SYNTHESIS AND CHARACTERIZATION OF PCL/CALCINED BONE COMPOSITES

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ABSTRACT

In this article, the hydroxyapatite and carbonate(HAC) nanoparticles were obtained by calcining the original pig bones, and the influence of different calcining temperature on morphology and size was investigated. Then, the polycaprolactone(PCL)/HAC composite materials were synthesized through in-situ polymerization with caprolactone(CL) and HAC. The effects of different weight ratios of HAC to CL on the performance of composites was discussed, and the morphology and microstructure of obtained composite materials were characterized by transmission electron microscopy(TEM), Fourier transform infrared(FT-IR) and (X-ray diffraction)XRD, etc. The results showed that HAC was uniformly dispersed in PCL matrix, and the composite material with HAC of 6 wt% was chosen for further application. This study provided a new way for synthesizing PCL/HAC materials that could be used for repairing and substituting for human damaged bone, which is significant in the theory and practice of bio-medicine.

Keywords: Calcined bone particles; Polycaprolactone; Hydroxyapatite; Composite.

1. INTRODUCTION

Biomaterials play a dramatically important role in tissue engineering, which is aimed to provide replacements and help to repair the damaged or lost tissues and organs as a consequence of disease, aging or accident¹. In order to meet multi-requirements of clinical applications, it is necessary to develop multi-component and multistructure biomaterials, which could combine the property advantages of included elements, i.e. mechanical properties, rate of biodegradation and bioactive function. This design provides new possibility in biomedical applications as new biomaterials.

The human bone contains hydroxyapatite(HA) and carbonate etc. Especially HA is widely used as bone implant materials in clinics in either sintered block or granular forms due to its excellent osteoconductivity^{2,3}. However, the mechanical properties and biodegradability of the application forms have some limitations. Thus in recent years, many studies on HA focus on its composites with organic polymers, especially those possessing biodegradability⁴⁻¹¹. The polymeric parts are metabolized and excreted, and the ceramic constituents are assimilated in the body. The brittleness of HA ceramics is also improved by mixing with tough organic polymers.

PCL is aliphatic, biodegradable polymer which is able to hydrolytic and enzymatic cleavage along polymer chains and is widely used for medical applications, pharmaceutical controlled release systems and in biodegradable packaging materials¹².

So far, the papers about preparation of PCL/HA composite materials have been reported¹³⁻¹⁷. However, PCL/HA composite materials were mostly prepared by blend¹⁸⁻²⁰, in the process of which HA were produced through hydrothermal synthesis²¹ or sol-gel²², etc. To the best of our knowledge, no work was reported about the synthesis of composites of PCL and HAC which obtained by calcining original pig bone. The preparation of HAC is very convenient for its simple source of raw materials. Also, HA particles obtained coexist with traces of calcium carbonate, etc., which are more similar to the inorganic components of human bones.

Here, we first report the synthesis of PCL/HAC composites via in-situ polymerization. The properties of composites also investigated. These prepared PCL/HAC composites may have better prospect application in repairing and replanting biomaterial.

2 EXPERIMENTAL

2.1. Chemicals: Caprolactone (CL) was purchased from Aldrich. alcohol, stannous caprylate, toluene, tetrahydrofuran (THF), etc. All agents and solvents used in this work were of analytic reagent (AR) grade. They were used without further purification. Pig bone was purchased from farmer's market.

2.2. Instruments: Fourier transform infrared (FT-IR) spectra were recorded on a Nexus 870 Fourier transform infrared spectroscopy (American Nicolet Co.), transmission electron microscopy (TEM) experiments were performed using a JEM-100SX microscope operated at 100 kV (Japan JEOL Co.), X-ray

diffraction (XRD) results were recorded on a D/max-cA X-ray diffractometer (Japan Rigaku Co.) with a scan speed of 10°(2θ)/min and a scan step of 0.020. Thermal properties of nanocomposite materials were recorded by Pyrir-1 differential scanning calorimetry (DSC) and Pyrir-1 thermo-gravimetric analysis (TGA) (American Perkin-Elmer Co.). The size distributions of HAC were recorded by Zetasizer 3000HSA Laser Particle Sizer (U.K. Malvern Co.).

2.3. Preparation of HAC and PCL/HAC composite materials: Firstly, the pig bone was boiled in distilled water for 12 h. After dried, the HAC were obtained from calcined bone at different temperature with different time. CL monomer and as-prepared HAC (the content of HAC were 3 wt%, 6 wt% and 9 wt%, respectively) were mixed and then ultrasonically dispersed. After added in the four neck flask, the mixture was heated with nitrogen prevention. The stannous caprylate was added into the system when the temperature of mixture reached 150 °C. Then the temperature of system was rise to 170 °C and kept it for 1.5 h. After the reaction finished, the composite materials were dissolved in THF and then dried in a vacuum drying box.

