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Porous Bioactive Composites from Marine Origin Based in Chitosan and Hydroxylapatite Particles

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Abstract. An optimal carrier for bone tissue engineering should be both a controlled release system and a scaffold. In the former role, the carrier must prevent rapid factor clearance and ideally meter out the growth factor in a predictable manner, allowing therapeutic doses to stimulate target cells for the appropriate duration. In the latter role, the material should act as a permissive environment into which bone cells would be attracted to migrate and begin the process of depositing bone matrix. Therefore the direct incorporation of growth factor in porous scaffolds should be a desirable goal. The inclusion of a bioactive ceramic on the scaffold design will confer to the systems a bone bonding behaviour that will guide bone formation. This work reports the development of composite chitosan/HA (from algal origin) porous structures produced by means of freeze-drying processing routes that can be further loaded with a biologically active agent. The developed bioactive 3D structures (completely from marine origin) have potential application as tissue engineering scaffolds and drug delivery systems due to their morphological and bioactive properties.

Introduction

Bone repair is a subject of intensive investigation in human health care. Owing to their physicochemical and biological properties, calcium phosphates have a considerable potential as remodeling implants, prosthetic bone replacement and drug delivery devices. Many efforts have been made towards the development of new bone substitutes materials. Among these hydroxylapatite/polymer composites have attracted much attention, since such composites may be osteoconductive due to the presence of hydroxylapatite and its similarity with bone inorganic part [1,2]. One interesting polymer to be used in composite biomaterials is chitosan, a polymer from marine origin, that has been widely used in biomedical applications, namely in scaffolds for skin tissue engineering, wound dressings and sustained drug release carriers. It is a biocompatible and biodegradable polymer, that can be hydrolysed by lysozyme (present in human body fluids) [3]. Chitosan possess a large number of hydroxyl groups all over its chain. The presence of OH^- groups on the surface seems to facilitate the connection of an apatite layer to a certain substrate, nucleated by a biomimetic treatment [4]. On the other hand, it has been observed that glucosamine has a beneficial effect on treatment and symptoms of osteoarthritis as it helps to regenerate joint cartilage [5]. Chitosan/HA composite 3D porous structures combine chitosan unique properties with those of HA and could lead to the design of adequate systems both for bone and for osteochondral tissue engineering applications. Due to the processing method proposed in this work, these composite 3D architectures can be further loaded with a biologically active agent in order to improve the cell differentiation, adhesion and proliferation.

Materials & Methods

Chitosan from crab shells (Sigma) with a degree of deacetylation higher than 85% was dissolved in acetic acid solution (1%wt). HA particles from algal origin were used as bioactive fillers [2]. In some cases, crosslinking reaction was carried out in a neutralised solution by means of adding a small amount of glutaraldehyde (GLU). To avoid the use of glutaraldehyde, an alternative method

was also developed being based on the chitosan precipitation in a NaOH solution. The incorporation of HA particles (10%wt) was performed in the solution that was immediately freezed, both to avoid the precipitation of particles and to improve adhesion between the polymeric and the ceramic phases. The samples were then freezed overnight at –85ºC and then freeze-dried for 3 days in a lyophilizator.

The materials were characterized morphologically by scanning electron microscopy (SEM). The swelling and degradation behaviours were evaluated in an isotonic saline solution (NaCl 0.154M, T=37ºC, pH=7.4). A preliminary *in-vitro* bioactivity study was carried out in a simulated body fluid (SBF) at physiological temperature and pH.

Results & Discussion

The proposed freeze-drying processing methodology allowed to obtain 3D porous structures with typical morphology shown in Figure 1. One interesting fact that must be stress out is the decreased pore size (200-20 μm) obtained for the materials precipitated with NaOH as shown in the figure. This could be very interesting in tailoring the water-uptake and consequently the drug release profile. Typical pores size for the crosslinked materials was in the 600-200 μm range.

