EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

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ESRF

ESRF	**Experiment title:** Early steps of bone mineralization in differentiating osteosarcoma SaOS2 cells investigated by XANES at the Ca-K edges	**Experiment number**: LS 3038
Beamline: ID 21	**Date of experiment**: from: 12/07/22 to: 16/07/22	**Date of report**: 10/09/22
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Report:

The main aim of this proposal was to study how the osteblasic differentiation stimuli affect the maturation process of mineral depositions released by osteosarcoma SaOS2 cells at 4 and 10 days after the osteoblastic induction. To this purpose μXANES at the Ca K-edges was used to obtain the chemical speciation of mineral depositions since Ca is one the constitutive and pivotal element of hexagonal hydroxyapatite crystal present in bone. This study will contribute to unveil the genesis of bone mineralization process in osteosarcoma which is still far from to be elucidated. Moreover, this research is a part of a larger project aimed to validate a new anticancer strategy, based on the differentiation of osteosarcoma cells towards a less aggressive phenotype. The application of this strategy will be fundamental to improve the chance to translate the new knowledge in osteosarcoma clinical practice and treatment.

The samples were prepared at the Biomedical Facility in ESRF and were constituted of frozen hydrated osteosarcoma SaOS2 cells which were induced to differentiate towards osteoblasts using a cocktail containing β-Glycero- phosphate, ascorbic acid, and vitamin D for 4 and 10 days. We succeeded in analysing treated and control samples at both 4 and 10 day after induction in all the conditions requested. We started the data analysis, which is quite complex: the fluorescence spectra must be analysed with PyMCA, to obtain the integrated intensity of several fluorescence lines, including calcium, for all the measured samples; in parallel we carried out the analysis of XANES spectra with the precious support of the beamline staff.

A significant analysis of this phenomenon requires a large amount of elemental concentration maps for each sample. Since very little is known about the chemical composition and progression of the extracellular Ca-polyphosphates depositions in osteosarcoma cells, and even less during their osteoblastic differentiation, we acquired more scans on different cells for each sample to increase the number of cells analysed. Acquisitions were performed in the intracellular and extracellular environment in order to further characterize the genesis and the evolution of nano-sized mineral depositions. Interesting considerations can be made from preliminary data analysed (Figure 1).

4-days control samples show both calcite and calcium Phosphate spectra in the extracellular environment. The same scenario can be appreciated after 10 days. In treated samples, these depositions are made of Caclium Phosphates (at the moment we cannot discriminate between calcium Phosphate and Hydroxyapatyte)

underlying the effectiveness of the differentiating treatement: osteosarcoma cells have been induced to differentiate towards a less aggressive phenotype as the final product of differentiation is a deposition made of CaP which is the main component of healthy bone tissue.

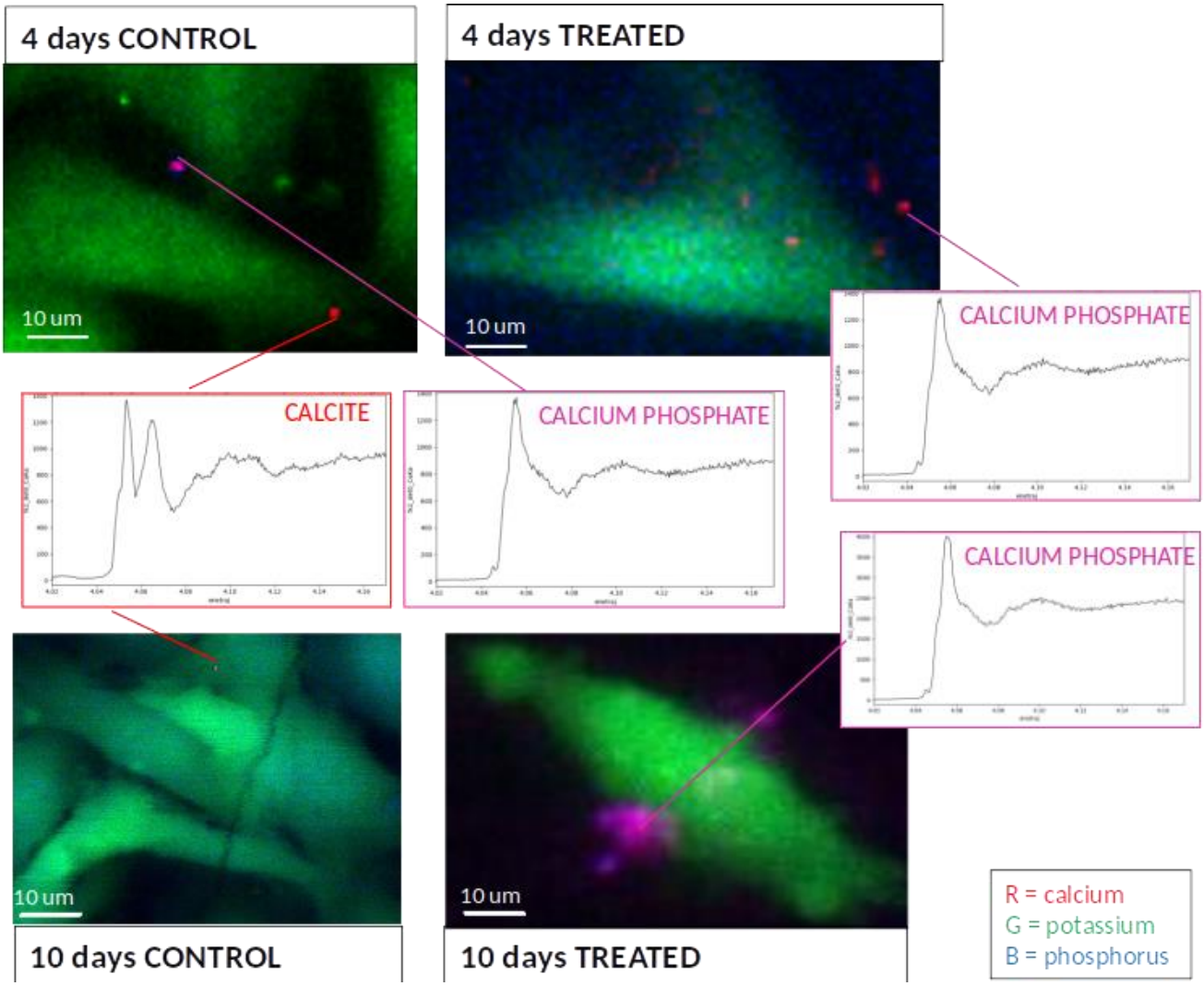

Figure 1: Fluorescence maps (merge of Calcium, Potassium and Phosphorus channels) and XANES spectra from Ca depositis of 4 and 10 days control samples and 4 and 10 days treated sample.

At the moment, a Principal Component Analysis on Calcium Phosphate and Hydroxyapatite spectra is ongoing. From this analysis we expect to have Hydrpxyapatyte spectra in 10 days treated samples and a final spect of calcium compounds evolution during differentiation.