Synthesis of Intercalated Lamellar Hydroxyapatite/Gelatin Nanocomposite for Bone Substitute Application

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ABSTRACT: Hydroxyapatite (HAp)/polymer composites have been widely investigated for bone substitute applications in recent years. Inspired by the arrangement of ordered organic and inorganic layers in natural bones and seashells, for the first time a novel intercalated nanocomposite of gelatin and lamellar HAp was prepared via solution intercalation process. X-ray diffraction (XRD) results showed that the basal spacing of HAp lamellas enlarged by 3.0 nm from 3.1 nm to 6.1 nm, indicating that the gelatin molecules had been intercalated into the gallery of lamellar HAp. The microstructures of pure lamellar HAp and intercalated gelatin/HAp nanocomposite were observed by transmission electron microscopy (TEM) analysis. Fourier transform infrared spectroscopy (FT-IR) analysis revealed that there were chemical interactions between gelatin molecules and HAp. Thermogravimetric analysis (TGA) results confirmed that thermal stability of the composites was enhanced. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 3089–3094, 2009

Key words: lamellar; gelatin; x-ray; FT-IR; thermogravimetric analysis

INTRODUCTION

Natural composite materials such as bones and seashells have been refined and perfected over millions of years of evolutionary selection. Thus, their microstructures and macroscopical properties possess many advantages over artificial materials. In recent years, Heuer and co-workers have conducted many investigations on the crossed-lamellar structure of seashells.[1–3] Liang et al. investigated the relationship between the mechanical properties and crossed-lamellar structure.[4] Fabrication of a biomimetic laminated clay/polymer nanocomposite by mimicking the synthesis processes of nacre was presented by Tang et al.[5] and Podsiadlo et al.[6]

The clay/polymer nanocomposites exhibited excellent properties such as high strength and stiffness, and improved thermal stabilization.[7] As of today, there is no report on the biocompatibility of clay/polymer nanocomposites.

Compared with clay materials, HAp exhibits excellent biocompatibility and osteoconductivity due to the similarity of its chemical composition to mineral component of natural bone tissues.[8,9] Gelatin, the denatured derivative of collagen, has the potential to be an engineered biomaterial. In practice, gelatin is currently used as a wound dressing due to its excellent biodegradability and cytocompatibility, and as a drug delivery carrier because of its sufficient plasticity and hydrogel properties.[10–12] It is desired to create a composite combining the advantages of gelatin and HAp. Traditionally, the HAp/gelatin nanocomposites are synthesized by dispersing HAp powders in the gelatin solution. However, this method tends to cause the aggregation of HAp particles and it may not exert any morphological control or any chemical interaction at organic/inorganic interface which makes it difficult to form a controlled structure.[10,13–15] Another method is the biomimetic approach, in which way the HAp/collagen composites have been synthesized to maintain the nanoscale HAp precipitates along the collagen fibers.[16–18] It is noted that the mineralization of unmodified collagen templates and of those modified with glucuronic acid and decorin results in the

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formation of different calcium phosphate phases.[19] Furthermore, the nucleation mechanism of fluorapatite on collagen fibers has been investigated by Kawska et al.[20] Recently, some novel methods were applied on preparation of HAp/gelatin composites. Ethirajan et al.[21] reported a novel biomimetic strategy which synthesized HAp inside of crosslinked gelatin particles using a miniemulsion process. Double-diffusion technique was also used for preparation of carbonated fluorapatite-gelatin nanocomposites.[22] However, to our best knowledge, there is no report on preparation of lamellar HAp/gelatin composites by intercalation method.

In recent years, many kinds of clay/polymer composites have been prepared through intercalation processes. The physical and chemical properties of the composites attained were significantly improved.[23–25] Herein, for the first time, solution intercalation technique was used on synthesis of HAp/gelatin composites. First, the highly ordered lamellar HAp was prepared via the template method. Then gelatin molecules were intercalated into the lamellar HAp through water solution. As a result, the HAp/gelatin composites with intercalated structure were obtained.