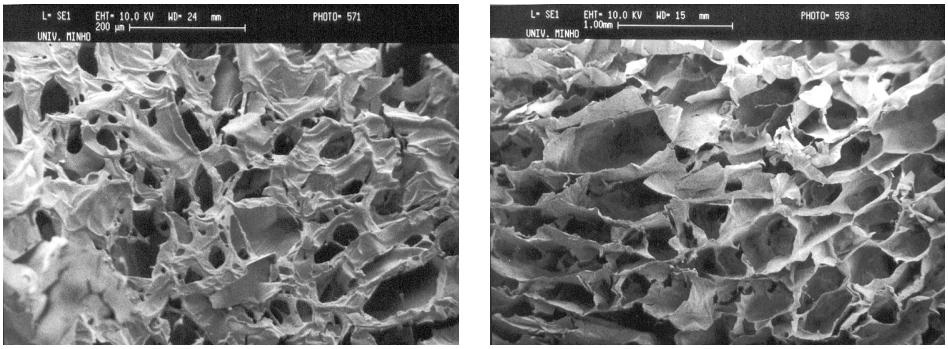

Figure 1. SEM micrographs showing the morphology and porosity of the chitosan 3D porous structures obtained with treatment with NaOH (left) and crosslinked with GLU (right). Please note the scale bars, respectively 200 μm and 1 mm.

With the developed methodology it was possible to obtain not only polymeric but also composite porous structures (with an extremely good adhesion between the ceramic and polymer as shown in Figure 2). The good interface that could be obtained may be seen on Figure 2.

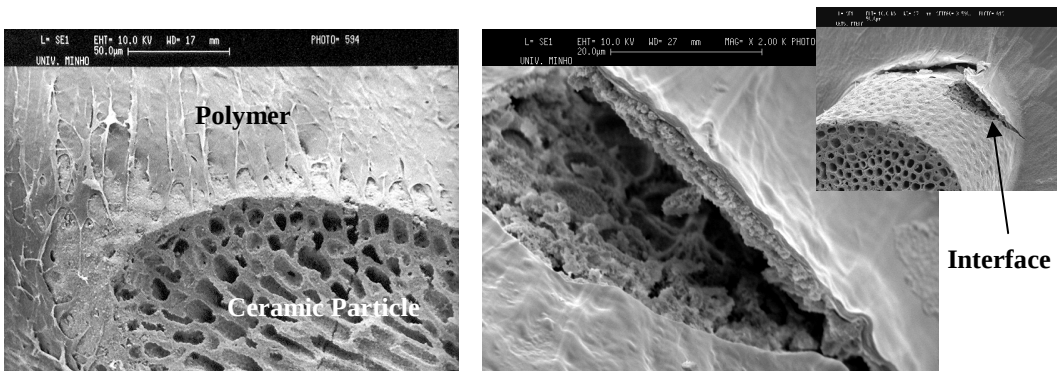

Figure 2. SEM micrographs showing the interface between the polymer and the ceramic phases.

The swelling degree of porous structures can be controlled by means of changing 3 different parameters: (i) the crosslinking degree, (ii) by treatment with NaOH and (iii) the amount of HA particles. The equilibrium hydration degree was reached after approximately 1 day and can varied from 800% up to 1500% (Fig.3). One additional interesting fact is that the treatment with NaOH

seems to be more effective in controlling the hydration degree when compared to crosslinking. The result could be related with protonated amine groups of chitosan, which readily dissolve in aqueous medium. It is also important to stress out that the precipitation with NaOH led to a decrease in both macro and microporosity that consequently leads to a lower water uptake equilibrium value due to the more difficult penetration of the medium into the bulk of the materials.

On what concerns to the degradation behaviour, the presence of HA particles decreases the weight loss in about 20%. It seems that crosslinking with GLU was not totally effective, as a higher value of weight loss was obtained for GLU treated materials.

