A NOVEL BONE SCAFFOLD MATERIAL BASED ON COLLAGEN/HYDROXYAPATITE/GELATIN COMPOSITE

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A novel bone scaffold with collagen-hydroxyapatite (CHA) and gelatin (Gel) was fabricated using biomimetic mineralization method, combineed with sonication dispersion, crosslinking, blending and lyophilization. The structure and properties of the scaffold were investigated. By the introduction of gelatin into collagen-hydroxyapatite, the scaffold is easy to be shaped with three-dimensional porous microstructure. Both gelatin and crosslinking affect the mechnical properties. The novel collagen-hydroxyapatite-gelatin composite could be a candidate of scaffold materials for bone tissue engineering

Keywords: collagen, hydroxyapatite, gelatin

INTRODUCTION

Nowadays, there is a large demand in biomedical bone materials because of various bone defects or damage caused by bone fractures, osteoporosis, osteoarthritis and so on. Bone tissue engineering rise in response to the proper time and conditions. This field means that a suitable material is either uesd to induce bone remodeling from surrounding tissue, or as a carrier or template to implant bone cells or other growth factors for the healing of bone morbidity. Therefore, bone tissue engineering materials must be non-toxic, low-immunogenic, biocompatible, biodegradable, and have a corresponding matching of the mechanical properties. Materials commonly used are metal materials, ceramic materials, natural or synthetic polymer materials and composite materials, such as titanium, calcium phosphate, bioactive glasses, collagen (Col), gelatin (Gel), polylactic acid (PLA), polycaprolactone (PCL) and many other materials (Du *et al.*, 1998; Yamaguchi *et al.*, 2001; Liao *et al.*, 2004; Masoud *et al.*, 2010).

Collagen and hydroxyapatite thought as organic and inorganic constituents of natural bone is one of the most worthy of the topics studied, the biocompatible, biodegradable, osteoinductive characteristics of which have drawn the attention and favor of researchers (Kikuchi *et al.*, 2008; Lickorish *et al.*, 2004; Tsai *et al.*, 2008). However, poor plasticity, difficult molding and the mechanical strength of the collagen/hydroxyapatite composite materials having significant differences with natural bone are challenging issues limiting its clinical application. For the preparation of a more ideal bone repair material, the laboratory conducted a series of studies, this paper presents the fabrication of novel collagen/hydroxyapatite/gelatin composite materials. The materials are composed of a natural matrix made of collagen-hydroxyapatite embedding gelatin, to furnish the right mechanical properties to implant position, and collagen as well as hydroxyapatite could act as chemo-attractor for cells and tissue. Different concentrations of the components are made. And as biological cross-linkers, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloricde (EDC)/N-hydroxysuccinimide (NHS) was chosen to modify materials comparable to materials without crosslinking.

MATERIALS AND METHODS

Preparation of Collagen-Hydroxyapatite-Gelatin (CHA-Gel) Composite

CHA powder was prepared by biomimetic method, which has been reported previously (He *et al.*, 2013). Briefy, collagen was dispersed in a 0.5 mol/L acetic acid aqueous solution. H_3PO_4 aqueous solution, $Ca(OH)_2$ aqueous solution were successively and gradually added into the reaction vessel with stirring frequently and starting materials for collagen-hydroxyapatite (Col-HA) composites were mixed at an 35/65 initial weight ratio by stoichiometry. The reaction temperature controlled using an oil bath and the pH of reaction solution controlled through NaOH aqueous solution by pH meter was set as 37°C and 7, respectively. Precipitates thus were obtained at the respective preparation temperature (37°C) for 48 h and were subsequently filtrated. After centrifugation, precipitates were freezed drying at −50°C under vacuum (GT2-Type-8, LYOTECH).

The CHA-Gel composite was prepared by blending, solvent-casting technique, biological crosslinking treatment and lyophilization method. CHA powder was added into gelatin solution and ultrasonically mixed (final composition Gel to CHA 10%, 15%, and 20% (wt/wt %)). Then the mixture were poured into a self-made polyethylene mold and dried in air at room temperature. This was kept in a ethanol solution containing 50 mM EDS and 25 mM NHS at at room temperature for 4 h to complete the crosslinking. Subsequently, the mixture was washed with 0.1mM Na_2HPO_4 solution and finally lyophilize at −50°C under vacuum for 24 h (GT2-Type-8, LYOTECH). The composite prepared in this way is denoted as CHA-Gel composite.

