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Study of synthesis of nano-hydroxyapatite using a silk fibroin template

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Abstract

Nano-hydroxyapatite (HA) was directly synthesized on a silk fibroin (SF) template using the property of SF being soluble in a concentrated CaCl₂ solution as a HA source of calcium at pH 7.4 and room temperature. The microstructure and bonding state were investigated by x-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry–thermogravimetry analysis (DSC-TG) and transmission electron microscopy (TEM). The results indicated that the HA crystals were poorly crystallized with a rod-like shape of 20–60 nm length and 10–20 nm diameter. Strong molecular interactions and chemical bonds might be present between SF and HA. There were other nucleation sites such as carbonyl (−C=O) and amine (−N−H−) groups on SF molecules besides the carboxyl (−COOH) and hydroxyl (−OH) groups previously reported. During the formation of HA, the coordination action between specific functional groups on SF and calcium ions (Ca²⁺) played an important role. The crystallinity of HA was improved and had an orientation growth along (0 0 2) at the presence of SF, resulting in a structure similar to natural bone. It was concluded that SF could regulate the structure and morphology of HA effectively.

1. Introduction

It is well known that bones are the products of natural minerals, which are mainly composed of inorganic hydroxyapatite (HA) nanocrystals and collagen. The c-axis of the HA nanocrystals is preferentially aligned parallel to the longitudinal direction of the collagen fibril, and HA exhibits poor crystallinity and nanostructure features as well as intimate bonding between inorganic and organic phases, which endows natural bones with a more dynamic response to their environment [1]. For the preparation of HA-based composites with a structure and components similar to natural bone, biomimetic synthesis on organic templates may well be the most attractive route. It is demonstrated that silk fibroin (SF) is a better biomaterial than collagen, and SF has a greater potential for application to clinical repair, tissue engineering and modified materials owing to excellent properties such as a rich source, good cellular affinity with plenty of hydrophilic polar groups, non-toxicity and good biocompatibility [2]. SF is a kind of degradable protein, and the products of degradation themselves can not only produce non-toxic side effects but also provide nutrients for skin and tissues. Moreover, SF proteins have an important role in regulating the mineralization of HA nanocrystals, and HA crystals can combine with SF to form nanocomposites by chemical interactions [3]. Generally, good interfacial bonding in organic/inorganic composites endows materials with excellent properties. Therefore, the combination of HA and SF shows potential to be explored as bone-repair materials and bone tissue engineering materials, but few studies on the HA/SF composite have been reported. In this work, we employed the property of SF of being soluble in a concentrated CaCl₂ solution as a HA source...
of calcium and further reacted it with (NH$_4$)$_2$HPO$_4$ to directly synthesize a nano-HA/SF composite; in the process, SF acted as an organic template guiding HA and was expected to show excellent bioaffinity, mechanical toughness and further improved osteoconductivity.

A combination of HA and SF could enhance the ability of the bone formation. In the composite, functional groups on the SF molecule provided nucleation sites for Ca$^{2+}$ and PO$_4^{3-}$, and the presence of HA was beneficial to the precipitation of bone-like apatite because of the crystal lattice match. Also, nano-HA synthesized by a template, providing more surface atoms, was more conducive to ion adsorption and further promoted deposition of bone-like apatite. Hence, a novel material with good overall properties might be obtained.

2. Materials and methods

The co-solution of SF/CaCl$_2$ was obtained by dissolving SF powders with reverse β-sheet structure into a solution (CaCl$_2$·CH$_3$OH·H$_2$O 1:2.8 in molar ratio) at 80 °C for 30 min to disintegrate SF and then diluted to the concentration of 0.2 M by adding distilled water. SF/CaCl$_2$ powders were obtained after the co-solution of SF/CaCl$_2$ was centrifuged and freeze-dried.

The raw materials of CaCl$_2$ and (NH$_4$)$_2$HPO$_4$ were weighed precisely to coincide with the stoichiometric HA (1.67 in molar ratio), and the HA/SF composites were prepared by adding a 300 ml (NH$_4$)$_2$HPO$_4$ solution (0.2 M) in a 500 ml co-solution of SF/CaCl$_2$. The reaction solution was stirred and titrated with ammonia in drops for 2 h at room temperature to maintain the pH value at 7.4, kept at room temperature for 24 h and then centrifuged three times using distilled water and ethanol to remove residual ions. Deposits were lyophilized to analyze their structure. The ratio of HA and SF by weight was set as 7:3.

The powders obtained were characterized by x-ray diffraction (XRD, D/max-rb), transmission electron microscopy (TEM, 1200EX), differential scanning calorimetry–thermogravimetry analysis (DSC-TG, Netzsch 409 PC) and Fourier transform infrared spectrometric analysis (FTIR, PE).

