EUROPEAN SYNCHROTRON RADIATION FACILITY

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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:** Gadolinium Photoactivation: Dose-enhancement induced by Gd nanoparticles versus Gd contrast agents	**Experiment number**: MD660
Beamline: ID17	**Date of experiment**: from: 06/07/2012 to: 09/07/2012	**Date of report**:
Shifts: 9	**Local contact(s)**: Thierry Brochard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Dr. Jean-Luc Ravanat
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Aim of the work :

The combined use of high Z elements and low energy radiations as those produced by synchrotron, enhances the potential of radiotherapy. For such a purpose, high-Z elements should be incorporated into the tumor that is then irradiated, increasing the dose received by the tumor compared to surrounding tissues. Recently, the potential use of nanoparticles has been highlighted (Hainfeld et al., 2008; Hainfeld et al., 2004).
The aims of the study are firstly to evaluate the importance of the Gd structure (nanoparticle *versus* molecular agents, such as contrast agents (Magnevist)) for tumoral cells radiosensitizing. Secondly we study the effects at lower energies for a better understanding of the fundamental mechanisms.

Experimental methods and parameters:

Samples preparation / irradiation:
F98 cells were cultured 3 days before irradiation. Depending on the irradiation's conditions, they were incubated during 5 hours at 37°C with Gd-NPs or not. The radiation source energy was tuned from 25 to 80 keV, to allow the Sensitive Enhancement Ratio (SER) dependence investigation. 7 energies were investigated: 25, 31, 40, 49.5, 51, 65 and 80 keV. In addition, we irradiated the samples at high energy (1.25 MeV – ^{60}Cobalt source – CEA Grenoble). Irradiated samples receive 4Gy whatever the condition.

Gadolinium concentrations measurement:
The intracellular gadolinium concentration was determined by ICP-MS, after F98 cells incubation with Gd-NPs (2.1 mg/ml, 30 minutes, 2h or 5h).
The distribution of the internalized Gd-NPs was observed by confocal microscopy. Gd-NPs are stained with FITC fluorophore.

Cell cycle measurement:
The cell cycle distribution was evaluated after incubation with the Gd-NPs. 6 samples were incubated during 5h with 2.1 mg Gd/ml (Gd-NPs). 3 of them were fixed (PFA 4%) just after incubation and the 3 others, 24h after the end of the incubation. The cell cycle was determined from DNA histograms measured by flow cytometry. DNA was stained with DAPI (1µg/ml) and after membrane permeation (Triton X100).

Cell proliferation measurement:
We measured the cells' proliferation after any treatment (irradiation alone, irradiation, in combination with Gd-NPs (2.1 mg/ml) or Magnevist Gd (2.1 mg/ml)), by performing a cell counting every 24h over a 7 days period after irradiation. The automatic cell counter (CEDEX XS – Roche) was used.

Cell survival measurement:
Clonogenic assays were used for measuring the cell capability to proliferate after a treatment (irradiation alone, irradiation, in combination with Gd-NPs (2.1 mg/ml) or Magnevist Gd (2.1 mg/ml)). The toxicity of the drugs alone was also studied.

Selected preliminary results:

Gadolinium concentrations measurement:
F98 incubation with 2.1 mg/ml Gd-NPs leads to an internalisation of the Gd by the cells. The Gd internalized concentration increases with the incubation time and reaches 0.6 mg Gd/ml after 5 hours.
We observe an accumulation of the Gd-NPs on the cells' membranes.

Cell cycle measurement:
The cells incubation with Gd-NPs leads to an accumulation in late S phase measurable just after the incubation. 24h after it, we measure a shift of the accumulation into the G2/M phase.

Cell proliferation measurement:
In order to evaluate the determine the effect of nanopaticles on F98 cells we measure their proliferation velocity after any treatment. Incubation with Gd-NPs leads to an inhibition of the cells' growth which is measurable until 3 days after incubation. The combination with irradiation increases this inhibition which is no more reversible (cells' death increase).

Cell survival measurement:
From cell survival measurement we have calculated the SER as the ratio between the survival fraction for cells irradiated without gadolinium and cells irradiated with gadolinium.

Gd-NPs lead to an increase in the cells'death for all studied photons' energies, but the increase is strongly dependant on this energy. The highest effect is measured at low energy and for 65 keV (corresponding to the highest Dose Enhancement Factor (DEF), calculated by Monte Carlo simulations. At high energy (1.25MeV) we still measure an increase of the cell death (less important than for low energy). As photoelectric effect is minoritary for this energy, the effect can not be attributed to Gd photoactivation. Gd-NPs increase the radiosensitivity of the cells. Between 49.5keV and 51keV we measure a slope which is significant of the k-edge effect. A part of the effect can thus be attributed to photoelectric effect.

Magnevist leads to an increase of the SER only above the Gd K-edge and for Synchrotron radiations (51, 65 and 80 keV). The SER energy dependence is directly comparable to the DEF.

Conclusions:

This experiment has shown the interest of using nanoparticles for enhancing the radiosenstivity of tumoral cells. With equivalent high Z atoms concentrations, nanoparticles lead to a higher SER than Magnevist. The internalization of the Gadolinium plays an important role in the effect. The cell cycle modification and the cell growth inhibition can give explanations to understand the increase of the F98 cells radiosensitivity.
Moreover, we have shown a strong energy dependence of the SER. Synchrotron radiations provide the more important effect illustrating the importance of using monochromatic and low energy x-rays beam for the technique.
Altogether the results of our experimental studies have high practical significance and will be published in the near future.
We are grateful and very satisfied by the help provided to us by the local contact.