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Ceramics International 33 (2007) 193–196

CERAMICS INTERNATIONAL

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Synthesis and sintering of nanocrystalline hydroxyapatite powders by gelatin-based precipitation method

Cai Shu [a,*], Yu Xianzhu [a], Xiao Zhangyin [a], Xu Guohua [b], Lv Hong [b], Yao Kangde [a]

[a] *School of Material Science and Engineering, Tianjin University, Tianjin 300072, PR China*
[b] *Shanghai Changzheng Hospital, Shanghai 200003, PR China*

Received 6 June 2005; received in revised form 27 June 2005; accepted 5 September 2005
Available online 29 November 2005

Abstract

The effect of gelatin on the nucleation, growth of carbonated hydroxyapatite (CHAp) crystals and subsequent influence on the microstructure of the sintered body was investigated. Nanocrystalline powders of carbonated hydroxyapatite (CHAp) were prepared from calcium nitrate tetrahydrate $Ca(NO_3)_2{\cdot}4H_2O$ and diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) in the presence of gelatin under aqueous solution of different concentrations. X-ray diffraction (XRD) results, combined with fourier transform-infrared spectroscopy (FTIR), indicate gelatin solution concentration being a predominant factor for inhibiting nucleation and growth that is believed to relate strongly to the interaction between Ca ions in the solution and R–$(COO)^-$ ions of gelatin molecules. FTIR collected on the powder with 4 wt.% gelatin after heat-treatment at 900 °C for 2 h in air exhibits single phase of CHAp. Scanning electron microscopy (SEM) shows that the powder obtained after heat-treatment at 900 °C is composed of nanocrystalline (50–100 nm) CHAp particles, and that the subsequently sintered bodies is highly porous.

Keywords: Nanocrystalline; Carbonated hydroxyapatite; Gelatin

1. Introduction

Hydroxyapatite (HAp) ceramics is a biocompatible and bioactive material that can be used to restore damaged human calcified tissue [1,2]. However, biological reaction to a HAp ceramics is strongly dependent on its chemical composition, phase purity, and microstructural properties (i.e., Ca:P ratios, particle size and porosity). Among the variety of HAp-based materials, carbonated HAp appears to be an excellent material for bioresorbable bone substitutes. Recent in vivo study indicated that the dissolution rate of sintered carbonated hydroxyapatite (CHAp) ceramics implanted subcutaneously was intermediate between tricalcium phosphate (β-TCP) and pure HAp [3]. Recently, many advanced methods that have been used for nanocrystalline CHAp powder synthesis, have become available and they have a variety of biomedical applications [4–7]. For example, chemical synthesis of HAp powders by using synthetic body fluids (SBF) solutions at physiological pH and temperature (i.e., 7.4 and 37 °C, respectively). Among the existing methods, in situ formation of nanoapatite crystals in presence of polymers is one of the most attractive routes. Synthesis of calcium phosphate in presence of cholesterol polyaspatic [8], stearic acid [9] was performed. These substances were found to promote crystallization and change crystal sHApe and possibly crystal structure of resultant powder precipitated, leaving an imprint of their original presence in the sintered bodies even after their disappearance at high temperature [10,11]. On the other hand, Kato et al. [4] studying a series of nanocrystalline hydroxyapatite/polymer composites via an in situ synthesis method, found that the crystallization of the hydroxyapatite was retarded in the presence of ionized polymers employed and they showed that the crystallization was concentration-dependent of polymers. Gelatin, an amphoteric polyelectrolyte because of a gelatin chain containing both anionic and cationic groups, can adsorb ions. The adsorption could be driven by electrostatic or/and by hydrophobic interaction, depending on the nature of surface and the medium. Moreover, gelatin chains can strongly interact with each other in water by hydrogen bond. Accordingly, the aim of this work is to study the effect of gelatin on nucleation and growth of nanocrystalline hydroxyapatite.

* Corresponding author. Tel.: +86 22 27890027; fax: +86 22 27404724.
E-mail address: caishu@tju.edu.cn (C. Shu).

