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Development of gelatin/HA membranes

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Abstract. The aim of this work is to develop membranes of gelatin/hydorxyapatite composite for using as mechanical barrier. Membranes with 4% of gelatin and 0.5-3% of hydroxyapatite were studied. The composite was obtained by heterogeneous co-precipitation method. X-ray diffractometry showed the formation of a pure phase of hydroxyapatite with excellent crystallinity. Using atomic force microscopy, the distribution of crystals of hydroxyapatite in the gelatin matrix was observed, with nanoparticles of about 50-100nm in a homogeneous mixture. After crosslinking process with glutaraldehyde at different concentrations (0.5, 1, 1.5 and 2%), a significant decrease of the swelling behavior was observed due to the increase of the amount of triple helix in the samples, which increases their stability in aqueous solution.

Introduction

One of the main obstacles for the success of bone cicatrisation is the very fast formation of conjunctive and epithelial tissue. The regeneration of tissue was questioned since the first works appearing in the literature [1], according to whom bone could be regenerated in a more predictable manner if isolated from the adjacent conjunctive tissue. The migration of these soft tissues could disturb or totally impede the osteogenesis on the defect or the surgical area. One of the drawbacks of this migration for bone development is the production of soluble factors by the fibroblasts, which can inhibit the differentiation of bone cells and osteogenesis. Physical sealing of an anatomic site for better cicatrisation of a specific kind of tissue and direct tissue regeneration can be achieved by using a mechanical barrier. In applications with hard tissue, in order to mimic the bone structure specifically organized at nanoscale, which consists in collagen fibers and mineralized apatite nanocrystals, a variety of systems were developed [2-6]. Among them, composites of apatite crystals and natural polymers received great attention because they can offer the benefits of composition, preserving the structural and biological functions of the damaged hard tissues in a more efficient way and more similarly to the natural system [1]. The collagen-based gelatin has a large number of biological functional groups and potential applications in tissue scaffolds [7,8]. In practice, gelatin is currently used in pharmaceutical products, because of its excellent cellular viability and the lack of antigenicity [7]. The availability of different forms and the low cost facilitates the selectivity and production at large scale.

Materials and Methods

A 4% aqueous solution of type-A gelatin was prepared, Bloom 280-300, at 60°C, adding afterward glycerin (20g/100g of gelatin) and an H_3PO_4 solution. The resulting gelatin/H_3PO_4 solution was then dripped in a calcium hydroxide solution under agitation at a constant temperature of 45°C, with a ratio Ca/P of 1.67. A constant pH was kept constant trough out the reaction (24hs) at a value of 7. The final suspension was then poured in a plastic mold obtaining membranes containing 4% of

gelatin and 0.5%, 1%, 2% and 3% of hydroxyapatite (HA), labeled as G4H05, G4H1, G4H2 and G4H3, respectively. The membranes reticulated with glutaraldehyde were obtained following the same procedure described before adding, after the 24-hour reaction, different concentration of glutaraldehyde solutions (0.5%, 1%, 1.5% and 2%) labeling them as G4H05G05, G4H05G1, G4H05G15 and G4H05G2 respectively. The crystalline phase of the membranes was analyzed by X-ray difractometry (XRD), Atomic Force Microscopy (AFM) and measurements of swelling of the membranes with glutaraldehyde.

Results and Discussion

To confirm that the gelatin solution did not alter the production of the HA in the co-precipitation process, the inorganic phase was characterized by XRD. Because the low concentration of HA in the membranes, which difficult the detection, the organic and inorganic phases were separated, characterizing the HA powder, Fig. 1. The diffractograms for the different quantities of apatite crystals corresponding to each sample, exhibited the HA phase only, with high degree of crystallinity, even without of thermal treatment after the precipitation.