Characterisation

FTIR spectra of CHA-Gel composites were obtained at room temperature using Bruker VERTEX 70 FTIR measurement (VERTEX 70, GER) in the range of 4000-400cm^{-1} using KBr pellets. And samples were subjected to X-ray diffraction (XD-3X, CHN) using CuKα radiation generated at 30 kV and 20 mA, the range of diffraction angle was 10°-90° 2θ. Fracture surface of samples were sputter coated with gold in a vacuum evaporator, and photographed using a scanning electron microscope (Quanta200, USA) using an accelerating voltage of 3 kV.

Mechanical Tests

Mechanical testes were carried out with a tensile tester (UTM2203, China). The tests were carried out at a crosshead speed of 1mm/min. Dumbbell specimens with size 4mm ×20mm were tested for tensile property according to China National Standard (GB/T 1040-3). Five same samples of each experiment with the average reported.

RESULTS AND DISCUSSION

In Figure 1(A), the main absorption bands of collagen, hydroxyapatite and gelatin appear. The main absorption bands corresponding to collagen are: amide A at 3424 cm^{-1}, amide B at 2931 cm^{-1}, amide I at 1700-1600 cm^{-1}, amide II at 1550-1500 cm^{-1} and amide III at 1300-1200 cm^{-1}. The main absorption bands of hydroxyapatite are: ν_1 mode of PO_4^{3-} at 964 cm^{-1}, ν_3 mode of PO_4^{3-}at 1096 cm^{-1} and 1035 cm^{-1} for the asymmetric HA, ν_4 mode of PO_4^{3-} at 601 cm^{-1} and 562 cm^{-1}, ν_2 mode of PO_4^{3-} at 470 cm^{-1}, and ν_5 mode of PO_4^{3-} at 420 cm^{-1}. The main absorption bands corresponding to gealtin are: N-H stretching vibration at 3500-3200 cm^{-1}, -CH vibration at 2845-2760 cm^{-1}, -C=O vibration at 1631 cm^{-1} and $-OH^{-}$absorption peaks at 1390-1450 cm^{-1}.

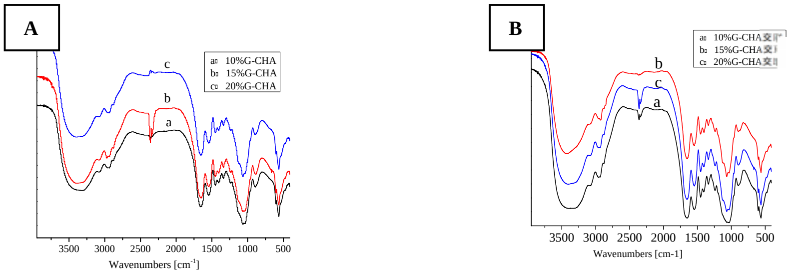

Figure 1. FTIR spectra of collagen/hydroxyapatite/gelatin composites
(A) uncrosslinked composites and (B) crosslinked composites

For CHA-Gel composite, the peaks' position of the starting CHA and Gel does not have red-shift change in the composite. This means that CHA and Gel still keeps their original structure in the composite. Comparison of composite using the cross-linkage agent EDC/NHS (Figure 1(B)), it is confirmed that the organic-inorganic bonding between CHA and Gel from the strong amide bands shows the frequency change: amide I band in the range of 1700-1600 cm^{-1} and amide II band in the range of 1550-1500 cm^{-1}. By effects of EDC/NHS, Gel molecules were chemical modified and affected the lattice interaction between organic molecules and HA interfaces. The -COO groups of Gel make a covalent bond with Ca^{2+} sites in the interfacial surface of HA.