0272-8842/$32.00
doi:10.1016/j.ceramint.2005.09.001

2. Experimental

The precipitation was prepared from calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) and diammonium hydrogen phosphate ($(NH_4)_2HPO_4$). Using aqueous ammonia to keep the pH of the reaction at around 10. The molar ratio of Ca to P was 1.67:1. gelatin was dissolved into the distilled water at 40 °C and added during the precipitation in order that their concentrations were 0, 1, 2, 3 and 4 wt.% of the synthetic HAp powder. The precipitation was washed several times to remove the excess of ammonia and ammonium by-products, then dried at 80 °C. The dried powder was lightly grounded to form a free-flowing powder, and then uniaxialy pressed at 10 MPa followed by cold isosatic pressing at 180 MPa. The green compacts were sintered at a temperature of 1200 °C for 4 h in air in a furnace.

The phases of the powders were determined by X-ray diffractometer (D/MAX-2500) with Cu Kα radiation at 40 kV and 100 mA. Specimens were scanned in the range of $20^\circ \leq 2\theta \leq 40^\circ$. Infrared absorption spectra of dried and calcined powder were measured by a Nicolet 5DX FTIR spectrometer. The calcined powders and fracture surface were observed by direct environment scanning electron microscopy (ESEM)(Model XL30Philips).

3. Results and discussion

3.1. Phase identification

Fig. 1 shows XRD patterns for the synthetic precipitate powders with different gelatin concentration. It is clear that all samples reveal characteristic peaks at 2θ of ∼26° and ∼32°. These peaks are almost identical to those in stoichiometric microcrystalline hydroxyapatite, but the powders with 3 and 4 wt.% gelatin showed broad weak peaks, indicating a poor crystalline HAp phase or crystallites getting smaller in dimension. Gelatin is a mixture of multiple polypeptide strands arising from the breakdown of ordered, helical mammalian proteins and collagens. A gelatin chain contains both anionic and cationic groups. In the preparation of nano-HAp powder at a temperature of about 40 °C, the pH 10 of the gelatin aqueous solution brings about the dissociation of ionic bonds because most of carboxyl groups are changed into a protonated form, resulting in the covalent interaction between Ca ions in the solution and $R–(COO)^-$ ions of gelatin molecules. With increasing gelatin concentration, the interaction sites of the gelatin macromolecules increase. It means increasing the amount of dissociated gelatin anions, i.e., carboxylic ion $R–(COO)^-$, in aqueous solution. Thus, a Ca–gelatin complex formed rapidly in aqueous solution further inhibits desired reaction between available Ca ions and phosphate ions to form crystalline HAp. This mechanism not only suppresses the formation of crystalline HAp, i.e., inhibition of nucleation, but also retards the growth of the HAp crystals. The complex nature of the interactions that take place between Ca ions and carboxylic ions can be explained by the FTIR spectra of the powders as shown in Fig. 2. The spectrum of the synthesized HAp powder without gelatin (A) shows characteristic peaks at 1092,1043, 962, 403 and 567 cm^{-1}, being assigned to PO_4^{3-} groups in the HAp and 3571 and 633 cm^{-1} correspond to the vibration of OH^-. For powder with 4 wt.% gelatin (B), the vibrational bands of OH^- at 3571 and 633 cm^{-1} decreased in intensity, showing the decrease in hydroxyl group. Meanwhile, some new bands can be observed in the spectrum. For example, bands at 1656 and 1544 cm^{-1} are attributed to amino and