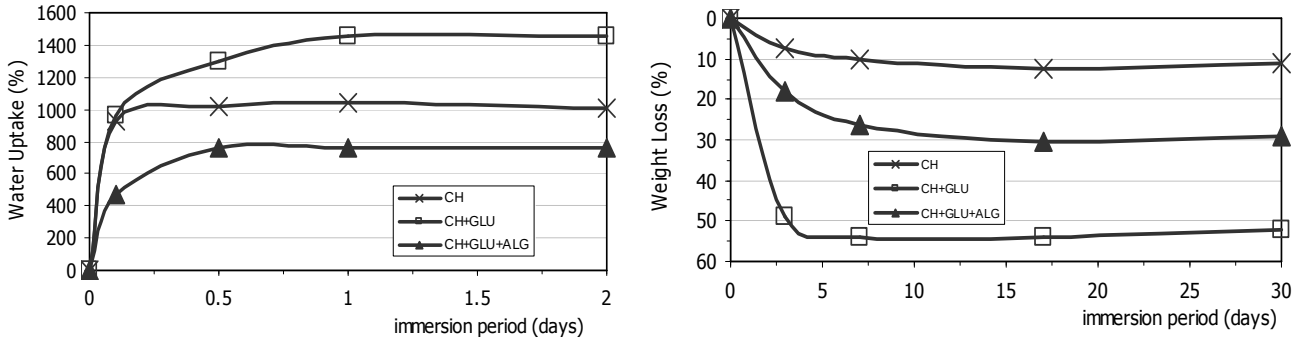

Figure 3. Swelling (left) and degradation (right) behaviour of porous structures: treated with NaOH (CH), crosslinked with glutaraldehyde (CH+GLU), with incorporation of HA particles (CH+GLU+HA).

Preliminary *in vitro* bioactive tests showed a formation of an apatite-like layer at the surface of porous structures (in the composite materials) due to the presence of HA particles. Another remarkable fact is the presence of Ca-P nucleous on unfilled chitosan samples treated with NaOH only after 3 days of immersion in SBF, that could be related with the incorporation of –OH groups at the surface of the chitosan based materials and their swelling ability. For these chitosan porous structures, it was possible to observe the formation of an apatite-like layer after 3 days of immersion in SBF even inside the pores as shown in Figure 4. After 45 days of immersion, both the polymeric and the composite porous materials were covered by a dense layer with the typical "cauliflower" morphology, except for the chitosan materials crosslinked with glutaraldehyde. This result is confirmed by ICP results (see fig. 6b) on which both the Ca and the P tend to be stable as function of time. One possible explanation can be related with the higher weight loss observed for this treatment. The non-totally effective crosslinking reaction leads to constant surface and pH changes that do not allow for the Ca-P layer formation. However, further studies are necessary to confirm this behaviour and improve the efficiency of the crosslinking reaction.

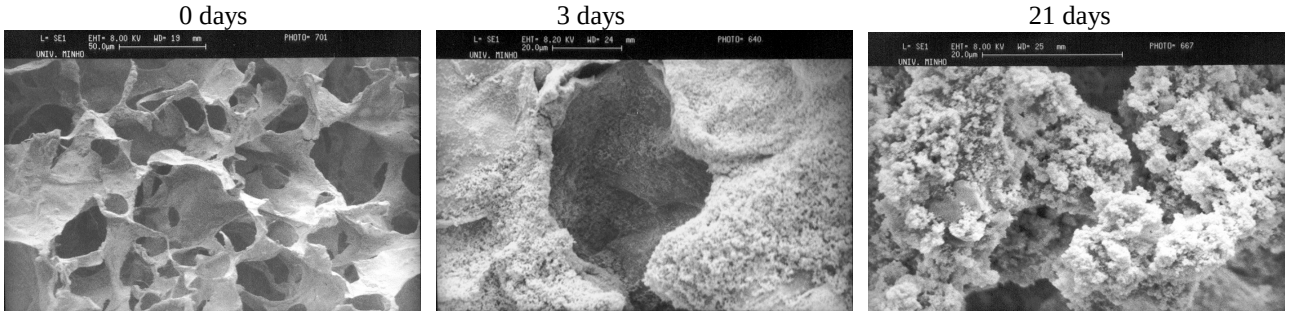

Figure 4. SEM micrographs showing the formation of an apatite-like layer in the surface of the chitosan porous structures treated with NaOH after the respective immersion period in SBF solution. As shown, the the treated chitosan structures are cleary bioactive.