The results of XRD patterns revealed that HA within CHA-Gel composite matched with the JCPDS PDF database pattern of hydroxyapatite, but reflected low crystallinity (Figure 2(A)). The reaction was not carried out at higher temperature. Consequently, the HA crystals formed were the immature fine crystallites. The crystallinity of composites was lower with higher Gel content, possibly due to a higher proportion of the amorphous Gel phase and the small size of the HA crystals. Comparison of patterns after crosslinking, some new peaks and steeper peak development indicate that the introduction of EDC/NHS may leads to reaction within compound and the compound is related with the interaction among Col, Gel and HA molecule. As commonly stated in literatures, the co-use of EDC and NHS greatly improved the molecular structure between Col and HA, or Gel and HA (Ofner III and Bubnis 1996; Wissink et al. 2001).

The morphology and microstructure of CHA-Gel composites are examined using SEM. And section micrographs of the synthesized CHA-Gel composites of different ratios without or with crosslinked are shown in Figure 3. It shows that CHA-Gel composite has a typical lamellar-like structure and is interconnected by micropores inside.

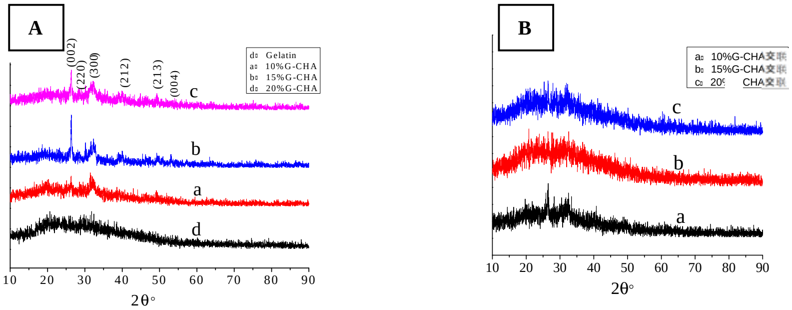

Figure 2. XRD spectra of collagen/hydroxyapatite/gelatin composites
(A) uncrosslinked composites and (B) crosslinked composites

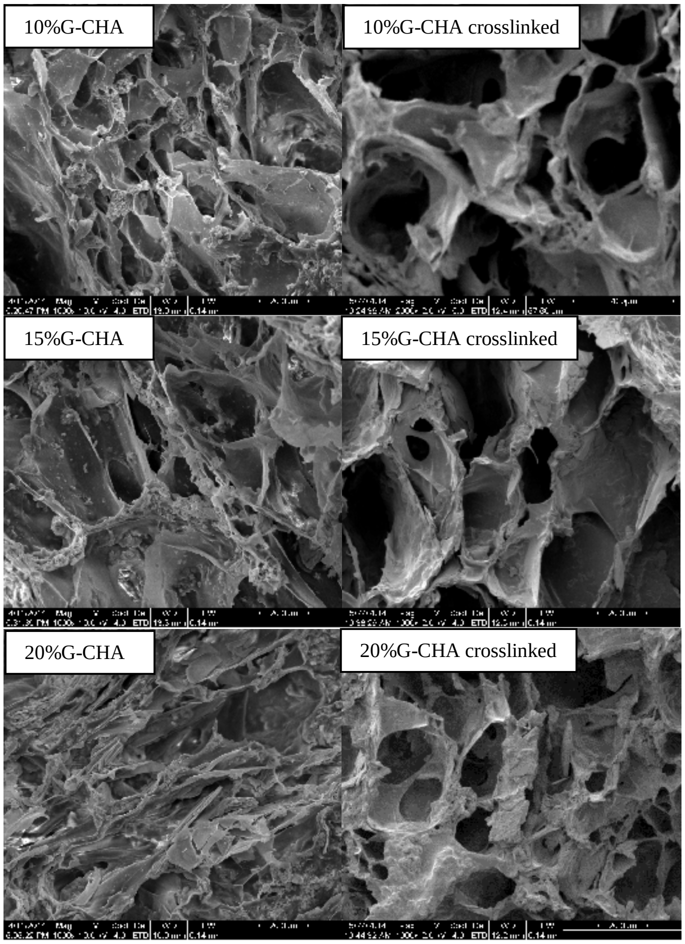

Figure 3. SEM micrographs of collagen/hydroxyapatite/gelatin composites (section)

The micropores in materials could greatly enlarge the surface contact area for protein adsorption. Hence this will enhance protein absorption on pore surface and inside pore to facilitate bone formation. With increasing content of gelatin, the pores of CHA-Gel composite appear to be partly fine adhesion beyond composite layers along their interfaces to each other.

