DOI: 10.1017/S1431927603446448
Microsc Microanal 9(Suppl 2), 2003

Characterization of a Collagen/Hydroxyapatite Biocomposite; Interaction with Human Osteoblasts *in vitro*

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Osteoconduction and osteoinduction are expected when biomaterials are used as scaffolds for bone tissue engineering. Several composites from natural origin have been proposed as bone grafts, including echinoderm spine, sea sponges, coral skeleton and nacre from mollusk shell [1]. Other materials are synthetic as hydroxyapatite, tricalcium phosphate and bioactive glass or "biomimetic", as porous vaterite microspheres generated by self-organizing media [1]. Composites including collagen/hydroxyapatite and biodegradable polimers have also been used [2]. The association of osteo-inductive factors and osteogenic stem cells with implantable materials, are important emerging research fields [3]. Among surface characteristics influencing the osteogenic process related to implanted biomaterials are surface energy, roughness and topography (with rough surfaces inducing osteoblast attachment), pore dimensions and interconnectivity of pores [4]. In particular, pore diameters in the range of 150-500μm induce bone mineralization [1].

In this work we investigated the effects of sintering temperature (600, 900 and 1100^{o}C) on the physicochemical and crystallographic properties of cortical bovine bone, by using transmission and scanning electron microscopy, X-ray diffraction, infrared spectroscopy and mass spectrometry. Samples were powdered (200 – 500μm) before analysis. Bovine bone HA calcined at 1100^{o}C and powdered (200 to 500μm) was mixed to type I collagen from bovine origin forming a biocomposite when mixed in different proportions (1:2.6 and 1:1 Coll/HA wet weight).

Electron microscopy and X-Ray diffraction showed that HA grains are larger and present higher crystallinity and unit cell parameters (*a* and *b* axes) for higher sintering temperatures. Infrared spectra of sintered samples showed that they were composed essentially of HA with minor additional groups such as calcium hydroxide, crystal water, free carbon dioxide and possibly brushite. Thermo controlled desorption of sintered samples using the mass spectrometer showed the presence of carbonate groups distributed at A and B sites of HA, and other weakly bound to its structure. However, while type A carbonate content decreased in HA, weakly bound and probably type B ones relatively increased with the increasing of sintering temperature. When a second desorption was performed in samples and the carbonate content analyzed by the mass spectrometer, it was observed a distribution similar to the previous one for the higher sintering temperature, indicating that HA probably trapped CO_2 from the environment during the cooling process.

Feret diamenter and *shape factor* were used to characterize the HA particles while the morphometric parameters Vv and Sv, obtained from the observation of polished sections of Epoxy resin embedded samples by scanning electron microscopy, were used to characterize the 3-D arrangement of the Coll/HA composite. The comparison of Vv and Sv mean values from samples with different Coll/HA proportions (1:2.6 and 1:1 wet weight) and procedures (*e.g.* dehydrated and non-dehydrated samples) indicated that a macroporosity may be defined for the biocomposite, and that this macroporosity is in the range of the ideal one (circa 50% in the present case which means voids in the interval 200 – 500μm approximately). The above characteristics were close for the two Coll/HA

proportions used and seemed to not change very much, after manipulation of the sample. These observations indicate that this biocomposite may be particularly useful as bone grafts.

Some authors believe that the bioactivity and ability to form a strong interface bone-material is related to the formation of carbonated HA on the calcium phosphate biomaterials surface. Organic and inorganic processes related to the interaction between substrate surface and serum, biologic fluids and/or cells may cause the dissolution of surface material and precipitation of micron-sized crystals of carbonated HA [5]. The formation of a carbonated HA crystals layer associated to the adsorption and incorporation of biological molecules and ions on the substrate surface, would mediate effects on the cells activity, including adhesion, proliferation and differentiation [6].

Human osteoblasts were used (International guidelines for the use of human cells were followed). The higher number of cells observed in cultures using HA particles sintered at 1100^{o}C when compared to the other sintering temperatures may be related to the presence of weakly bound carbonates to the structure, as these carbonates would dissolve and reprecipitate carbonated hydroxyapatite in the presnce of serum in culture.

Figure 1 shows an 11 day culture of human osteoblast onto Coll/HA biocomposite (1:1; 1100^{o}C). Cell adhered both to HA particle and to type I collagen fibers.

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[7] Financial Support: CNPq, Faperj and Institutos do Milênio/CNPq.

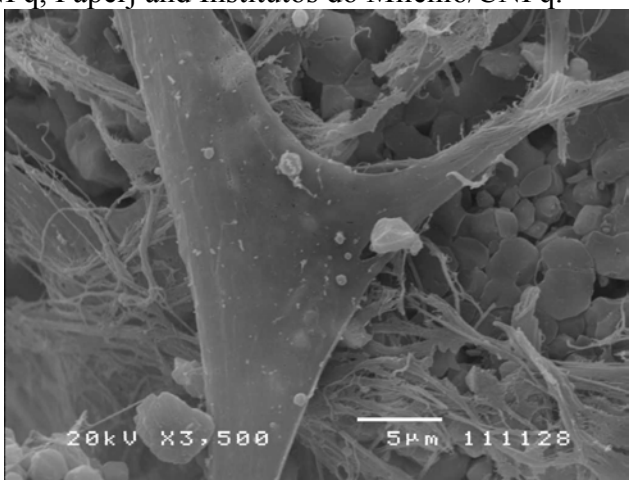

Figure 1: A human osteoblast adhered to Coll/HA composite in an 11 day culture. Collagen fibers are seen, as well as HA grains caused by the sintering process. Bar = 5µm.

https://doi.org/10.1017/S1431927603446448 Published online by Cambridge University Press