SYNTHESIS OF NANOSPHERICAL AND ULTRALONG FIBROUS HYDROXYAPATITE AND REINFORCEMENT OF BIODEGRADABLE CHITOSAN/HYDROXYAPATITE COMPOSITE

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Morphologies of hydroxyapatite (HAp) powders have influence on the mechanical properties of HAp/polymer composites. In this paper we reported a synthetic route for nanospherical and ultralong fibrous HAp powders and compared the influence of HAp morphologies on composite mechanical properties. HAp fibers with the length of \(250\ \mu\text{m}\) along c-axis direction and nanospheres with the diameter of \(80\ \text{nm}\) were produced, respectively, in the acidic solution containing glutamic acid and in the alkaline solution containing polyacrylic acid. The ultralong HAp fibers synthesized were used to reinforce biodegradable chitosan biomaterials with the significant improvement of bending strength because of the pull-out effect of long fibers.

Keywords: Hydroxyapatite fiber; chitosan; bending strength.

1. Introduction

Hydroxyapatite (HAp), a prototype mineral in calcified vertebrate bone, has been widely synthesized and used as biocompatible and osteoconductive substitute in the field of biomedical materials.\(^1\) However, HAp powders, used for the treatment of bone defects, have the problem that they easily migrate from the implanted sites. Combining HAp with polymer gives composite materials and can compensate for the drawback of HAp powders.\(^2,3\) Among many kinds of polymers, chitosan has attracted great interests due to its special characteristics, such as the predictable degradability, a minimal foreign body reaction, an intrinsic antibacterial nature, and so forth.\(^4\)–\(^7\) However, chitosan has the same drawback as other polymer biomaterials, the low mechanical properties.\(^2,8,9\) Successful biomaterials need not only good biological response but also a range of mechanical properties to hypothetically match that of the surrounding host tissue.\(^9\) Therefore, the combination of chitosan and HAp had been expected to address the problems which occurred in their separate forms and even to endue composites with some hardly obtainable properties. However, more often, the inclusion of HAp led to the decrease of

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Table 1. Preparation parameters and morphologies of products.\(^a\)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>CA/Ca/Cp/pH</th>
<th>Additives</th>
<th>Morphologies</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>712/5.58/2.48/3.5</td>
<td>Glutamic acid</td>
<td>Ultralong fibers</td>
</tr>
<tr>
<td>R2</td>
<td>10/6.31/2.50/10</td>
<td>Polyacrylic acid (MW4000)</td>
<td>Nanospheres</td>
</tr>
</tbody>
</table>

\(^a\)CA, Ca, and Cp represent the added amount (unit: gram) of additives, Ca(NO\(_3\))\(_2\)-4H\(_2\)O, and NaH\(_2\)PO\(_4\)-2H\(_2\)O, respectively.

composite strength,\(^10\) so chitosan/HAp composites with the remarkable mechanical properties are not yet available.

Actually, the morphologies of HAp powders play an important role in the mechanical properties of the polymer/HAp composites.\(^9\) Thus, the morphologically-controlled syntheses of HAp powders are necessary for exploring the possibility of improving the mechanical properties of chitosan composite biomaterials. Although there were some reports on preparing HAp powders with different morphologies,\(^11\)\(^-\)\(^13\) the controllability over HAp morphologies, such as the aspect ratio of HAp powders, is limited and not ideal for reinforcing chitosan.

In this paper, we developed a novel method to synthesize ultralong fibrous and nanospherical HAp with glutamic acid and polyacrylic acid as the crystal growth modifier under hydrothermal conditions. The as-synthesized HAp powders were characterized by various methods and showed good uniformity. The biodegradable chitosan biomaterials were reinforced by the resultant HAp powders with two morphologies and as a result, ultralong HAp fibers improved the bending strength of chitosan/HAp composites because of the pull-out effect of long fibers.