MATERIALS AND METHODS

Materials

The materials used in the present work included bovine gelatin, calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$), sodium hydroxide (NaOH), sodium dodecyl sulphonate (SDS, $C_{12}H_{25}SO_3Na$), ammonium hydrogen phosphate ($(NH_4)_2HPO_4$), ethanol, and deionized water. All chemicals used were of analytical grade and were used without further purification. The ethanol was provided by Kewei Company, Tianjin, China. All other reagents were provided by Acros® organic company (Belgium).

Preparation of lamellar HAp

Under magnetic stirring (2000 r/min), 0.5 g of SDS was mixed with 15 mL of deionized water and 30 mL of ethanol. The solution was then heated to 60°C at which the mixture of 11.8 g of $Ca(NO_3)_2{\cdot}4H_2O$ in 15 mL of deionized water was added, followed by the addition of 3.96 g of $(NH_4)_2HPO_4$ in 30 mL of deionized water and 30 mL of ethanol. Then 20 mL of 2.5*M* NaOH solution and 20 mL of ethanol were added. The mixture was refluxed at 83°C for 14 h. Then the precipitate was deposited at room temperature for 12 days. After being centrifuged and immersed in water for 3 days, the precipitate was centrifuged again and dried at 60°C to get the lamellar HAp.

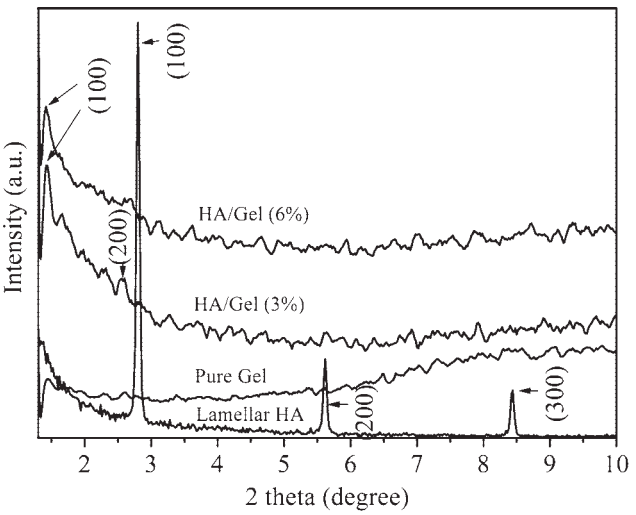

Figure 1 Small-angle XRD patterns of HAp/gelatin (6%) composite (a), HAp/gelatin (3%) composite (b), pure gelatin, (c) and lamellar HAp (d).

Preparation of HAp/gelatin composites

After dispersing 2 g of lamellar HAp powder with 10 mL water, the HAp suspension was poured into a flask. Two definite concentrations of gelatin solution (3% and 6% (w/w)) were prepared. Under magnetic stirring (2000 r/min), two resulting emulsions of HAp were preheated to 70°C to which 3% and 6% gelatin solutions were added separately. After stirring (2000 r/min) at 75°C for 4 h, the solutions were poured into Petri dishes and dried at 60°C.

Characterization

The changes in basal spacing of HAp/gelatin composites were measured by X-ray diffraction (XRD) (Rigaku D/Max-2500, Cu Kα, $\lambda = 0.15406$ nm, 40.0 kV, 100.0 mA, from 1° to 60° and step 8°/min). Transmission electron microscopy (TEM) experiments were performed on a JEOL 2000FX TEM operating at 160 kV accelerating voltage. FT-IR analysis (BIO-RAD FTS6000, KBr pellet 1 : 50–100, 4000–400 cm^{-1}) was applied to investigate the chemical interactions of gelatin and HAp. The thermal stability of HAp/gelatin composites was investigated by thermogravimetry analysis (TGA) with a heating rate of 10°C/min under N_2 atmosphere.