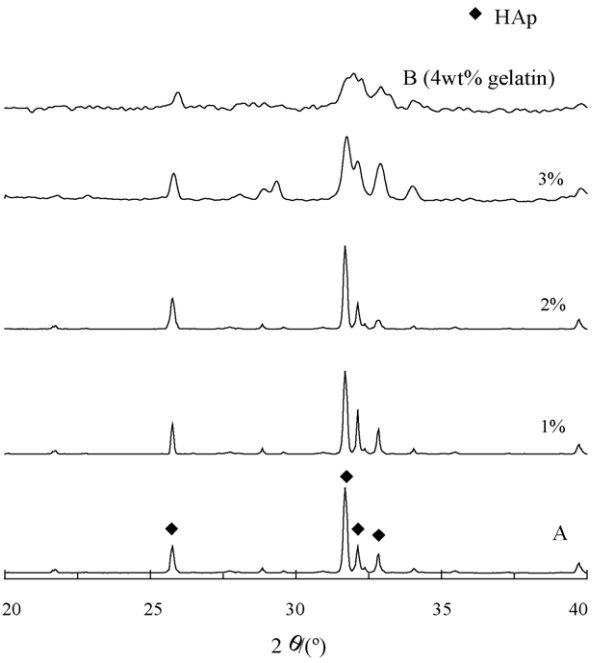

Fig. 1. XRD patterns of precipitated powder with different concentrations of gelatin.

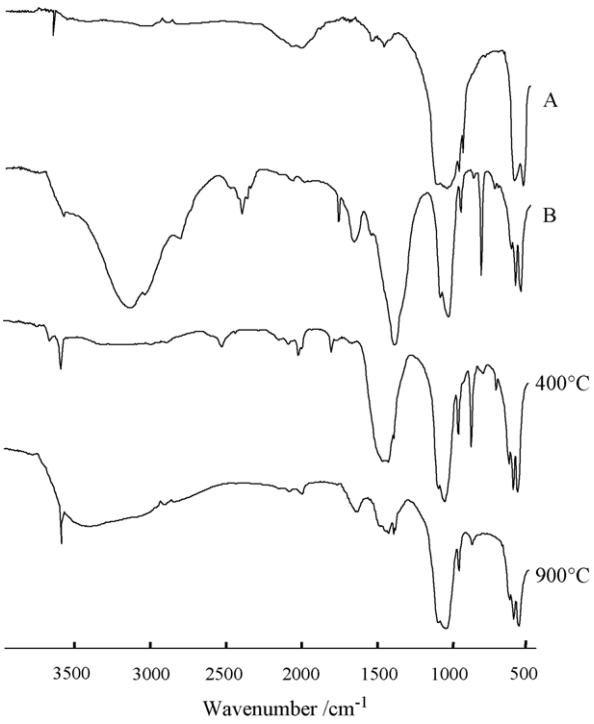

Fig. 2. FTIR spectra of the dried powders heat treatment at different temperatures.

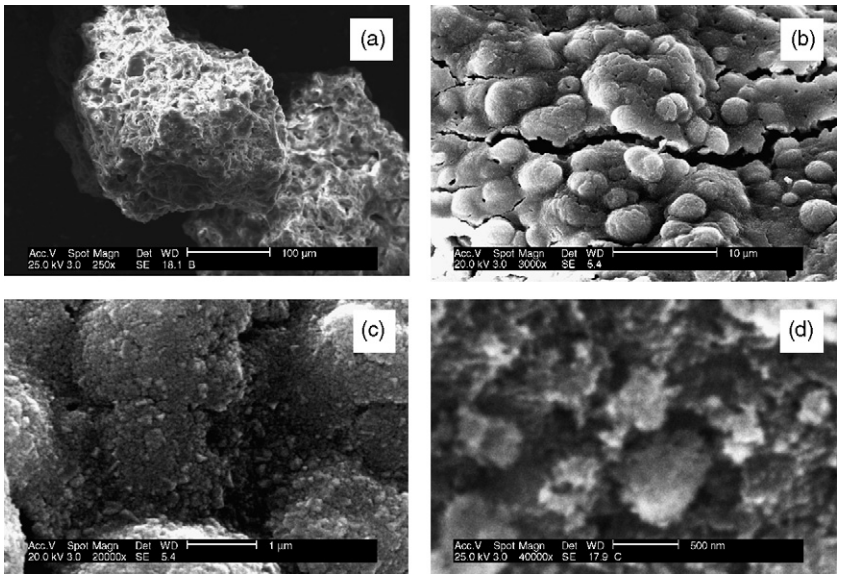

Fig. 3. SEM micrographs of the precipitated powders with 4 wt.% gelatin calcined at different temperatures. (a) precipitated powder, (b and c) calcined at 400 °C, (d) calcined at 900 °C, respectively.

carbonyl in the gelatin and 1390 cm^{-1}correspond to the vibration of $(COO)^-$, together with a weak O–H stretching peak observed at 3644 cm^{-1}. It is possible to account for this phenomenon in terms of the interaction degree of Ca ions with R–$(COO)^-$ in solution to form Ca–gelatin complex.