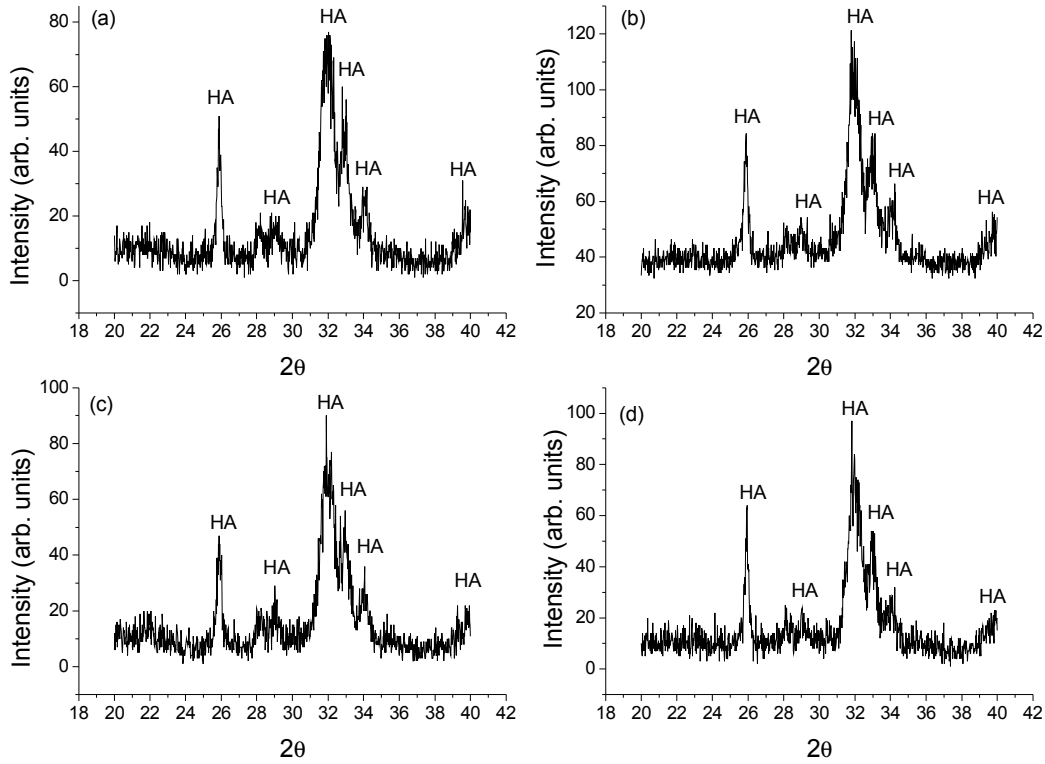

Figure 1: X-ray diffractogram of the gelatin membranes with different quantities of HA: (a) G4H05G05; (b) G4H05G1; (c) G4H05G15 and (d) G4H05G2.

Figure 2 shows the AFM micrographs of the gelatin/HA composites. The presence of inorganic material can be observed within the polymeric environment of the gelatin/HA membranes with different amounts of mineral phase and nanometric crystals with sizes between 50nm and 100nm. These values are in good agreement with the literature, for a co-precipitation process at the used temperature. The increase of the crystal size, Fig. 2b, of the G4H1 sample (200nm) can be explained by an uncontrolled increase of the temperature during the co-precipitation process, indicating that this could be a strategy to control the particle size (not studied here). The growth of crystals in aqueous solution is generally based on a diffusion process, i.e., a thermally activated process [9]. According to Chang et al. [9], during the growth of HA crystals in the gelatin matrix, with increasing temperatures, there is a competition between the interaction organic-inorganic phase

and thermodynamics. Below 50°C the molecules of gelatin are degraded more strongly. Above this temperature, the number of sites for nucleation of HA crystals is extremely limited and then larger crystals are induced. Consequently, since the HA crystals were growth at 45 °C, the reaction in this work is induced by thermodynamics. The HA crystals are well dispersed, leading to plane membranes. The homogeneity of the particle distribution within the gelatin allows evaluating the interaction of both components. In the G4H05G15 sample, it can be observed particles with the same nanometric size of the sample without reticulation. The spherical shape of the HA crystals is justified by the fact that this geometry has the lower area/volume ratio and then a lower surface free energy which corresponds to the most stable morphology.