2.4. Principle of in-situ polymerization of PCL/HAC composites: The synthesis principle of PCL/HAC composite by in-situ polymerization is shown in reaction equation (1):

The hydroxyl groups on the surface of HA (HO-R) in HAC were surface-grafted with PCL by ring-opening polymerization of CL, which inhibited the growth of PCL molecules.

3. RESULTS AND DISCUSSION

Fig. 1 displays the size distributions of HAC prepared by calcining bone. In Fig. 1a, HAC was prepared at different treatment temperature (600 °C, 800 °C, 900 °C, 1000 °C) for 2 h, respectively. Larger HAC nanoparticles were obtained at 600 °C (red line), their diameter are mainly concentrated at 500 nm. HAC with wide distribution (100-1500 nm) were received at 800 °C (green line) and 1000 °C (black line) are shown in Fig. 1a. It shows small size and concentrated distribution (190 nm) of HAC obtained at 900 °C (blue line). It's concluded that the 900 °C is a optimal calcining temperature for the preparation of uniform HAC. Fig. 1b shows the size distribution of HAC obtained from calcined bone at 900 °C with different time (1 h, 3 h). Compared with Fig. 1a (blue line) obtained for 2 h, the HAC achieved with a wide distribution at 900 °C for 1h (black line) and 3 h (red line). The average diameters of HAC are increased. It is demonstrated that the optimal calcined time of pig bone at 900 °C for the preparation of HAC is 2 h. So the HAC obtained from calcined bone at 900 °C with 2 h was chosen in the following preparation of PCL/HAC composites.

Fig. 1 Size distribution of HAC obtained from calcined bone at different treatment temperature and time. (a) different temperature for 2 h; (b) different time at 900 °C

Fig. 2 TEM images of HAC obtained from calcined bone at 900 °C for 2 h

TEM images of HAC obtained from calcined bone at 900 °C for 2 h are shown in Fig. 2. From the images, the spherical HAC were produced from calcined bone, and the average size of HAC was 190 nm. The result corresponds to the size distributions of Fig. 1a (blue line).

Fig. 3 FT-IR spectrum and XRD pattern of HAC obtained at 900 °C for 2 h. Inset shows the FTIR spectrum of original bone. (a) FT-IR spectrum of HAC obtained at 900 °C for 2 h; (b) XRD pattern of HAC obtained at 900 °C for 2 h

Fig. 3a presents FTIR spectrum of HAC obtained at 900 °C with 2 h. It is observed that the bands appearing at 1047 cm⁻¹ (P-O stretching vibration peak), 607 cm⁻¹ and 571 cm⁻¹ (P-O bending vibration peak) correspond to HA²³. We can further see that, O-H stretching vibration peak is observed at 3571 cm⁻¹ in Fig. 3a, and the same in the original bone shown in inset due to the presence of water. Through the comparison of the HAC FTIR spectrum and the inset of original bone FTIR spectrum, the C-H (2950 cm⁻¹) absorption peak disappeared in HAC, it indicates that the organism of bone was burned out. In addition, it can be seen that the bands pertain to the CO₃²⁻ functional group at 1545 (A-type) and 1400 cm⁻¹ (B-type), indicating the substitution of CO₃²⁻ ions into the HA²⁴ in the original bone and HAC. It proves that HAC has a closer chemical component with that of biomaterials of human calcified tissue. Fig. 3b shows XRD pattern of HAC. The peaks at 2θ values of 25.88°, 31.74°, 32.87°, 34.05°, 39.76°, 46.66°, and 49.46° are consistent with the (002), (211), (300), (202), (130), (222), and (230) Bragg reflections of HA in HAC, respectively. A diffraction peak at 37.5° belonged to CaO is observed²⁵. Some miscellaneous peaks could be found in Fig. 3b such as at 29.63° and 31.32° are assigned to CaCO₃ and Ca₃(PO₄)₂. It could be concluded that a small quantity of calcium carbonate and calcium phosphate were obtained, and the HA is a main inorganic component of HAC.

Fig. 4 TEM images of PCL/HAC composites contained different weight of HAC. (a) 3 wt %; (b, c) 6 wt %; (d) 9 wt %

Fig. 4 displays the morphology of PCL/HAC composite materials with different weight of HAC from 3 wt% to 9 wt%. Many spherical particles of the PCL/HAC composite with the size of 100-250 nm are found. Among them, the content 6 wt% of HAC composite dispersed orderly. A clearly TEM image of the obtained PCL/HAC composite materials core-shell microspheres (Fig. 4c) shown that the dark HAC spherical particles are individually coated with a gray PCL shell with a thickness about 20-50 nm. The images confirm the dispersion of HAC in the PCL matrix.