2. Experimental Details

2.1. Powders syntheses

All chemicals were purchased from Beijing Chemicals Corp. China and used as received without further purification. The appropriate amounts of analytical grade reagents in Table 1 were dissolved into 900 ml deionized water which was heated to \(~60^\circ\)C and vigorously stirred. The pH value was adjusted by the aqueous NaOH solution. The as-obtained solution was sealed into an autoclave immediately and hydrothermally treated for 48 hours at 160°C. After cooling down to room temperature, the white precipitate deposited in the bottom of the autoclave was collected and washed several times with deionized water and absolute ethanol respectively.

2.2. Composites preparation

Varied amounts of HAp and chitosan were dispersed in deionized water and ultrasonically treated for 5 min by an ultrasonic cell disruptor (JY92-2D, Zhejiang Xinzhi Corp., China). After adding 2 wt% acetic acid, the suspension was stirred
by a magnetic rod until chitosan was completely dissolved. The resulting slurry was held for 3 hours in vacuum to drive off bubbles. A 10 wt% KOH solution was used to mold chitosan/HAp rods. After soaking in the KOH solution for three days, solidified rods were carefully washed by deionized water several times and air-dried at 40°C for 3 days.

2.3. Materials characterization

The size and morphologies of the as-obtained powders were characterized by a field emission scanning electron microscope (FESEM, JSM-6700F, JEOL, Japan). Phases were identified in an X-ray diffractometer (XRD, X-Pert, PANalytical, The Netherlands) with Cu Ko radiation (40 kV, 30 mA). Fourier transform infrared (FTIR) spectra were recorded using a KBr pellet technique in a FTIR spectrometer (Equinox 55, Bruker, Germany) over 400 ~ 4000 cm⁻¹ at a resolution of 4 cm⁻¹. The Ca/P ratio of HAp powders was determined by the method of X-ray diffraction quantitative analysis,¹⁴ in which the mixture of pure stoichiometric HAp and β-tricalcium phosphate (β-TCP) in various compositions, after calcinated, was quantified to make a standard curve of the characteristic peak intensity ratio of β-TCP to HAp with reference to composition and the Ca/P ratio of the as-synthesized HAp was calculated through comparing the characteristic peak intensity ratio of thermally decomposed products with the standard curve. The bending strength of composite samples was measured by the three-point bending method in a universal testing machine (WDW-10, Tianchen Testing Machine Corp., China) at a crosshead speed of 2 mm/min. Ten samples were tested for each composition and Young’s modulus was determined from the slope of initial linear elastic portion in stress-strain curves.

3. Results and Discussion

Figure 1 shows the FESEM images of the powders which were prepared by the two reactions in Table 1. Obviously, the R1 reaction produced ultralong fibers with the
Fig. 2. XRD patterns of the powders obtained in the reactions (a) R1, (b) R2, and (c) JCPDS #9-432.

diameter of 0.5 ~ 1 µm and the length of ~250 µm (the aspect ratio, 250 ~ 500), and the R2 reaction led to the well-dispersed nanospheres with the size of about 80 nm. It can be seen in Fig. 1 that both powders obtained have good uniformity.

Figure 2 shows the XRD patterns of the as-synthesized fibers and nanospheres. As compared to the JCPDS card #9-432 (Fig. 2(c)), it was found that the as-synthesized powders were of apatite structure (P6\(\overline{3}\)/m space group) without any impurity and however, the relative peak intensity of fibrous HAp differed from that of nanospherical powder.

The FTIR spectrum of fibrous HAp in Fig. 3(a) shows the typical PO absorption bands, including the antisymmetric PO stretching modes at 1095 and 1037 cm\(^{-1}\), the symmetric PO stretching mode at 962 cm\(^{-1}\) and the OPO bending modes at 604 and 567 cm\(^{-1}\).\(^{15}\) The absorption bands at 631 cm\(^{-1}\) and 3573 cm\(^{-1}\) were assigned to the OH librational and stretching modes of HAp crystals, respectively and the weak band at 875 cm\(^{-1}\) was attributed to the HPO\(_4^{2-}\) group in the fibrous HAp crystals.\(^{16}\)