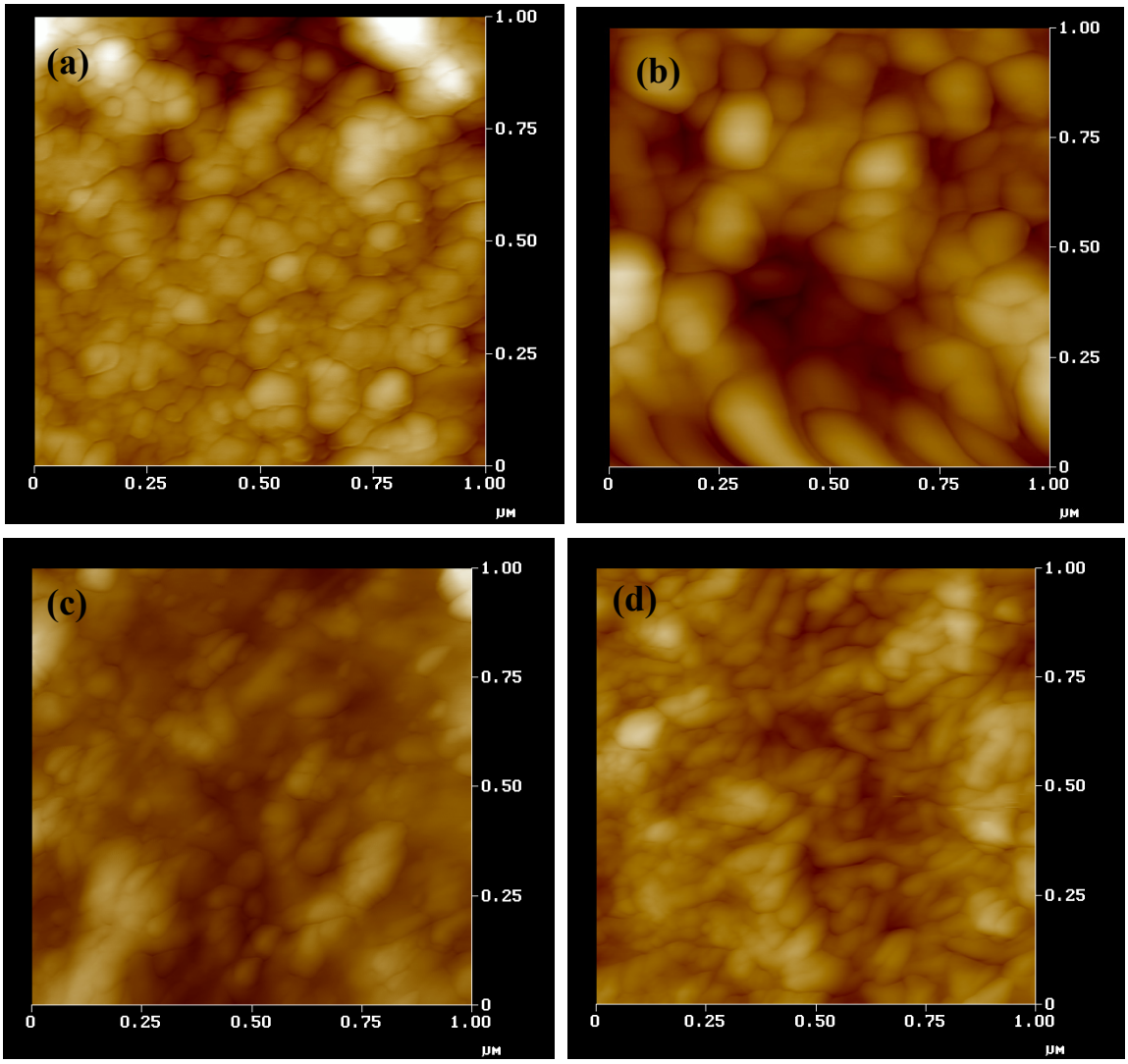

Figure 2: AFM micrographs of the gelatin membranes with different quantities of HA: (a) G4H05, (b) G4H1, (c) G4H2 and (d) G4H3.

Significant differences in the swelling behavior of the membranes were observed with the crosslinking, even using extremely low glutaraldehyde concentrations, Fig. 3. A decreasing swelling was observed for increasing glutaraldehyde concentration, for the reticulated membranes, resulting in more stability in aqueous solution. Gelatin membranes are extremely soluble and, because of this, few minutes of storage in physiological solution are sufficient to induce considerable swelling of the membranes, as shown in Fig. 3. Whereas the membrane without reticulation (G4H05) showed a swelling percentage of 500% in 45 minutes of immersion in phosphate buffer solution (pH 7.4), the reticulated sample (G4H05G2) with glutaraldehyde

concentration of 2%, in the same period, showed a swelling percentage of 200% only. This fact indicates that the decrease in swelling is due to an increase of the triple helix of the samples [10].

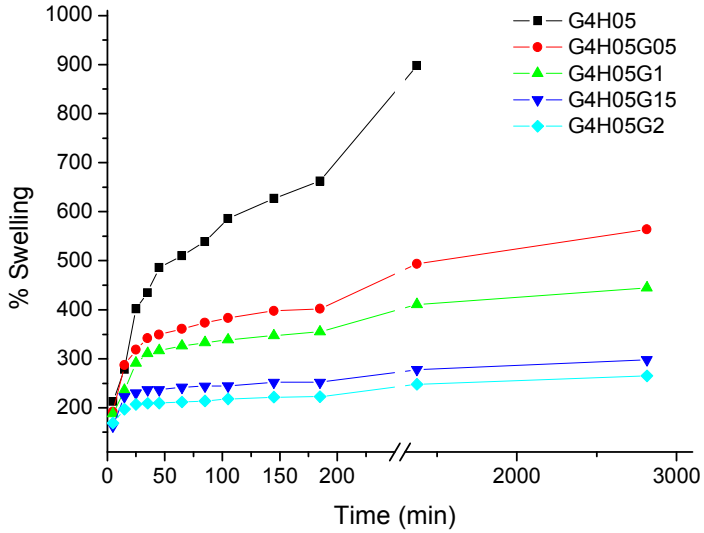

Figure 3: Swelling behavior of the gelatin membranes reticulated with different concentrations of glutaraldehyde (0.5%, 1%, 1.5% e 2%).

Conclusions

A very promising method of obtaining membranes with an organic matrix and inorganic nanoparticles was studied. The membranes exhibited a uniform and homogeneous particle distribution. According to the swelling test, very low glutaraldehyde concentrations allowed reticulating process resulting in more stable membranes in aqueous solutions. The samples exhibited ideal stiffness and excellent flexibility due to the addition of plastificant and the reticulation process, with the best properties observed for the G4H05G2 sample.

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