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

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

	Experiment title: Quantitative mapping of Ca, P and Zn during osteosarcoma cell differentiation: intracellular genesis of nano-sized mineral depositions and	**ESRF number**: LS3031
Beamline: ID16A	**Date of experiment**: from: 29/06/22 to: 4/07/22	**Date of report**: 19/09/22
Shifts: 15	**Local contact(s)**: Dmitry Karpov	*Received at ESRF:*

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Report:

The aim of this proposal is to study the intracellular genesis of the nano-sized mineral depositions and their evolution in the extracellular matrix, mapping the concentration of Ca, P and Zn during osteoblastic differentiation of osteosarcoma SaOS2 cells (at 4 and 10 days). With this experiment we expect to obtain useful information about the role of Ca, P and Zn in bone mineralization process of osteosarcoma cells. To this purpose, X-ray Fluorescence Microscopy (XRFM) measurements and x-ray phase contrast holo-tomography acquisitions were performed to obtain 2D Ca, P and Zn concentration maps at high spatial resolution. Ca, Zn, K and P fluorescence intensity maps are derived from XRFM acquisitions while the cellular volume is obtained by the holo-tomography at nanoscale.

The samples were prepared in the ID16A laboratory 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. For all samples, a 50 nm or 70 nm resolution XRFM measurement and an holo-tomography were performed at least on one cell. In treated samples, a 50 nm resolution from 5 different cells XRFM was acquired in correspondence as well as 3 different cells for control samples. 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 phase reconstruction using the programs elaborated by the beamline staff, but obtaining reliable quantitative phase retrieval on all the samples requires further efforts and hence, tomographic reconstruction is still in progress. When both fluorescence spectra analysis and phase retrieval procedure will be completed, fluorescence intensity maps derived through XRFM will be normalized with the cellular volume obtained by the holo-tomography allowing us to reach the projected elemental concentration map of the sample. In the following, we show examples of fluorescence intensity maps of cells from control and treated samples at 4 and 10 days (Figure 1, 2 and 3).

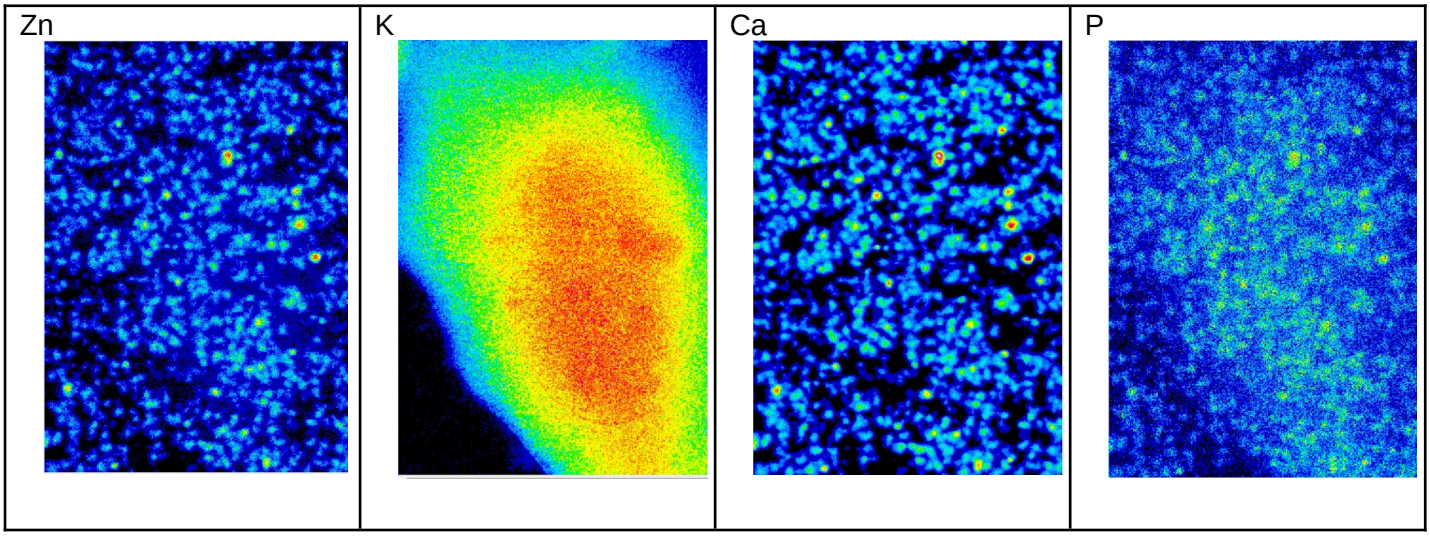

Figure 1: From left to right: fluorescence maps of Zn, K, Ca and P of a 10 days treated sample.