Figures 3(b) and 3(c) show the FTIR spectra of pure chitosan and chitosan/HAp composite. The absorption bands around 2920, 1655, 1593, and 1160 cm\(^{-1}\) were attributed to methylene (-CH\(_2\)), amide I carbonyl (\(-\equiv\text{C}=\text{O}\)), amino (\(-\equiv\text{NH}_2\)) and bridge oxygen (\(-\equiv\text{O}=\text{C}\)) in chitosan.\(^{2,17}\) The occurrence of absorption band at 1579 cm\(^{-1}\) was consistent with the previous report which precipitated chitosan film in aqueous NaOH solution and assigned it to amine II (\(-\equiv\text{NH}\)).\(^{18}\)

The mechanical properties of the as-prepared chitosan/HAp rods were determined in a universal testing machine with reference to the Chinese and British
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Fig. 3. FTIR spectra of the (a) fibrous HAp, (b) chitosan, and (c) chitosan/fibrous HAp composite.

national standards (GB 14208-93 and BS 3597-2-03). Figure 4(a) shows that, when the HAp fiber content was increased to 12.3 wt%, the bending strength of chitosan/HAp composite rose to 87.3 MPa in comparison to 72.1 MPa of pure chitosan measured under the same conditions. With further increasing HAp fiber content, the bending strength dropped abruptly. However, the elastic modulus (Fig. 4(b)) calculated from the linear section of stress-strain curves continuously increased from 1.2 to 4.7 GPa when the HAp content varied from 0 to 28.1 wt%.

Because the ultralong fibers were easily laid parallel to the bottom surface of the XRD sample stage when preparing the samples and formed the preferential orientation, the XRD pattern in Fig. 2(a) shows that the strongest peak is (300), which differs from the (211) peak in JCPDS card #9-432. The intensity of (100), (200), (300), (210), (310) and (410) planes, parallel to the [001] direction, gained significant increase and the intensity of other planes, such as (211), (112), (202), (102), (111), (222), (213) and so forth, relatively decreased, indicating that fibers may be elongated along c-axis direction. The relative peak intensity of nanospheres in Fig. 2(b) was similar to the standard card, which was in agreement with the orientation influenced by the spherical morphology.

By comparing the synthetic conditions, it can be seen that the significant variation of HAp morphologies was mainly related to both the addition of additives and the change of pH values. Bigi et al.\textsuperscript{19} and our previous reports\textsuperscript{20} showed that the formation of whisker-like HAp crystals in acidic solutions had been attributed...
to the hydrolysis from the interlayered structure of octacalcium phosphate (OCP) and apatite to HAp. The preferential adsorption of glutamate ions on (100) surfaces of OCP layers increased the tendency toward the anisotropic growth of HAp crystals,\textsuperscript{20} which led to the formation of ultralong HAp fibers along the [001] direction. HAp nanospheres resulted from the quick nucleation owing to the high pH value and furthermore, the adsorption of polyacrylic acid retarded the growth of apatite crystals.\textsuperscript{21,22}

To determine the Ca/P molar ratio, HAp powders were calcinated at 1100°C for 2 hours and the Ca/P ratio was quantified by the X-ray diffraction quantitative analysis according to Raynaud’s report.\textsuperscript{14} As shown in Fig. 5, HAp fibers and nanospheres decomposed into two phases, stoichiometric HAp and $\beta$-TCP, indicating that both R1 and R2 reactions produced non-stoichiometric HAp with the low Ca/P ratio. According to the relative peak intensity ratio of $\beta$-TCP (02 10) and HAp (002) planes,\textsuperscript{14} the Ca/P ratios of fibers and nanospheres were respectively determined to be 1.60 and 1.65. Since the FTIR analysis identified the HPO$_4^{2-}$ group in HAp fibers, the chemical formula of fibrous HAp may be calculated as Ca$_{9.6}$(HPO$_4$)$_{0.4}$(PO$_4$)$_{5.6}$(OH)$_{1.6}$. This Ca-deficient hydroxyapatite is of greater value than stoichiometric HAp because of it relatively good bioabsorbability,\textsuperscript{16} which is in agreement with the purpose of designing biodegradable polymer-containing composite.\textsuperscript{23}