On the other hand, the powder with 4 wt.% gelatin showed the significant characteristics of carbonated hydroxyapatite, which are revealed by the carbonate bands at 1460 and 1422 and 880 cm^{-1}. This is due to the substitution of phosphate groups (PO_4^{3-}) by carbonate (CO_3^{2-}) species. The presence of the carbonate ions can be evidenced by the spectra, proving the formation of carbonated hydroxyapatite. The carbonated hydroxyapatite bands exist up to 400 °C. When the temperature was increased, these carbonate bands became less intense and bands that corresponded to the stretching (3650 cm^{-1}) and 630 cm^{-1} of the OH^- groups became more intense. However, the powder that was heated at 900 °C still had poor carbonate bands, indicating a small deviation from the ideal Ca/P ratios of pure HAp.

3.2. Morphology of powders and microstructure of sintered samples

The SEM micrographs of the as-dried powder with 4 wt.% gelatin and the powders obtained after heat treatment at different temperatures are shown in Fig. 3. The as-dried powders appear to be highly agglomerated. It is possible that the small particles were embedded in each agglomerated cluster caused by the formation of the triple helix conformation between the gelatin molecular chains because gelatin chains can strongly interact with each other in water by hydrogen bond between amino and carboxylic groups on the chain backbone. It is notable that carboxyl group is not stable and breaks down under the heating or humidity conditions. So the dried powder was very easy to sop up and deliquescence also resulting in the serious agglomeration of powders. When the heat treatment temperature reached 400 °C, interestingly, an imprint of the spherical composite remained in the microstructure of the calcined powders after gelatin molecules had vanished. Fig. 3d exhibits the morphology of powders undergone to heat treatment at 900 °C. It appears that there are many spherical agglomerations and crystallines of nanosize with a tendency to agglomerate leaving nanopores in between. These nanocrystallines of HAp have been sintered slightly during the processes of further decomposition and crystallization. When the green compacts made from the powder containing 4 wt.% gelatin were sintered at 1200 °C, its SEM picture of fracture surface is shown in Fig. 4. There are many micropores with irregular shape. The formation of pores is beneficial, as they would permit the circulation of the physiological fluid throughout them.

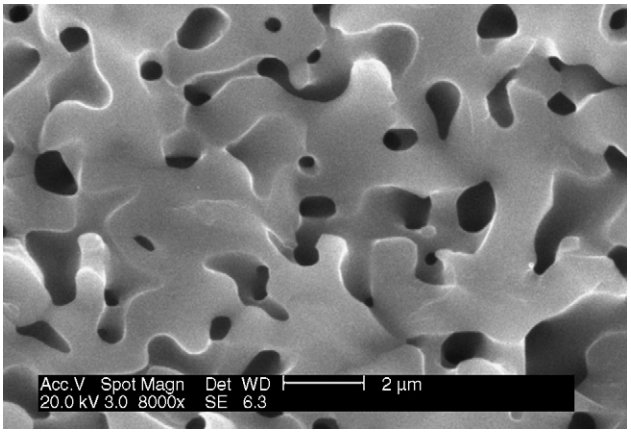

Fig. 4. SEM micrograph of the fracture surface for samples sintered at 1200 °C.

4. Conclusion

Carbonated hydroxyapatite nanocrystallines (CHAp) were synthesized in the presence of gelatin molecules. The structural

development of the spherical CHAp nanocomposites was manipulated up to the temperature of 900 °C. Experimental results indicate gelatin solution concentration being a predominant factor for inhibiting nucleation and growth that is believed to relate strongly to the covalent interaction between Ca ions in the solution and R–$(COO)^-$ ions of gelatin molecules. It also influences the Ca/P ratio, and subsequently affects the microstructure of the sintered body.

Acknowledgements

Authors are grateful to the Natural Science Foundation of China (Grant No. 30170954) and the Ministry of Science and Technology for their financial support to this research via 863 Grant 2002AA332080.

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