On the other hand, interconnected porosity depends on cross-linking of composites. It is showed that the microstructure of CHA-Gel composites before and after cross-linking were also compared in Fig. 3. In composites crosslinked, the pores obtained are more homogeneous. Moreover, CHA-Gel composites crosslinked show a increased degree of interconnected porosity and a lower density with respect to their corresponding uncrosslinked counterparts. The high porosity and low density of these composite are also benefit to increasing the number of cells adhering to the scaffolds on implantation, and shortening the time for bone tissue formation (Zhang *et al.*, 2003).

Figure 4 reports the tensile strength and elongatian at break of uncrosslinked and crosslinked CHA-Gel composites. Generally, with increasing gelatin form 10 to 20wt%, the tensile strength and elongatian at break of composites decrease. The addition of gelatin into composite should have incresse the tensile strength. However, lyophilization of materials makes gelation in dry condition lose its elasticity. The tensile strength decreases sharply from 90.13±1.58MPa to 61.74±1.76MPa and the differences among elongation at break are not significant. After crosslinking, the trend of elongatian of crosslinked CHA-Gel composites at break also drops . However, the tensile strength enhance with increasing the percentage of gelatin. Such a phenomenon illustrates that the crosslinking causes the interaction within composites, to a certain extent, it promotes bongding between gelatin and CHA, or between CHA-Gel and crosslinker.

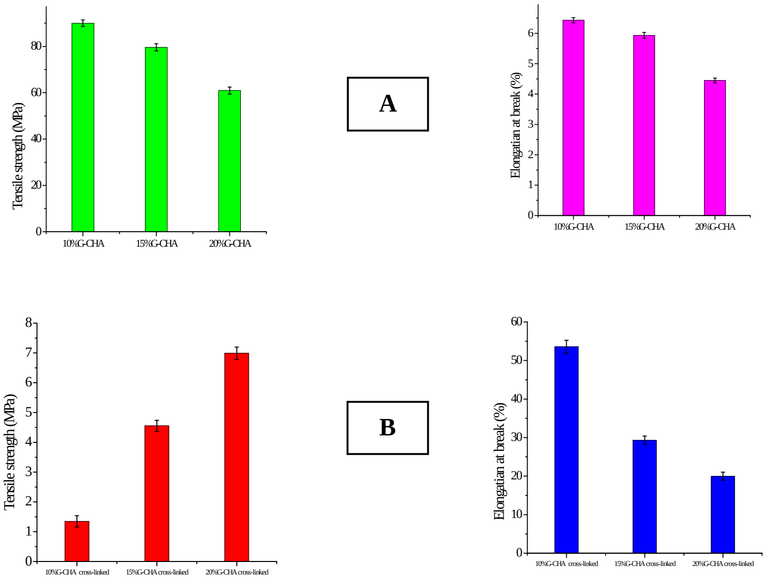

Figure 4. Mechanical properties of collagen/hydroxyapatite/gelatin composites
(A) uncrosslinked composites and (B) crosslinked composites

For both crosslinked and uncrosslinked CHA-Gel composites, the mechanical response is also affected by sponge porosity degree together with pore size and pore orientation. As reported in SEM analysis above, with respect to their corresponding uncrosslinked counterparts, crosslinking makes materials' pore more homogeneous, the sizes of pores more bigger, porosity degree higher and the density lower. The microstructure of CHA-Gel composites also give primary information on their mechanical property.

CONCLUSIONS

The CHA-Gel composites were prepared by layer solvent casting combined with lyophilizationand crosslinkingas a biomedical material. The gelatin produced a more homogeneous porous structure at millimeter scale with high porosity degree and low density. The modification of EDC/NHS crosslingker has significant effects on the mechanical property of composites. With crosslinking, the elongation at break increases sharply. However, the tensile strength decreased. .

Acknowledgements

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