3. Results and discussion

3.1. XRD analysis

X-ray diffraction patterns are shown in figure 1; the diffraction peaks such as (0 0 2), (2 1 1), (3 0 0), (2 0 2), (3 1 0), (2 2 2), (3 0 2) in the HA/SF composite could be clearly seen, but they significantly broadened and overlapped in comparison with the peaks of sintered HA at 800 °C, which indicated that the inorganic component of the composite was identified as nano-crystalline and belonged to a poorly crystallized HA [4]. However, the crystallinity improved relative to a single HA, which was concerned with more regularity and order of nucleation on SF surfaces.

Comparing figure 1(b) with figure 1(a), we can see that the peak intensity of the (0 0 2) plane in the composite situated at 2θ angles of 25.84° was lower than that of the (3 0 0) plane at 32° (figure 1(b)), indicating that HA grew faster along the (002) crystal plane, so the growth along the (300) plane vertical to (002) was restrained and resulted in a higher diffraction intensity in the (300) direction. Meanwhile, the diffraction angle of HA (002) shifted to a low diffraction region relative to that of single HA (26.04°), suggesting that the crystal parameter c elongated. The HA size in the composite along the c-axis calculated by the Scherrer formula was 27.15 nm, while it was 25.56 nm in a single HA. This demonstrated that the HA crystallites in a composite showed more anisotropic growth along the c-axis; thus the preferential orientation of HA crystallites along the c-axis was promoted owing to the presence of SF.

3.2. FTIR analysis

The FTIR spectra of different powders are shown in figure 2. The amide A band at 3294 cm$^{-1}$ corresponded to the −N=H stretching vibration, and the amide I, II, III bands (at 1633 cm$^{-1}$, 1517 cm$^{-1}$ and 1231 cm$^{-1}$) were attributed to the carbonyl (−C=O) stretching mode, combination peak of the main N=H in-plane bending and the C−H stretching vibration, coupled peak of the main C−N stretching and the −N=H in-plane bending vibration, respectively (figure 2(a)).

As can be observed, the positions of the amide A, I, II, III bands shifted and the vibration intensity apparently decreased with the addition of CaCl$_2$ (figure 2(b)), which implied that both the −C−O and −N=H groups on SF molecules had been preferentially coordinated with calcium ions (Ca$^{2+}$) in the CaCl$_2$ solution. Ca$^{2+}$ was liable to coordinate with −COO− and weak to coordinate with nitrogen atoms. But at higher pH values (>6.5), nitrogen atoms could form a complex with Ca$^{2+}$ because of the dissociation of protonated amino groups [5]. Rhee reported that −COOH in protein coordinated with Ca$^{2+}$ [6]. About 16.5 mol% of hydroxyl and 2.9 mol% of carboxyl groups on SF molecules were the first type of nucleation sites reported before [7]. When studied properly, a chemical interaction was found to be present between the peptide linkage and Ca$^{2+}$. In SF molecules, the number of −C−O and −N=H groups was large, so −C−O and −N=H groups were the
mainly coordinated ones. The interaction between the peptide linkage and Ca$^{2+}$ should be the coordination of $-\text{C} \cdots \text{O}$ and $-\text{N} \cdots \text{H}$ groups with Ca$^{2+}$, and coordination action spots were just on the amide links. Accordingly, it was concluded that $-\text{C} \cdots \text{O}$ and $-\text{N} \cdots \text{H}$− groups on SF surfaces were another type of nucleation site of HA crystals in addition to the $-\text{COOH}$ and $-\text{OH}$ groups.

The group vibration of SF in HA/SF was restrained because HA crystals nucleated uniformly on the group nucleation sites and wrapped them entirely or partly. Figures 2(a) and (c) show that the intensity of the amide A band weakened and broadened in HA/SF, the amide I and II bands decreased appreciably and amide III band almost disappeared. It was due to the fact that only the groups unwrapped or wrapped partly presented FTIR spectra. The position of the amide A band shifted from 3294 cm$^{-1}$ to 3301 cm$^{-1}$, the amide I band corresponding to $-\text{C} \cdots \text{O}$ shifted by 26 cm$^{-1}$ (1633–1659 cm$^{-1}$), and the amide II band, observed at 1517 cm$^{-1}$ of SF, shifted to 1531 cm$^{-1}$; the blue shift of amide bands proved that the strong chemical interactions existed between HA and SF, and the $-\text{C} \cdots \text{O}$ and $-\text{N} \cdots \text{H}$ bonds in the lattice chain were weakened in the mixture due to the formation of new coordination bonds between Ca$^{2+}$, $-\text{C} \cdots \text{O}$ and $-\text{N} \cdots \text{H}$ groups. In addition, the $-\text{OH}$ stretching vibration of n-HA decreased and the peak position at 1038 cm$^{-1}$ belonging to PO$_{4}^{3-}$ of n-HA was also different from that of the composite. These results indicated that some molecular interactions such as electrostatic force and the hydroxyl bond together with the coordination bond were present between n-HA and SF in the composite.