RESULTS AND DISCUSSION

XRD analysis

Figure 1 gives small-angle ($2\theta < 10°$) XRD patterns of various samples obtained in this work. The patterns of pure lamellar HAp showed three intense diffraction peaks at 2θ value of 2.87°, 5.67°, and 8.49°. According to Brag's formula ($\lambda = 2d\sin\theta$), the

TABLE I
Peaks and d Spacings of Lamellar HAp and HAp/Gelatin Composites

Samples	2θ (°)			d (nm)		
	100	200	300	100	200	300
HA	2.87	5.67	8.49	3.07	1.55	1.03
HA/gelatin (3%) composite	1.44	2.56	–	6.12	3.40	–
HA/gelatin (6%) composite	1.43	–	–	6.17	–	–

d spacing was identified as: $d_{100} = 3.07$ nm, $d_{200} = 1.55$ nm, $d_{300} = 1.03$ nm. The three intense diffraction peaks indicated the existence of a long range ordered layered mesostructured phase with a periodical spacing of about 3.10 nm.[26] Similarly, Zhang et al.[27] successfully synthesized lamellar HAp uniform layer spacing of 3.64 nm with a MAP (phosphoric acid monododecyl ester) template. It is assumed that the different d spacing of these two lamellar HAp is attributed to the various templates used in the two experiments. Table I shows the diffraction data of HAp and two composites. The d spacing of composites was about 6.1 nm which was nearly doubled as compared to pure HAp (3.1 nm), indicating that the gelatin chains were intercalated into the gallery of the HAp platelets during solution intercalation process. In other words, intercalated HAp/gelatin nanocomposites were obtained.[15,28,29] As shown in Figure 1(a,b), the d_{200} peak observed in the HAp/gelatin (3%) composite disappeared in the HAp/gelatin (6%) composite. This finding indicated that a higher concentration of gelatin intercalated corresponed to a lower ordered structure of composite obtained. Figure 2 shows wide-angle ($2\theta > 10°$) XRD patterns of pure gelatin, pure HAp, and HAp/gelatin composites. It was found that the patterns of the HAp/gelatin composites contained all the characteristic peaks of gelatin and HAp, which indicated the existence of the two components in the composites.

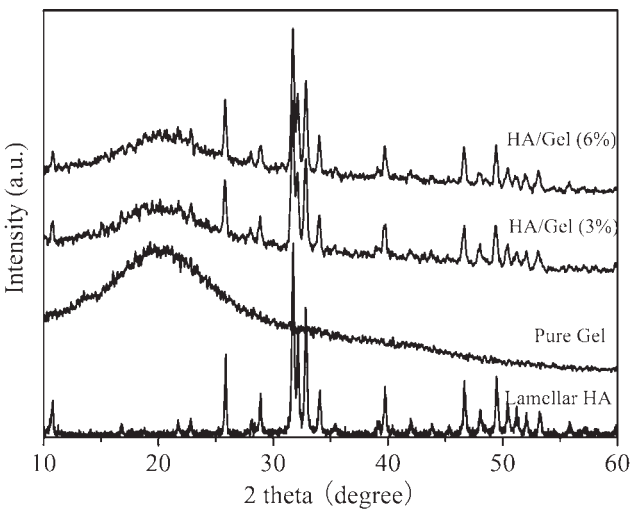

Figure 2 Wide-angle XRD patterns of HAp/gelatin (6%) composite (a), HAp/gelatin (3%) composite (b), pure gelatin, (c) and lamellar HAp (d).

The schematic structures of lamellar HAp and HAp/gelatin composites are illustrated in Figure 3. Gelatin molecules were intercalated into the laminated HAp [Fig. 3(a)] via solution intercalation. As shown in Figure 3(b), the intercalated gelatin molecules enlarged the spacing of HAp platelets and interacted with sodium dodecyl sulphonate (SDS) existing in the inner surfaces of HAp platelets. As a result, an organic/inorganic layered composite was obtained.

