	Experiment title: Identification of Reactive oxygen species produced by nanoscintillators during synchrotron radiation therapy	Experiment number: MD1296
Beamline: ID17	**Date of experiment**: from: 10/09/2021 at 8:00 am to: 12/09/2021 at 8 am	**Date of report**: 21/09/2021
Shifts: 6	**Local contact(s)**: REQUARDT Herwig	*Received at ESRF:*

Experimentalists who contributed to the experiments:

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Report:

The aim of this proposal was to investigate by which mechanisms scintillating nanoparticles potentiate synchrotron radiotherapy. Radioluminescent nanoparticles, or nanoscintillators, are high Z-element nanoparticles that down-convert ionizing radiations into visible light. Whereas scintillators are commonly developed for detection purposes, nanoscintillators have emerged as potential radiotherapeutics to potentiate radiation therapy by inducing deep-tissue photodynamic therapy (PDT) and radiation dose-enhancement (RDE). While the RDE effect results from an excess of energy deposited by photo- and Auger electrons that are created when X-rays interact with heavy-elements, PDT relies on photosensitizers that generate cytotoxic species upon light activation. As light penetrates weakly in tissues, PDT is limited to superficial tumors. To reach deep tumors, nanoscintillators can be used to locally convert X-rays into visible light and remotely induce PDT. When using nanoscintillators/photosensitizer conjugates to enhance radiotherapy efficacy, the therapeutic benefit may thus come from two contributions: PDT and RDE. In order to refine the design of future nanoconjugates, it is crucial to fully understand the underlying mechanisms and quantify the relative efficacy of each contribution. This project aims at implementing a new strategy that will allow to distinguish between the PDT and the RDE contribution.

Spectroscopy experiments:

We used an experimental method based on chemical probes that present a fluorescence when they react with reactive oxygen species. We selected the APF (Aminophenyl fluorescein) that is mostly sensitive to OH• radicals and singlet oxygen. The photoluminescence of the APF was measured between 525 nm and 580 nm upon a light excitation at 490 nm. The light was collected using an optical fiber placed right after a long-path filter that cuts the light below 500 nm. The signal was monochromatized in a spectrophotometer and measured by a CCD camera. The spectra were then post-processed to estimate the relative quantity of ROS that was produced.

We investigated the effect of the X-ray energy by irradiating 1 keV below and 1 keV above the K-edge of the heavy element present in the nanoparticles and we measured the APF photoluminescence after delivering increasing doses of radiations to the samples. Doses ranging from 0 to 64 Gy were delivered.
We observed that more ROS were created in presence of the nanoscintillators compared to the control sample containing only the solvant (phosphate buffer saline). More interestingly, we observed a stronger effect 1 keV above the K-edge compared to 1 keV below the K-edge of the heavy element, which is an unequivocal demonstration that the observed effect is related to photoelectric interactions between the X-rays and the heavy-elements present in the nanoparticles.

***In vitro* experiments:**

Additionnally, we used this beamtime to repeat one experiment we previously performed *in vitro* (proposal MD1260) and for which we had a bacterial contamination in one of the experimental repeat. Therefore, we reproduced this experiment to achieve a proper number of experimental repeats to secure data for publication.
In this experiment, we grew adherent 3D microtumors of pancreatic tumor cells in 24 well-plates and incubated the cells before irradiating the plates with either:

- LuAG nanoparticles
- LuAG@PpIX nanoparticles
- PpIX alone
- no nanoparticles

Two X-ray energies were investigated: 1 keV below the Lu K-edge and 1 keV above the Lu K-edge. We delivered increasing doses of radiations ranging from 0 to 8 Gy. After irradiation, the plates were maintained in culture for an extra 6 days and a viability assay was then performed to assess the efficacy of each treatment condition.

This experiment will allow us to analyze the viability of the microtumors as a function of the X-ray energy and as a function of the radiation dose. In addition, the viability of the spheroids will be analyzed as a function of the microtumor area, in order to correlate the efficacy of the treatment with the size of the microtumors.

Data analysis is still ongoing.