Therefore, it can be concluded from the FESEM, XRD, and FTIR analyses that ultralong HAp fibers were synthesized in a simple additive-assisted hydrothermal process and HAp morphologies could be controlled from ultralong fibers to nanospheres through adding glutamic acid or polyacrylic acid and adjusting...
experimental parameters. Suchanek et al.\textsuperscript{12} reviewed the syntheses of morphological controlled whiskers, of which the longest whisker was 30 ~ 100 μm. Ioku et al.\textsuperscript{13} reported the 150 μm-long HAp fiber with the aspect ratio of 20 ~ 60. However, such a remarkable variation of the morphologies of HAp powders from ~ 250 μm long fibers to ~ 80 nm-sized nanospheres has not been reported. Inorganic fibers can usually be used to reinforce polymer and however, there are no reports about HAp fiber-reinforced chitosan biomaterials. Therefore, ultralong HAp fibers obtained were investigated to reinforce chitosan and the reinforcement effects were compared with the HAp nanospheres synthesized.

In the XRD pattern of chitosan/HAp composites (Fig. 6), the strong (300) peaks, as compared to the (211) peak, implied that the route in which chitosan was dissolved in the acetic acid solution and then precipitated in the aqueous alkaline solution, did not lead to the disappearance of fibrous morphology of HAp crystals. It indicated that the ultralong HAp fibers derived from the above hydrothermal reaction in low pH values could resist the acidic environment of the blending route in acetic acid solutions, which were used to dissolve chitosan.

Previous studies\textsuperscript{8,10,24} showed the inclusion of HAp into chitosan via blending methods led to the decrease of the mechanical properties. But the results in Fig. 4 showed that the simple blending method can improve the mechanical properties. The reason for the improvement of the bending strength could be sought from the FESEM images of fracture faces. Figure 7(a) shows that the clear and smooth surface was formed in pure chitosan during crack propagation. Conversely, Fig. 7(b) shows that chitosan/fibrous HAp composite formed the curved and coarse
fracture surface. Especially, the pull-out of HAp fibers could absorb a part of fracture energy and contributed to the improvement of mechanical properties. The comparison experiments using HAp nanospheres led to the decrease of bending strength as shown in Fig. 8, which was in agreement with previous reports.\textsuperscript{10} The reinforcement failure of nanospherical HAp owing to the lack of the morphology of long fibers proved from the opposite side that the improvement of the mechanical properties of chitosan/fibrous HAp was resulted from the pull-out effect. Although there were investigations\textsuperscript{8,10} which had thought the interface adhesion between HAp and chitosan weak, Yamaguchi et al.\textsuperscript{2} reported calcium ions could be interacted
with amino group of chitosan and Yin et al.\textsuperscript{25} considered that HAp powder could be bound to chitosan-gel composites. Thus, it was inferred that the pull-out of fibrous HAp appropriately transferred the strain across the interface between HAp and chitosan when fracturing. The drop of the bending strength of the composite with high content of HAp fibers may be caused by the dispersion problems because of interlacing between ultralong fibers.

4. Conclusions

Ultralong fibrous and nanospherical HAp powders were successfully synthesized via an additive-assisted hydrothermal reaction. FESEM, XRD, and FTIR analyses were performed to identify that the fibers with \( \sim 250 \mu m \) along \( c \)-axis direction and \( 0.5 \sim 1 \mu m \) in diameter were yielded in the acidic solution with glutamic acid and nanospheres with the size of \( \sim 80 \text{ nm} \) produced in the alkaline solution with polyacrylic acid. Via a simple blending route, the chitosan/HAp composites were prepared by using the as-synthesized HAp powders with two kinds of morphologies. Fibrous HAp significantly improved the bending strength of chitosan from 72.1 to 87.3 MPa, while nanospherical HAp decreased the mechanical properties. The FE-SEM observation confirmed that the pull-out of HAp fibers contributed to the improvement of mechanical properties. Prospectively speaking, the morphologically-controlled HAp obtained has wide applications in the field of biomaterials.

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