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A novel chitosan/hydroxyapatite biocomposite for tissue engineering applications.

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Abstract – Porous scaffold biomaterials provide a framework for cell adhesion, proliferation and creation an extracellular matrix. These scaffolds are considered to be suitable biomaterials for bone regeneration. Chitosan/hydroxyapatite biocomposite scaffold, CNHAP, was prepared by nucleation *in situ* of hydroxyapatite in neutral chitosan matrix. The morphology of this composite was similar to the bone tissue, with a distribution of HAP spherical particles of medium size of ~20nm. The biological tests showed pre-osteoblast adhesion and differentiation on the surface. Therefore, the CNHAP scaffold showed promising biological behavior and may therefore find applications in tissue engineering for bone defects.

Hydroxyapatite (HAP) and chitosan (CN) have been widely employed to develop suitable 3D supports for applications tissue engineering of bone and cartilage [1,2]. Tissue engineering is widely understood as the use of a combination of cells, engineering and material with particular importance the interaction between cell and the support material. Besides the choice of appropriate materials and the optimization of their mechanical properties and architecture as biological scaffolds, tissue engineering strategies require the use of cells, which can be explored at clinical practice as an effective tool for tissue regeneration.

In this study, a novel 3D biocomposite (CNHAP) derived from CN and HAP was prepared as a hydrogel with good mechanical property, from high molecular mass chitosan and the *in situ* growth of hydroxyapatite. The biocomposite was characterized by X ray diffraction and scanning electron microscopy. The biological characterization was evaluated by assessing cellular activity *in vitro* on CNHAP, by evaluating the cell viability (MTT reduction), cell adhesion/proliferation (Violet crystal) and osteoblast differentiation (alkaline phosphatase activity measurement).

The CNHAP scaffold presented the upper side with a flat surface and the bottom with a roughness surface. SEM micrographs of the CNHAP scaffold fracture surface, showed a distribution of the HAP spherical particles, of ~20 nm, uniformly dispersed in the chitosan matrix. Both CNHAP scaffold surface (flat and roughness) were submitted to biological tests. Both of them showed pre-osteoblast adhesion (Fig.1A), proliferation and differentiation (Fig.1B) of cells on the surface. However, the roughness surface showed a better performance in all evaluated parameters. XRD has revealed that this biocomposite showed HAP poorly crystalline particles. In addition, the results of biological tests showed that the CNHAP composite provided an adequate support for the attachment, proliferation and differentiation of MC3T3-E1 pre-osteoblast cells into mature osteoblasts. The CNHAP scaffolds presented a promising biological behavior and may therefore find applications in tissue engineering for bone defects.

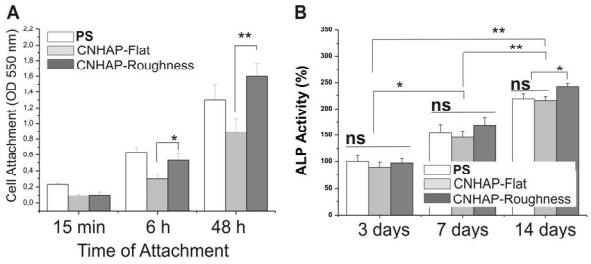

Figure 1: Biological assessments. **(A)** Osteoblast adhesion and proliferation; **(B)** ALP activity was used as osteoblast differentiation biomarker. (ns) no statistically difference; (*) p<0.05; (**) p<0.01. Polystyrene (PS) surfaces were used as a control group.

References

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