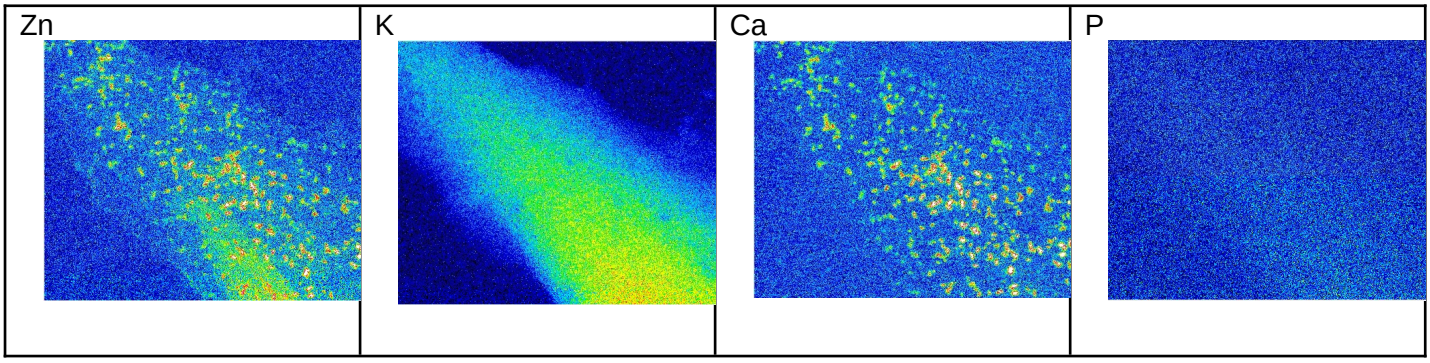

Figure 2: From left to right: fluorescence maps of Zn, K, Ca and P of a 4 days treated sample.

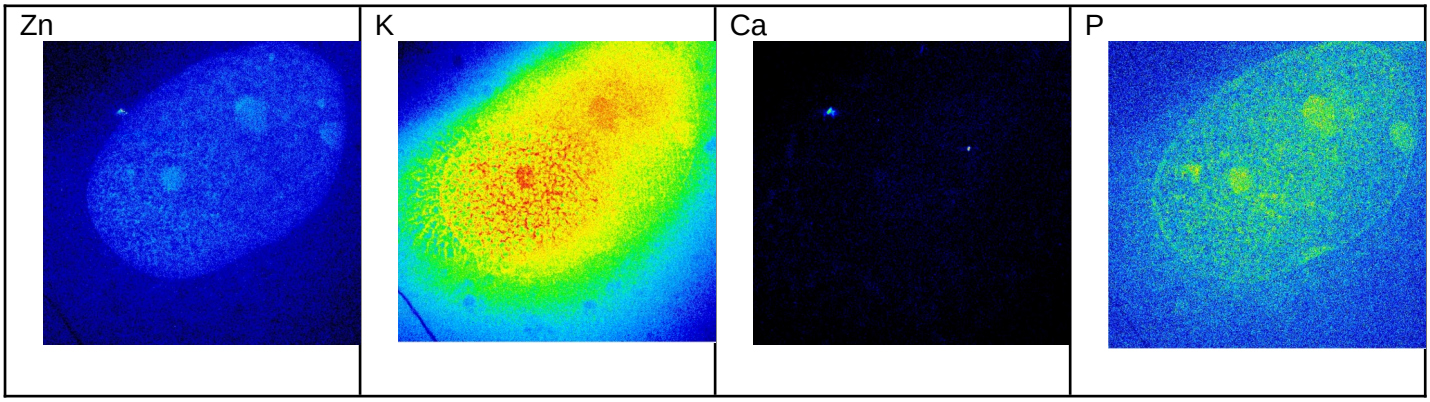

Figure 3: From left to right: fluorescence maps of Zn, K, Ca and P of a 10 days control sample.

Interestingly, Ca depositions were observed even at 4 day of osteoblastic differentiation and at 10 days after treatment very high Ca intensity values were registered. In treated samples a co-localization of Ca and P can be easily noticed: this co-localization probably higlight the fact that Ca deposits in treated samples are made of Calcium Phosphates compounds. Thanks to beamtime at ALBA synchrotron we have characterized ca deposits at these conditions hand we have observed that depositions are made of Calcium Phosphates in treated samples higlighting the effectiveness of the treatement. Results from ALBA experiment are under publication at the moment. On the other side, no co-localization of ca and P can be appreciated in control samples. In this case, ca deposits could be made of a Calcium Carbonate compound (as observed from XANES spectra acquired at ALBA).

From a previous work [1], we found out an increase of the number of mineral depositions with a lower volume in differentiating cells with respect to control cells; the same condition can be observed in our data acquisition. 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.

1) PICONE, Giovanna, et al. Analysis of intracellular magnesium and mineral depositions during osteogenic commitment of 3d cultured saos2 cells. International journal of molecular sciences, 2020, 21.7: 2368.