Ultrastructural study of biomineralization process in human bone marrow mesenchymal stem cells during the osteoblastic differentiation

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Biomineralization (BM) is the process by which organisms form organized mineral crystals during tissue regeneration. During the BM process ions in solution are converted to biominerals thanks to chemical-physical transformations performed by the cellular activity. The process creates complex composite materials, made of organic and inorganic compounds. On the nanometric scale the highly specialized organic matrix of collagen microfibrils seems to direct the formation of nanosized platelet-hydroxyapatite (HA) oriented parallel to the collagen fibril axis [1-2]. Alongside these organic components, a mineral precursor amorphous, calcium phosphate is believed to play a fundamental role in the growth of HA nanocrystals. At the cellular level, the formation, maintenance, and repair of bone is based on the complex crosstalk between osteoclasts, osteoblasts and osteocytes[3]. Osteoblasts differentiate from bone mesenchymal stem cells (bMSC) and promote bone deposition [4].
Calcium is a crucial mineral for the bone and is present in the extracellular mineralized matrix as an integral component of hydroxyapatite crystals. Very little is known about the intracellular Ca concentration, distribution and homeostasis in bMSC, and even less about the progression of the extracellular Ca-phosphates and polyphosphates deposition during osteoblast differentiation. Several ionic substitutions in the HA structure are important also in BM; among these Zinc has drawn considerable attention due to its inhibitory activity to osteoclastic bone resorption [5].
Thanks to the advent of synchrotron radiation sources, which provide high intense coherent X-ray beams, it is now possible to study at nanoscale the cellular content and the extracellular deposition of the elements involved in the HA formation.
The goal of this study is to measure the intracellular Ca concentration and the extracellular Ca, P and Zn deposition in bMSC induced to osteoblast differentiation. Human bMSC have been isolated from the bone marrow of healthy individuals. bMSC have been exposed to a differentiating cocktail containing β-Glycero-phosphate, 50 µg/ml ascorbic acid, and vitamin D. Samples have been taken at day 0 (as control) and at 4, 10 days of differentiation towards osteoblast. Cryogenic sample preservation was used, and frozen hydrated cells have been studied at the beamline ID16A-NI at the ESRF synchrotron. In particular, we combined X-ray Fluorescence Microscopy (XRFM) measurements with x-ray phase contrast nano-tomography, to obtain 2D Ca, P and Zn concentration maps at high spatial resolution (down to 15nm). Moreover, to overcome the misleading interpretation coming from 2D elemental maps, we acquired x-ray fluorescence tomography to better localize in the space the deposition of Ca, P and Zn.
The preliminary results (Figure 1) of the 2D fluorescence shown an early spot deposition of Ca already at 4 days. It is worthy to note that in correspondence of Ca deposition a P and Zn accumulation is present as well, Moreover, the extracellular deposition of Ca at 10 days is massive since the bMSC osteoblast differentiation is almost complete and the co-localization of Ca with P and Zn is still evident.
These results strongly suggest the presence of some phosphate or polyphosphate compound of Ca precursors of the HA formation. Further analysis for the visualization of the 3D results are still ongoing.

REFERENCES:
[1] Nudelman F. et al., Nat Mater 9, 1004 (2010).
[2] Scaglione S. et al., Scientific Reports 2, 1-6 (2012).
[3] Kular J. et al., ClinicalBiochemistry, 45, 12 (2012).
[4] Abdallah BM. et al., Gene Therapy, 15 (2008)
[5] Bhattacharjee P. et al., Journal of Asian Ceramic Societies 2, 44-51 (2014).

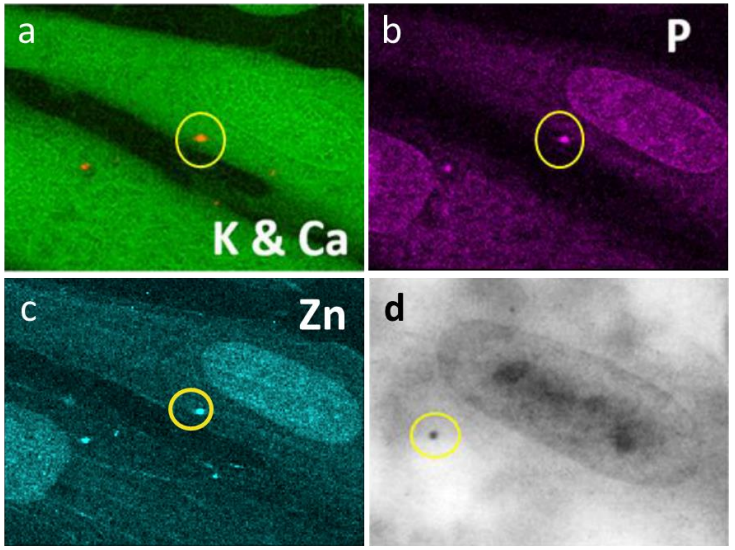

Figure 1. shows the deposition of Ca, P and Zn after 4 days of differentiation in bMSC (Panel a, b and c). Panel d, particular of phase contrast reconstruction of the same bMSC

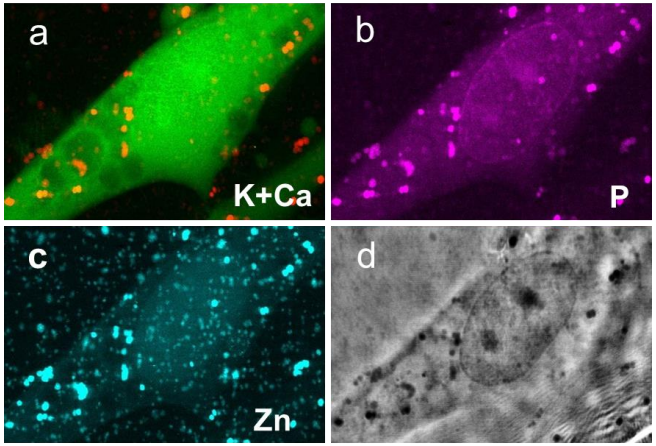

Figure 2. shows the deposition of Ca, P and Zn after 10 days of differentiation in bMSC (Panel a, b and c). Panel d, phase contrast reconstruction of the same bMSC