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Novel hydroxyapatite nanorods crystal growth in silk fibroin/sodium alginate nanofiber hydrogel

Jinfa Ming*, Shiyu Bie, Zhijuan Jiang, Peng Wang, Baoqi Zuo

National Engineering Laboratory for Modern Silk, College of Textile and Clothing Engineering, Soochow University, Suzhou 215123, China

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ABSTRACT

Silk fibroin/sodium alginate (SF/SA) nanofiber hydrogels were used as organic template to control hydroxyapatite (HAp) crystal growth at room temperature. Scanning electron microscopy demonstrated rectangular column and size-controllable HAp nanorods formed, and the crystalline structure of nanorods crystals was confirmed by energy dispersive X-ray spectroscopy, X-ray diffraction, and Fourier transform infrared spectroscopy. Time-dependent experimental results also exhibited HAp nanorods with rectangular column growth in the mineralization process. SF/SA nanofiber hydrogels had an important impact on the morphology of crystals due to the strong electrostatic interaction between hybrid molecular and Ca^{2+} ions. The regulating formation mechanism of HAp nanorods by SF/SA nanofiber hydrogels may extend the understanding of the potential for using biomimetic principles to synthesize bone-like composite materials.

1. Introduction

Hydroxyapatite (HAp), a class of calcium phosphate-based material, has been used for a variety of biomedical applications, including bone substitutes [1], matrices for drug release control [2], etc. Many chemical methods have emerged for the preparation of HAp with control over their size and morphology, such as co-precipitation [3], solid-state reactions [4], hydrothermal method [5,6], sol–gel synthesis [7,8] and reflux method [9]. These methods, however, mostly prepare irregular forms of powder [10]. Presently, organic templates are found to control the nucleation and growth of inorganic crystals with controllable morphology [11,12]. Moreover, in several works, the mineral growth environment occurred in gel state [13]. In our study, we used SF/SA nanofiber hydrogels to regulate and control HAp crystal growth. SF protein and SA polysaccharides can form a nanofiber hydrogel biopolymer system, which can better mimic the real mineralization system of bone more than a single protein system [14]. At the same time, in this nanofiber hydrogel system, SF and SA molecules can easily coordinate the divalent cations (i.e., Ca^{2+}) through the ionic interaction between the carboxylic acid groups located on the polymer molecular chain and the chelating cation [15]. These chelating ions provide the location site of crystal and facilitate the crystal growth in hydrogel microenvironment.

Here, we report novel SF/SA nanofiber hydrogels as organic template to synthesize rectangular column and size-controllable HAp nanorods at room temperature. The effect of time-dependent growth study on the morphology of HAp has been investigated.

2. Materials and methods

Bombyx mori silk was purchased from Zhejiang, China. Calcium chloride, diammonium hydrogen phosphate, ammonium hydroxide, and ethanol (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) were of analytical grade and used without further purification. All solutions were prepared with deionized water.

SF solution was prepared following the procedure described previously [15]. Briefly, *Bombyx mori* silk fibers were degummed three times in 0.05 wt% Na_2CO_3 solution at 100 °C for 30 min, rinsed thoroughly and dried. The extracted SF was dissolved in a mixture of solvent composed of LiBr/ethanol/H_2O (44/45/11, wt/wt/wt) at 70 °C for 4 h, yielding a 10 g dL^{-1} solution. SF solution (2.0 wt%) was obtained after dialysis for 4 days and filtration. SA (2 g) was dissolved in deionized water to obtain a uniform 0.5 wt% SA solution at room temperature. Then, SF and SA aqueous solutions with 70/30 ratio were mixed by stirring, and the concentration of the mixture solution was controlled at 1.0 wt%. The mixed solution was stored overnight at 5 °C to avoid and premature precipitation of the protein, which occurred at room temperature. Finally, hydrogels were prepared by adding 1 mL blended solution in 24 well plates (Coring, USA). The solutions

* Corresponding author. Tel.: +86 512 67061157; fax: +86 512 67246786.
E-mail address: jinfa.ming@gmail.com (J. Ming).