TEM analysis

For further support of our XRD analysis, TEM was used to observe the microstructures of pure lamellar HAp and gelatin/HAp composite. Figure 4(a) shows

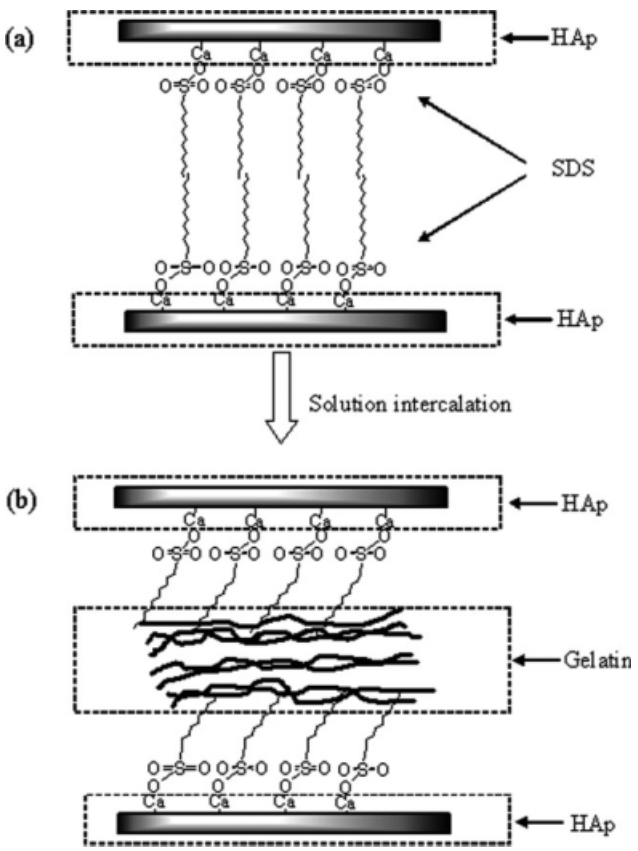

Figure 3 The schematic structure of lamellar HAp (a) and gelatin/HAp composites (b).

