Analysis of the new bone tissue formed onto hydroxyapatite-alginate and sintered hydroxyapatite in vivo

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Abstract – Micrometer sized spheres of hydroxyapatite-alginate (HA-Alg) and sintered hydroxyapatite (HA-Sint) were used as scaffolds for bone tissue engineering. Critical defects in rat calvaria were filled with the biomaterials and the new mineralized tissue formed around the biomaterials after 90 and 120 days was analyzed by TEM, SAED and EDS. HA-Alg promoted bone formation more intensely than HA-Sint. However, the mineralized layer adjacent to HA-Alg presented lower Ca/P ratio when compared with similar regions of HA-Sint, which showed a Ca/P ratio close to the one found for mineralized collagen.

In the last few decades different biomaterials were produced aiming to improve the regeneration process in both space (complete regeneration of a lesion) and time (reducing healing time). In this work we produced two kinds of biomaterials for bone engineering: Hydroxyapatite-Alginate (HA-Alg) composite, and Sintered-Hydroxyapatite (HA-Sint), in the shape of small spheres (≈ 400 μm). Nanoparticles of hydroxyapatite were synthesized and mixed with sodium alginate. Afterwards the sample was dropped in a solution of $CaCl_2$ in order to produce a composite containing polymers of calcium alginate mixed with nanoparticles of hydroxyapatite [1]. After this step, one of the samples was sintered at 1100 ^{o}C (for decomposition of the alginate), while the other was air dried and stored at room temperature. The spheres of both materials were introduced in critical defects (≈ 8 mm) in rat calvaria. In a previous work we reported that HA-Alg promoted intensive formation of new bone tissue when compared with HA-Sint [1]. However, a detailed characterization of the new mineralized tissue surrounding the biomaterials at a nanometer scale had not been performed. Samples from the experimental time periods of 90 (HA-Sint) and 120 (HA-Alg) days were ultrathin-sectioned and analyzed by TEM. The areas chosen for analysis were those where the biomaterials and the new mineralized tissues were in close contact.

The new mineralized tissue surrounding HA-Sint sample was homogeneously distributed (Fig 1A) and was also present inside the bulk (Fig 1B). EDS analysis of this region (Fig 1C) showed a Ca/P ratio resembling the one from the collagen region (Fig 1D; EDS spectrum not shown). Differently, HA-Alg sample (Fig. 2A) showed a smaller Ca/P ratio for the tissue just surrounding the biomaterial (Fig 2B). Although it has been found that HA-Alg has promoted bone mineralization more intensely than HA-Sint, in this work we show that the nature of the mineralized tissue just surrounding the biomaterials may differ. This may be related to the direct interaction of cells with the nanoparticles of the two materials which presented different degrees of sinterization and/or disaggregation. Our results show the importance of characterizing the mineralized tissue around implant materials as a mean of optimizing bone healing process and biomaterials design.

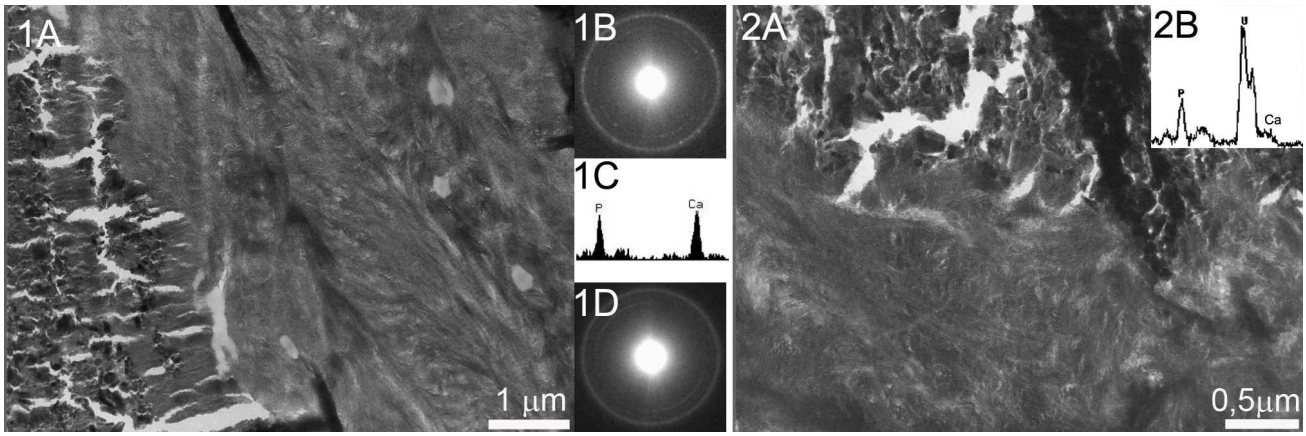

Figure 1: TEM of HA-Sint. A) Small dark regions at left: hydroxyapatite grains; right side of the figure: new mineralized tissue. B) SAED of the left side of the figure showing that it contains both HA nanocrystals (dots) and new mineralized tissue (thin and difuse rings); C) EDS from the region adjacent to HA-Sint biomaterial; D) SAED from mineralized collagen: typical image showing dispersion of [002] (inner ring). Figure 2: TEM of HA-Alg. A) Biomaterial (top); new formed tissue (bottom). B) EDS spectrum from a region that surrounds the biomaterial. Note that, differently from the previous case, calcium is absent, or present in a very low concentration.

[1] F.L. De Paula, I. Barreto, M. Rocha-Leão, R. Borojevic, A. Rossi, F. Rosa and M. Farina, Front. Mater. Sci. China 3 (2009) 101007.

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