Design Of Bone, Cartilage And Osteochondral Tissue Engineering Chitosan-Based Produced By A Particle Aggregation Methodology

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Statement of Purpose: In this work it is presented a new processing route for the production of bone, cartilage and osteochondral tissue engineering scaffolds using aggregation of previously developed chitosan-based microspheres. For osteogenic approaches, composite scaffolds were also developed. For osteochondral applications, bilayered chitosan based scaffolds were designed and characterized.

Materials and Methods: Chitosan was dissolved in acetic acid to obtain a 2%wt solution. For the production of composite particles, hydroxylapatite (HA) was homogeneously dispersed in the solution. The solutions were then extruded trough a syringe at constant rate into a precipitation bath where microspheres with regular diameter were formed. For the crosslinked scaffolds, the microspheres were submitted to a reaction with glutaraldehyde. The particles were then pressed into moulds and left to dry in an oven for 3 days. The scaffolds morphology was analysed by Scanning Electron Microscopy (SEM) and micro-Computed Tomography (μ-CT) analysis. A 2D histomorphometric analysis was performed. By means of using the Mimics® software from Materialise (Belgium), it was possible to build 3D models representing the scaffolds structures. The mechanical properties of the materials were tested on a compressive solicitation mode in an Instron universal machine in a controlled environment. The hydration behaviour was accessed in different pHs mediums in order to study the potential responsive behaviour of the developed systems.

Results and Discussion: By using the precipitation method, it was possible to obtain spherical chitosan-based particles with a mean diameter in the range of 500 to 800 μm. The typical morphology of the developed scaffolds is shown in Fig. 1. The cross-section (1.A) confirms the formation of pores in the bulk of the material showing the scaffolds interconnectivity. Furthermore, bilayered scaffolds were also produced as shown in Figure 1.B.

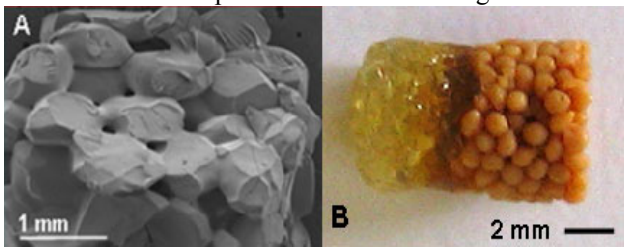

Figure 1: SEM (A) and stereolight microscopy (B) of polymeric and bilayered chitosan-based scaffolds.

Cross-section observation and μ-CT analysis confirmed the formation of pores in the bulk of the materials showing the scaffolds interconnectivity (Fig 1A and 1C). For the osteochondral applications, bilayered materials 3D virtual models were also built as shown in Fig. 1.C. The 2D histomorphometric analysis allowed for the characterization of the HA distribution and the porosity along the developed scaffolds (Fig 1.B and 1.D). It was possible to observed that the incorporation of HA decreased in some extent the porosity of the scaffolds.

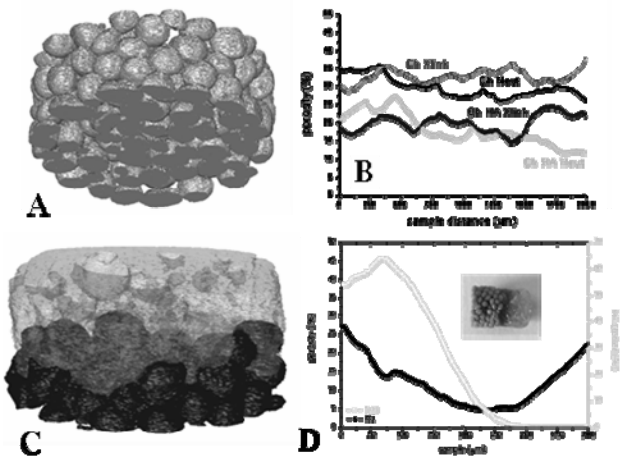

Figure 2: μ-CT and 2D histomorphometric analysis of polymeric and bilayered chitosan-based scaffolds.

Concerning the water uptake, it was possible to observe the pH responsive behaviour of the developed scaffolds. In general, the degree of swelling is very high at pH 5, as compared to that of pH 7.4 and 9, due to the inherent hydrophobicity of the chitosan dominating at high pH values. In the neutralized samples, the hydration degree reached values of 160-170%. However, and if desirable, this hydration behaviour can be controlled by crosslinking or incorporation of HA filler, as it was verified since a much lower value of water uptake was obtained (80-110%). For the conditions concerning the compressive properties, scaffolds have shown a very good mechanical behaviour. The highest modulus was obtained for composite crosslinked scaffolds with a value of 449±7 MPa. For the polymeric scaffolds, a modulus of 132±7 MPa (neutralized) and 230±26 MPa (crosslinked) could be obtained.

Conclusions: The obtained morphology, the responsive water uptake behaviour and the promising mechanical properties, make chitosan scaffolds processed by a novel particle aggregation processing route a very promising system to be applied in tissue engineering applications of bone, cartilage and osteochondral defects.

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