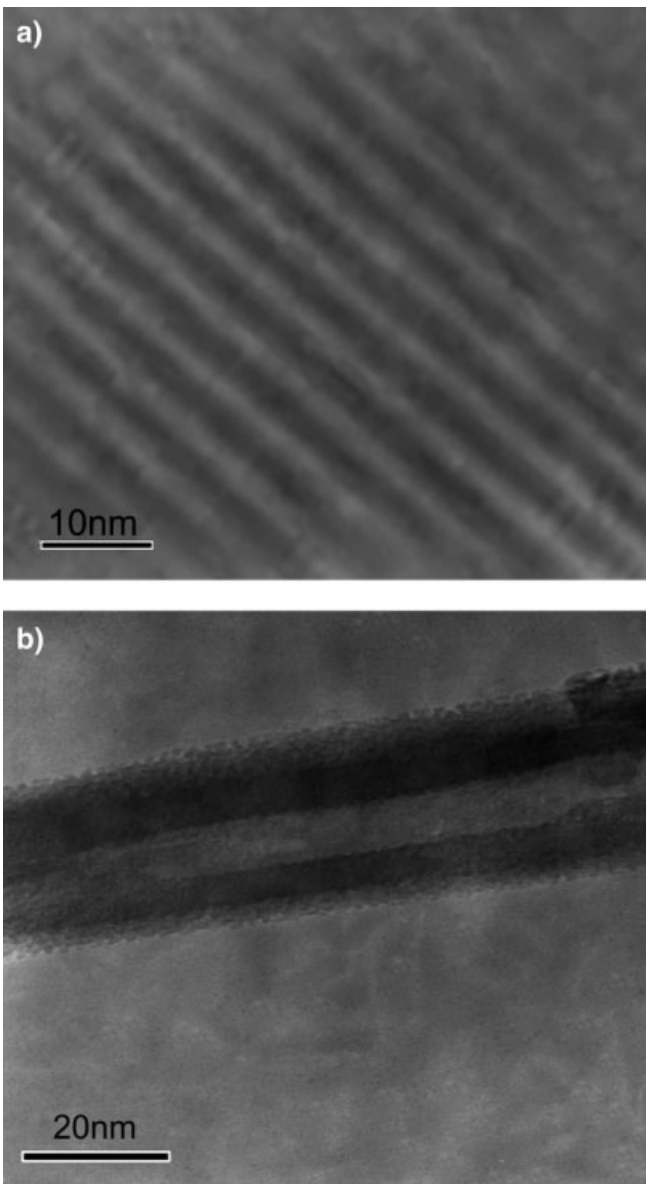

Figure 4 TEM images of pure lamellar HAp (a) and gelatin/HAp composite (b).

the TEM micrograph of pure lamellar HAp. It can be seen that the inorganic layer and the template layer were arranged alternately in one dimension to form a long range ordered lamellar structure. The *d* spacing of pure HAp is about 3 nm which is well according to the XRD analysis. Figure 4(b) shows the nanostructure of gelatin/HAp nanocomposite, the *d* spacing of the composite is about double to that of pure HAp attributed to the intercalation gelatin molecules, which meets good agreement with the XRD results. Moreover, it is observed that the composite did not possess the long range ordered structure as pure HAp due to the interruption of the intercalation process. It can further explain the disappearance of the diffraction peaks of (200) and (300) in the composite.

FT-IR analysis

Figure 5 shows the FT-IR spectra of samples obtained in the present study. The spectrum of gelatin was typical with the amide bands observed at 1652 cm^{-1}, 1539 cm^{-1}, and 1252 cm^{-1}.[30]

Fig. 5a shows the FT-IR spectrum of HAp. There were four vibrational modes for phosphate ions (PO_4^{3-}), two of which appeared at 1039 $cm^{-1}$and 962 cm^{-1},[31] the other two appeared at 564 cm^{-1} and 472 cm^{-1}.[32] Together with the phosphate ion bands indicating the existence of HAp, a hydroxyl (—OH) stretching vibrational band was observed at 3569 cm^{-1} and it belonged to the hydroxyl group along the c-column of HAp lattice.[33] The carbon-hydrogen (C—H) bands appeared at 2923 cm^{-1} and 2853 cm^{-1} indicated the existence of surfactant in the inner surfaces of the lamellar HAp. Note that there was an absorption band (1383 cm^{-1}) which could be assigned to NO_3^-,[26] considering that calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$) was used as the starting material in this work. The presence of NO_3^- could be attributed to the lamellar structure of HAp. Unlike conventional HAp, NO_3^- was entrapped in the space between HAp lamellas in this case, which in turn could confirm the formation of lamellar HAp.

After the intercalation process, as shown in Figure 5(b,c), all the phosphate ionic (PO_4^{3-}) bands weakened sharply compared with the original ones and this indicated the interaction of HAp and gelatin. The absence of the stretching vibration of hydroxyl group at 3569 cm^{-1} was attributed to the formation of chemical bonds between hydroxyl and gelatin. Furthermore, the disappearance of the NO_3^- absorption band (1383 cm^{-1}) in the composites demonstrated that the gelatin molecules have been

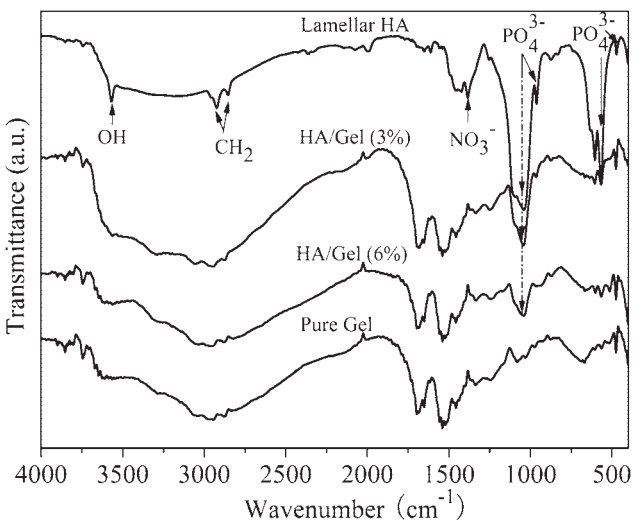

Figure 5 FT-IR of lamellar HAp (a), HAp/gelatin (3%) composite (b), HAp/gelatin (6%) composite (c), and pure gelatin (d).

