m12.o03

X-ray microdiffraction for engineered bone study: scaffold resorption analysis

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Keywords: X-ray microanalysis, biomaterials design, interface characterization

One of the most recent therapeutic strategies for the reconstruction of damaged large bony segments includes the tissue engineering approach. It takes advantage of the patient's own cells, which are isolated, expanded in vitro, loaded onto a bioceramic scaffold and reimplanted into the lesion site. Bone marrow stromal cells (BMSC) are the most commonly used cell type. In this therapeutic approach the scaffold, where the cells are loaded on, plays a crucial role for the determination of the spatial organization of the new bone and the bone-biomaterial integration. In particular, the detailed analysis of the interface between bone and scaffold is fundamental in order to study the influence of chemical composition and geometry on the regeneration of the new bone formation and on the scaffold resorption.
In this study we evaluated the behaviour of SkeliteTM (Millenium Biologix Corp., Kingston, Canada), a clinically available scaffold based on hydroxyapatite (HA) and Silicon-stabilized Tricalcium Phosphate (Si-TCP). Previous analysis by computed tomography (CT) revealed a progressive disappearance of the scaffold with the implantation time and its subsequent replacement with highly mineralized lamellar bone. However the resorption mechanism was not clearly understood in details. In this work bone formation and scaffold remodeling were evaluated by high spatial resolution (<1 micron) Wide Angle X-ray Scattering and Small and X-ray Scattering on sections of embedded implants retrieved from animals sacrificed at different times. With a special software we obtained microscopic images displaying the spatial variation of different structural features, thus allowing to map the mineralization intensity and bone orientation degree around the scaffold pore. The remarkable organization of the bone crystals and collagen fibres with respect to the scaffold geometry, reported in this work, appears to be the essential key to impose the 3-D desired architecture to the growing bone. A quantitative study of the diffraction data acquired at the interface, showed that the progressive scaffold resorption and bone formation are coupled to a significant depletion of Si-TCP with respect to the HA. Similar experiments where scaffolds were implanted in the absence of osteogenic cells, revealed that neither bone formation nor changes in scaffold chemical composition took place, thus indicating that SkeliteTM scaffold resorption and bone formation are interrelated processes. These results indicate the great potentialities of crystallographic analysis taken at high spatial resolution.

m12.o04

The organization of the organic structural framework in the enamel biomineralization processes

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Keywords: biomineralization, enamel, nanostructures

The growth of crystals within a preformed organic structural framework (the organic matrix) is a basic mode of skeletal formation adopted by many different organisms. Protein self-assembly into ordered structures is a critical step towards the control of mineral deposition in biomineralizing systems such as bone, teeth and mollusc shells. [1]
Mammalian tooth enamel is the hardest tissue in the vertebrate body and is a secretory product of cells of epithelial origin called ameloblasts. Enamel mineralization is a dynamic process that includes protein secretion, matrix assembly and initiation and growth of the crystals within an amelogenin-rich matrix. The assembly of the mineralized enamel matrix continues through the transition stage during which ameloblast activity is drastically reduced and the bulk of the protein matrix is eventually processed during the maturation stage, concomitant with the rapid growth and maturation of the mineral. Supra-molecular self-assembly of the dental enamel protein amelogenin into nanospheres has been recognized to be a key factor in controlling the oriented and elongated growth of carbonated apatite crystals during dental enamel biomineralization. We report the formation of birefringent micro-ribbon structures that were generated through the supramolecular assembly of amelogenin nanospheres. These micro-ribbons have diffraction patterns that clearly indicate a periodic structure of crystalline units along the long axis. Linear arrays of nanospheres were observed as intermediate states prior to the micro-ribbon formation. The induction and c-axial orientated organization of apatite crystals parallel to the long axes of the micro ribbons were observed. [2]

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