Fig. 5 XRD patterns and FT-IR spectrum of PCL/HAC composites containing different weight of HAC: (a) XRD pattern of PCL/HAC composites containing 3 wt% HAC; (b) XRD pattern of PCL/HAC composites containing 6 wt% HAC; (c) XRD pattern of PCL/HAC composites containing 9 wt% HAC; (d) FT-IR spectrum of PCL/HAC composites containing different weight of HAC

Fig. 5a, b and c give XRD patterns of PCL/HAC composite materials with different weight of HAC, respectively. As seen from XRD patterns, the corresponding main strong peaks which belong to the diffraction of PCL are located at 21.28° and 23.5° . But the peaks of HA are weaker because the content of HAC in composite materials is lower. Compared the inserts of Fig. 5a, b, c with Fig. 3b, the location of diffraction peaks are basically same, suggesting three groups of composite materials all contain HA. Fig. 5d demonstrates FT-IR spectra of PCL/HAC composite materials containing different weight of HAC. The FT-IR spectra of three products are similar. The C-H stretching vibration peaks located at 2944 cm^{-1} , 2865 cm^{-1} , and 1243 cm^{-1} , meanwhile the C-O stretching vibration and C-H bending vibration peaks at 1726 cm^{-1} and 1470 cm^{-1} indicate the formation of PCL. The stretching bands shown at 3445 cm^{-1} (OH end groups), together with 571 cm^{-1} , 604 cm^{-1} and 1046 cm^{-1} are due to the presence of HA in HAC. This is further proved and validated that the three groups of PCL/HAC composites were successfully synthesized via in-situ polymerization of CL and HAC.

Fig. 6 DSC and TGA curves of PCL/HAC composites containing different weight of HAC. (a) DSC curves; (b) TGA curves.

Fig. 6 shows DSC and TGA curves of PCL/HAC composites containing different weight of HAC. From Fig. 6a, it can be seen clearly that the melting temperature of PCL decreased obviously with increasing weight of HAC (the melting temperature of pure PCL is 56.1 °C), which suggested the crystal perfection and grain size of PCL were affected by HAC because that increasing weight of HAC can decline crystal perfection of PCL and inhibit growth of PCL molecules. As the decrease of molecular weight of composite materials, the grain size of PCL and the melting temperature of PCL/HAC composite materials compared with pure PCL crystalline was decreased. Moreover, TGA curves of PCL/HAC composites in the air atmosphere from room temperature to 550 °C are shown in Fig. 6b. The thermal degradation temperature of PCL/HAC composite materials concentrated from 300 °C to 500 °C. The initial decomposed temperature of composites declined gradually with increasing the weight of HAC.

In Table 1, the maximum load, tension strength, elastic modulus yield strength and breaking elongation rate of PCL/HAC composite materials decreased gradually with increasing the weight of HAC (Sample I > II > III). It could be concluded that the mechanical properties of composite materials with HAC of 3 wt% is best for the growth inhibition of PCL molecules, which was enhanced by increasing the hydroxyl groups of HA in HAC, resulting in decrease of molecular weight of composite materials, and declining the mechanical properties of composite material. So the higher intensity and rigidity of composite materials could be produced by decreasing the weight of HAC. However, the component of composite materials with lower weight of HAC would further different from that of human bone. The mechanical properties of composite material with HAC of 6 wt% are closer to that of composite material with HAC of 3 wt%, so the comprehensive performance of composite material with HAC of 6 wt% would choose for further application.

Table 1 Tensile stress-strain data of PCL/HAC composites with different weight of HAC.

Sample I 3 wt%
Sample II 6 wt%
Sample III 9 wt%

Sample	Cross section (mm ²)	Maximum load (N)	Tension strength (MPa)	Elastic modulus (MPa)	Yield strength (MPa)	Breaking elongation rate (%)
I	13.38	261.33	19.53	2106.1	19.50	688.72
II	13.20	254.89	19.31	1656.9	19.31	517.35
III	9.00	104.63	11.63	453.5	0.00	0.63

4. CONCLUSIONS

The HAC were obtained by calcining the natural pig bone in this work. Then, the PCL/HAC composite materials were synthesized through in situ polymerization of CL and produced HAC. And the morphology and microstructure of obtained HAC and composites were characterized by TEM images, FT-IR spectra and XRD patterns, etc. The results show that smaller size (190 nm) and uniform distribution of HAC were obtained by calcining bone at 900°C for 2 h. HAC was uniformly dispersed in PCL matrix. The molecular weight of PCL in composites and the mechanical properties of PCL/HAC decreased with the weight percentage of HAC increases. Finally, the comprehensive performance of composite material with HAC of 6 wt% is better. The study provides a new way to synthesize repairing and substituting materials for human damaged bone.

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