When comparing the FTIR spectrum in figure 2(d) with that in figure 2(a), it can be seen that the intensity and position of the demineralized HA/SF complex were similar to those of pure SF and three original amide bands rebound, which indicated that the bonding between HA and SF was reversible and the amide bond structure was not destroyed, and further confirmed that HA crystals nucleated on the groups and then grew to wrap them entirely or partly accordingly.

Each of the HA, SF and HA/SF powders was placed in chloroform (1.476 g cm$^{-3}$). The photographs obtained after intense agitation and ultrasonic dispersion are shown in figure 3. The density of HA/SF powders was between HA (3.07 g cm$^{-3}$) and SF (1.2 g cm$^{-3}$). As can be seen, HA powders were below the liquid level because the density of HA was greater than that of chloroform (figure 3(a)), SF powders floated completely on the liquid owing to a lower density of SF than chloroform (figure 3(b)), deposition of HA/SF powders below chloroform showed that the density of the composite powders was consistent and that an interaction existed between HA and SF, the immersion of SF into HA structure and formation of a HA/SF complex reflected the characteristics of the biological mineralization.

DSC-TG analysis of HA/SF complexes and SF show that there were clear absorption and exothermic changes. The TG curves show that the weight loss at 80 °C and 190 °C was caused by the loss of free water and bound water, respectively. The weight loss between 240 and 500 °C was responsible for thermal decomposition of SF. The starting temperature of the fastest decomposition of SF ranged from 294 °C (figure 4(a)) to 323 °C (figure 4(b)), which suggested that a strong chemical bond was formed between HA and SF. SF was involved in the process of formation of HA and the two closely integrated together.

From the TEM photographs of HA and HA/SF composites displayed in figures 5(a) and (c), it could be estimated that the size of HA crystals in the composite was approximately 10–20 nm in diameter and 20–60 nm in length, which was coarser than that of pure HA crystals. It could also be observed that the needle-like HA crystals distributed homogeneously.
in the SF matrix and combined closely with SF in the composite.

Figure 5(b) shows the selected area electron diffraction (SAED) pattern of HA/SF with a polycrystalline ring; the inner ring (0 0 2) was present and divided into several arcs compared with SAED of HA (figure 5(d)), indicating that HA grew with preferred growth along the (0 0 2) direction parallel to the longitudinal direction of SF and was similar to natural bone. In addition, the diffraction ring of HA in the composite was clearer and sharper than that of single HA, which was consistent with the XRD results.

3.6. Synthesis mechanism of HA/SF

In the process of synthesizing HA/SF, the structure, charges and three-dimensional chemical complementarity between SF template molecules and ions in the HA crystal are the most important factors in determining selective nucleation and growth.

In this study, the pH value was 7.4 (greater than the isoelectric point of SF, pH 4.5); SF fibers first precipitated from the solution and stretched into β-sheet structure; hence, more hydrophilic groups such as −NH− and −COO− got exposed to the surface and adsorbed inorganic ions (e.g. Ca2+). The SF molecules transformed into two-dimensional surfaces loading Ca2+. The Ca2+ surface electronic state with a combination of two PO43− groups of HA and −NH− and −COO− groups of SF is shown in figure 6. According to double-layer theory, when the PO43− ions were added, Ca2+ on the SF molecule attracted the PO43− and OH− groups by electrostatic interaction as a driving force to further gather more Ca2+, until the concentration was favorable for heterogeneous nucleation of HA. In this process, HA nucleation was inevitably affected by the interfacial structure. Only when a certain interplanar distance of HA matched with the space of amino acid molecule such as aspartic acid (ASP) in SF, HA particles could grow longer in this direction (figure 7)[8]. Because of the formation of SF–metallic ion chelate, Ca2+ was fixed on the functional groups. Presumably because the distance between β-ASP (0.15 nm) is close to that of Ca2+-Ca2+ along the c-axis direction in HA(0.13–0.165 nm), reflecting the special demand for complementarity between the crystal and the organic molecules, which led to a higher degree of local
supersaturation and decreased activation energy with a prior nucleation and growth orientation in the \(c\)-axis direction. HA and SF organically cross-linked together to form a structure similar to natural bone.

### 4. Conclusions

1. SF, as an organic template, can effectively regulate the structure and morphology of HA with growth orientation along the \(c\)-axis. In the synthesis mechanism HA nucleates on the functional groups of SF molecules and the coordination and electrostatic force play an important role.
2. The composite particles of nano-HA/SF with poor crystallinity show needle- or rod-like shapes with 20–60 nm length and 10–20 nm width, a structure similar to natural bone. It is a promising material for bone tissue engineering.

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### References


