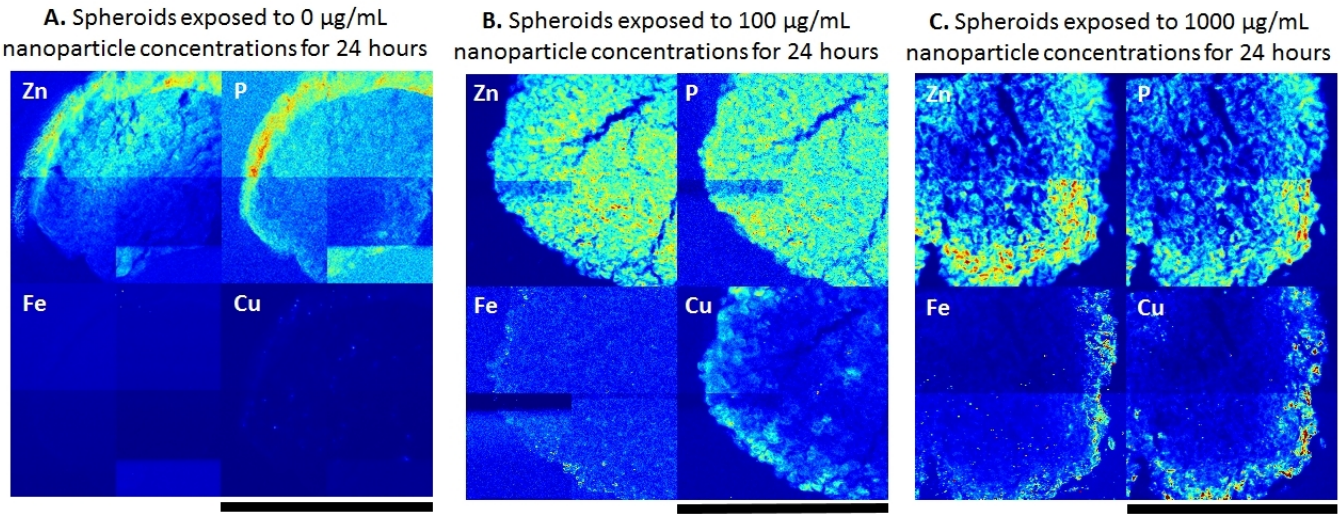

Figure 1: Zinc, phosphorus, iron and copper elemental distribution maps of tissue sections given by control and spheroid exposed to different concentration of Fe_2O_3 nanoparticles. Scale bars are 80 µm (**A**) and 100 µm (**B** & **C**).

The elemental distribution maps (Figure 1) indicate that most of the nanoparticles by are accumulated at the periphery of the spheroid and the penetration is of about 10-20 µm in depth that seems to increase with higher exposure concentrations. Nanoparticles accumulation also seems to increase in this manner. It is therefore required to see if penetration changes with different surface functionalisation. An interesting response was seen by copper, overlapping and close to the areas were iron was detected. It is known that proteins containing copper complexes are involved the externalisation of cellular iron and intracellular redox reactions. These responses have to be looked as they might be a form of protective mechanism against the exposed nanoparticles. More insight would be therefore acquired by observing the responses given by other iron nanoparticles of different ionic composition such as Fe_3O_4 and determine possible causes for this effect.

A complete and in depth report will be submitted in due time, with the required data analysis.