Biologically Inspired Scaffold Mimicking Trabecular Bone in a Rabbit Spinal Fusion Model

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Statement of Purpose: Several medical applications for bone forming materials have emerged in America's aging and enlarging population, including osteoporosis, fracture healing, and spine fusion applications. However, no reliable osteogenic device has been developed and applied clinically with satisfactory cost, efficacy, and safety [1]. In this study we developed and fully characterized an innovative multi-substituted hydroxyapatite/collagen composite scaffold, to mimic trabecular bone. The osteoinductive potential of this material was assessed *in vitro* with human mesenchymal stem cells (hMSC), and ultimately tested in a spinal fusion model in rabbit, without the addition of any biologic.

Methods: To fabricate the scaffold, composed of magnesium carbonate hydroxyapatite/collagen 70/30 wt% composite (MCHA/Coll), a biologically inspired mineralization process was followed. MCHA crystals were directly nucleated on type I collagen fibers during their self-assembling. The scaffold was characterized by XRD, FTIR, ICP-OES, TGA and SEM. hMSC were seeded on MCHA/Coll scaffolds and viability, cell growth and osteogenic differentiation were investigated, using collagen scaffolds with or without inducing media as positive and negative controls, respectively. Ultimately, MCHA/Coll were implanted in a spinal fusion model in rabbit, to study the kinetic of bone formation mediated by the material *per se in vivo*, compared to decorticated bone, as the current standard of care, and bone formation was followed by DynaCT. No cells or other biologics were added to the scaffolds that were implanted.

Results: We found that hMSC viability was not altered when seeded on MCHA/Coll scaffolds, however cells growth was reduced when on the scaffolds, respect to the controls. This was due to their quick differentiation towards the osteogenic lineage, which did not occur in control scaffolds. In fact, it was found a significant up-regulation of *RUX2*, *BGLAP*, *SPP1* and *ALP* by hMSC when seeded on MCHA/Coll. Also, it was observed that the up-regulation of these genes in hMSC cultivated on control collagen scaffolds, in presence of osteogenic media, did not match that of cells seeded on MCHA/Coll with regular media, demonstrating the higher osteoinductive potential of our materials, respect to any biological stimuli. The formation of an intimate bound of the organic matrix (which acted as a template for the inorganic phase) and MCHA was demonstrated by the shift found in the FTIR spectra in the range of the stretching of the asymmetric carbonyl, probable site of interaction of the apatite with the collagen. This was explained by an efficient mimicry of the bone composition as elucidated by XRD, which reported the production of a multisubstituted biomimetic apatite phase nucleated on the organic matrix, which resembled that of human trabecular bone. The presence of the doping ions magnesium and carbonate was confirmed and quantified by ICP-OES, revealing a Ca/P typical of not stoichiometric apatite, and a substitution of ~5% of calcium by magnesium ions, similar to that of newly formed bone. The TGA showed that also the ratio between inorganic and organic phase of MCHA/Coll matched that of human trabecular bone, with an overall 53% of MCHA. SEM micrographs further demonstrated the formation of a composite, in which the mineral phase is intimately associated with the collagen, as shown in fig. 1, and which looked very similar to the natural tissue.

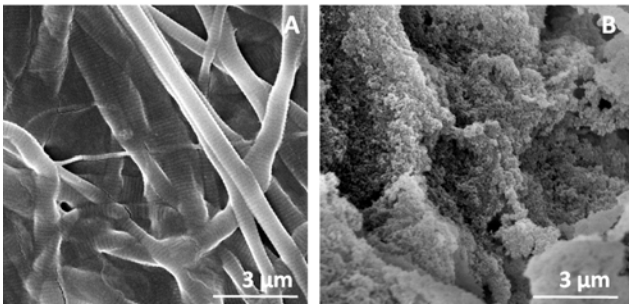

Figure 1. SEM micrograph of collagen scaffold and

Finally, the *in vivo* results showed that the material was able to produce spongy bone-like tissue in the spinal fusion model already after 2 weeks (quantified by DynaCT, *per* values above 200HU). Such newly formed bone was found to start evolving in more mature bone tissue, as assessed by the quantification of tissue mass with values above 500 HU at the DynaCT. This correlated with the fact that our material closely resembled (both morphologically and chemically) newly formed trabecular bone. In fact, newly formed bone has a faster turnover *in vivo*, explaining the faster regeneration. This is due mainly to the presence of the doping ions, which destabilize apatite's lattice, thus speeding up its degradation.

Conclusions: Our study resulted significant as our material displayed a higher level of biomimicry for young trabecular bone and faster bone formation respect to currently available therapeutics, without the use of biologics, making this material a safer, simpler and cheaper approach for bone formation.

References: Carragee, EJ. The Spine Journal 11.6 (2011): 471-491.