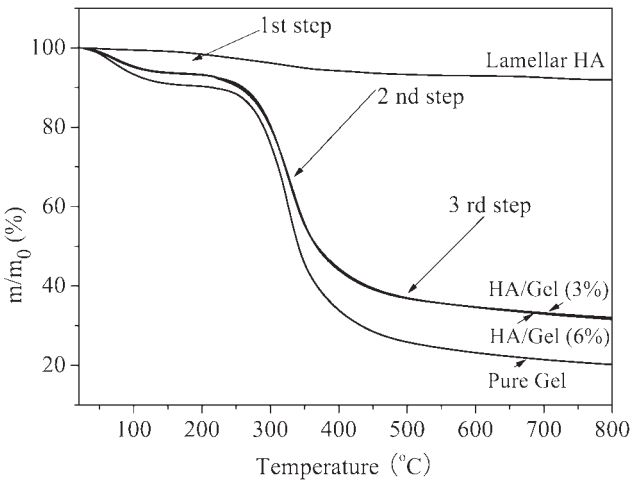

Figure 6 TGA curves of HAp, gelatin and two gelatin/HAp composites.

intercalated into the HAp lamellas allowing the NO_3^- to escape. The intensity of the C—H bands of SDS surfactant at 2923 and 2853 cm^{-1} reduced sharply and this could be attributed to the hydrophobic forces between the C—H chain of SDS and aliphatic residues in gelatin.[34,35] Because SDS only existed in the inner surfaces of HAp lamellas, it could further confirm the successful intercalation of gelatin molecules.

TG analysis

It is generally accepted that polymer/montmorillonite systems exhibit good thermal stability.[36,37] However, to the best knowledge of the authors, the effect of lamellar HAp on the thermal stability of its composites has not been reported. In this work, the thermal stability of lamellar HAp/gelatin composites was investigated by thermogravimetry analysis under nitrogen atmosphere. The TG curves for pure gelatin, HAp and HAp/gelatin (3%, 6%) composites are illustrated in Figure 6. The TG curve of pure HAp showed three distinct stages. The first one of about 0.7% weight loss at 17–120°C was attributed to the removal of H_2O contained in the samples. The second weight loss (approximating 6.3%) at 121–583°C was due to the decomposition of the SDS. The third step of weight loss of 1.0% at 584–900°C was due to the decomposition of phosphorite (Ca/P < 1.67).[21] The TG curves of gelatin and HAp/gelatin composites showed a similar trend of weight loss. The loss at 17–150°C was attributed to the decomposition of the free water evaporation. The huge loss at 150–450°C was due to the decomposition of gelatin chains. However, compared to pure gelatin, the gelatin/HAp composites showed a distinct delay in mass loss within the temperature range of protein chain degradation (150–450°C) and this indicated that the thermal stability of gelatin was enhanced by the addition of lamellar HAp.

CONCLUSION

Inspired by the delicate lamellar microstructure of natural biocomposites, gelatin molecules were intercalated into the lamellar HAp via the solution intercalation process to form an organic/inorganic layered structure. The d_{100} spacing of HAp/gelatin composites was 6.1 nm which enlarged by 3.0 nm compared to the original HAp. FT-IR results confirmed that the hydroxyl group of HAp and carbon-hydrogen chains of SDS had chemical interactions with hydrophile groups and leucine in gelatin, respectively. The thermal mobility of gelatin molecules was suppressed by the intercalation of HAp lamellas, thus the thermal stability of the HAp/gelatin composites was improved.

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