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Characterization of Ca-depositions evolution by cryo-XANES and cryo-STX in SaOS-2 osteosarcoma cells

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OBJECTIVE: This study aims at characterizing mineral depositions in the early phase of bone biomineralization in SaOS-2 osteosarcoma cell line. Cryo-soft X-ray tomography (Cryo-STX) and cryo-XANES at the CaL2,3 edges imaging were combined to investigate the evolution of the Ca-depositions during differentiation. A deeper knowledge of the early phase of bone formation and its mineral nanostructure could elucidate the relationship between osteoblastic differentiation and osteosarcoma, opening new perspectives in the treatment of this disease.
MATERIALS and METHODS: We characterized minerals produced by SaOS-2, osteoblast-like cells with high mineralizing activity [1]. SaOS-2 cells were subject to osteogenic medium according to Pasini et al. [2]. The combination of cryo-STX with cryo-XANES at the CaL2,3 edges allowed to identify respectively the spatial distribution and the chemical state of mineral depositions in frozen-hydrated cells at 4 and 10 days after osteoblastic induction [3, 4].
RESULTS: Thanks to this emerging technique, we appreciated the evolution of Ca depositions from calcium phosphate (Ca3(PO4)2) to hydroxyapatite (HA) from 4 days to 10 days after osteoblastic differentiation. The acquired spectra and tomography showed the presence of few calcite (CaCO3) deposits on the ground plane in 4 days control samples. SaOS-2 after 4 days of differentiation revealed an increase in depositions number and the evolution of their chemical composition to calcium phosphate. While deposits mainly laid on the ground plane of the samples, the increasing presence of intracellular vesicles containing calcium carbonate compounds was depicted (see Figure 1). Intriguingly, at 10 days after differentiation, vesicles increased in number as well as depositions whose chemical state evolved to hydroxyapatite.
CONCLUSIONS: The presence of Ca-phosphate and HA crystals after osteogenic induction could suggest a restoration of the biomineralization process due to the induction of SaOS-2 cells towards a less aggressive phenotype even at 4 days. Furthermore, vesicles could play a central role in the genesis within the cell and the propagation of minerals in the extracellular matrix: at the moment their potential remains unveiled, especially in humans.

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