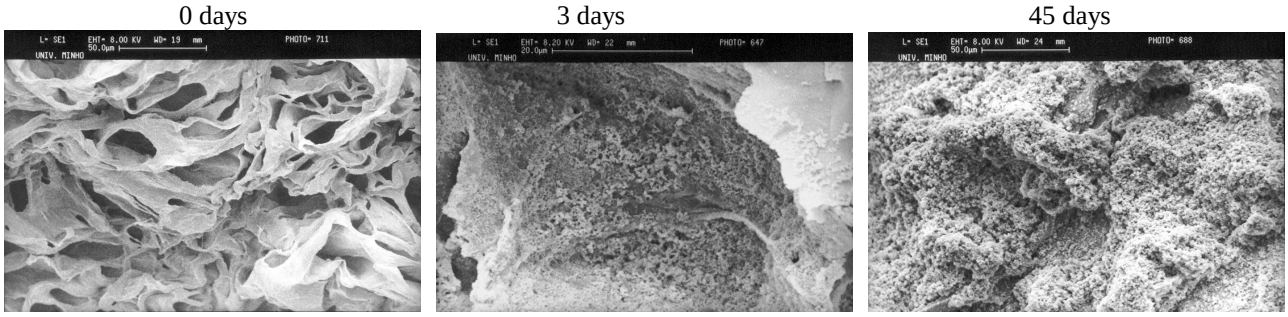

Figure 5. SEM micrographs showing the formation of an apatite-like layer in the surface of the chitosan composite porous structures treated with NaOH after the respective immersion period in SBF solution.

The ICP results have confirmed the SEM observations. It is possible to observe the decrease in the Ca and P concentrations in the solution (Fig. 6) indicating the materials uptaking of Ca and P ions leading to the precipitation and growth of the Ca-P layer. The slight increase in the Ca concentration in the crosslinked materials can be related once again with the higher weight loss. The release of non-crosslinked chitosan for the solution can cause this Ca increase since this materials is from crab shell origin.

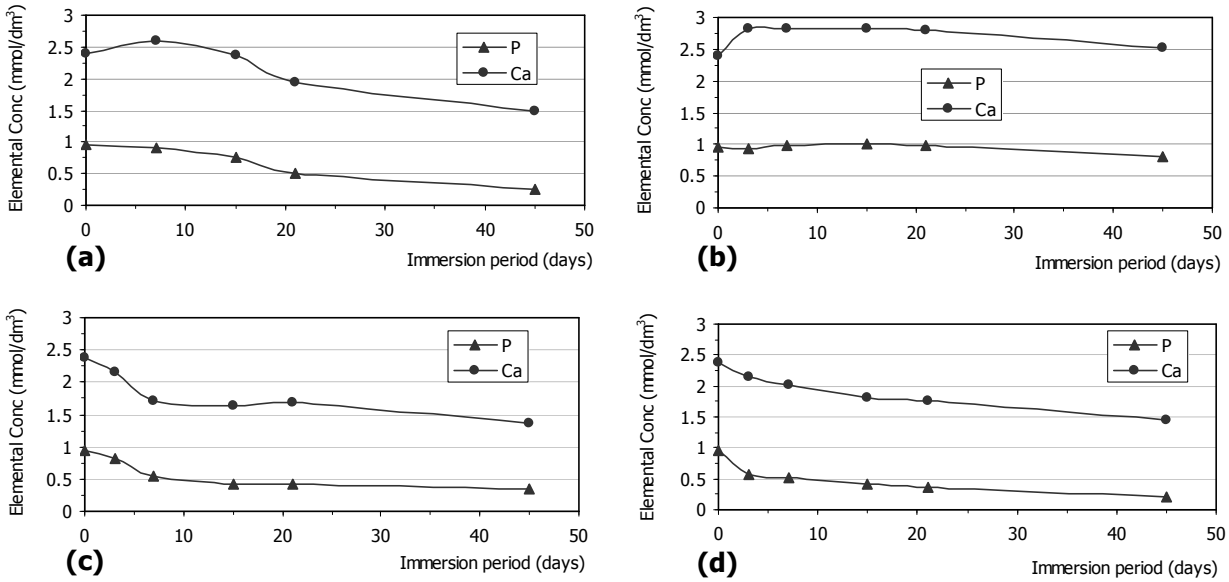

Figure 6. Evolution of Ca and P elemental concentration (ICP results) in SBF solution in function of immersion period of (a) chitosan treated with NaOH, (b) crosslinked with glutaraldehyde, (c) composite treated with NaOH and (d) composite crosslinked with glutaraldehyde.

Conclusions

Bioactive composite (and fully chitosan) porous structures totally from marine origin could be developed. The results have shown that the materials' properties can be controlled either by using a crosslinking reaction or by precipitation with NaOH. It was possible to incorporate porous HA particles without changing their morphology. The developed materials present a range of properties that might allow for their use on bone engineering applications and as materials for filling bone and dentistry defects.

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