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were allowed to gel in an incubator at 37 °C. In addition, SA hydrogels were gelled by adding 10 mM Ca^{2+} solution. The morphology of all hydrogels was interconnected nanofiber networks (Fig. 1).

Nanofiber hydrogels were used as templates to mineral HAp crystals. The mineralization process was as follows: first, hydrogels were treated in 75% (v/v) ethanol for 30 min to prepare the water-insoluble hydrogels. The water-insoluble hydrogels were immersed directly in $CaCl_2$ solution (2.22 g $CaCl_2$ dissolved in 100 mL deionized water) for 1 h at room temperature. Then, the samples were immersed in $(NH_4)_2HPO_4$ solution (1.578 g $(NH_4)_2HPO_4$ dissolved in 100 mL deionized water). Ammonium hydroxide was added to adjust the pH to 8. After being reacted for 48 h at room temperature, the final samples were rinsed with distilled water and lyophilization for characterization.

After mineralization, the samples were directly lyophilized. This lyophilization process does not change the existential state of HAp crystal in nanofiber hydrogels. At the same time, the lyophilized samples were fractured in liquid nitrogen for preparing SEM samples. The morphology of samples was observed by scanning electron microscopy (SEM, S4800, and Hitachi) with an energy dispersive X-ray spectroscopy (EDS) analyzer. The crystal structures of crystals were analyzed with an X-ray diffraction instrument (X Pert-Pro MPD, PANalytical, Netherlands) in a transmittance mode and FTIR on Nicolet5700 (Thermal Nicolet Company, USA) in an absorbance mode.

3. Results and discussion

The morphology of HAp induced by different templates is shown in Fig. 2. As control sample, Fig. 2A–d showed the needle-shaped HAp crystals without organic templates at room temperature. For the XRD patterns, the sample displayed characteristic 2θ peaks appearing at 25.7°, 31.8°, 33.8°, 39.8°, 46.8°, 49.5°, and 53.2°, corresponding to the diffraction planes (002), (211), (202), (310), (222), (213), and (004) of the HAp crystallites (Fig. 2B–d), respectively, which was consistent with the crystalline nature of HAp in the literature [16]. For SF hydrogel template, flower-type HAp crystals were obtained and the shape of the individual crystal was more flakelike as opposed to the needle-like crystal (Fig. 2A–a). However, Fig. 2A–b showed HAp nanorods with typical width of 345.20 ± 96.59 nm and lengths up to 5 μm, when SF/SA nanofiber hydrogels were added. In addition, Fig. 2A–c depicted the SEM image of spherical-like HAp crystals, which was controlled by SA hydrogel template. The crystalline structures of crystals prepared by different organic templates at mineralization 1 h were examined by XRD (Fig. 2B). The diffraction of all samples was seen at 2θ values (002), (211), (202), (310), (222), (213), and (004) (Fig. 2B), which was consistent with HAp sample preparing by solution-precipitation method without template (Fig. 2B–d). At the same time, these HAp crystals were further proved by EDS (Fig. 2C).

Fig. 3 showed SEM images of HAp crystals prepared by SF/SA nanofiber hydrogel templates at different mineral times. From the SEM micrograph of Fig. 3a, it depicted the crystal sample was composed of many regular nanorods at mineralization 1 h at room temperature. The crystal structure of nanorods was HAp crystals, which was confirmed by XRD and FTIR (Fig. 4). The diffraction peaks such as (002), (211), (310), (222), (213), and (004) were seen in Fig. 4A–a. At the same time, peaks at 560–610 cm^{-1} and 1000–1100 cm^{-1} were attributed to phosphate groups in HAp (Fig. 4B–a). When the mineralization time increased 6 h, HAp nanorods with about 310.93 ± 84.46 nm in width were grown (Fig. 3b). With the mineralization time increasing to 48 h, the elongated nanorods crystals were observed (Fig. 3c–e). The crystalline structure of HAp crystals was also examined using XRD and FTIR (Fig. 4). For the XRD patterns, the samples exhibited the diffraction planes (002), (211), (310), (222), (213), and (004) of HAp crystallites. Therefore, in the mineralization process, the morphology of HAp crystals was not influenced by mineralization times at room temperature.

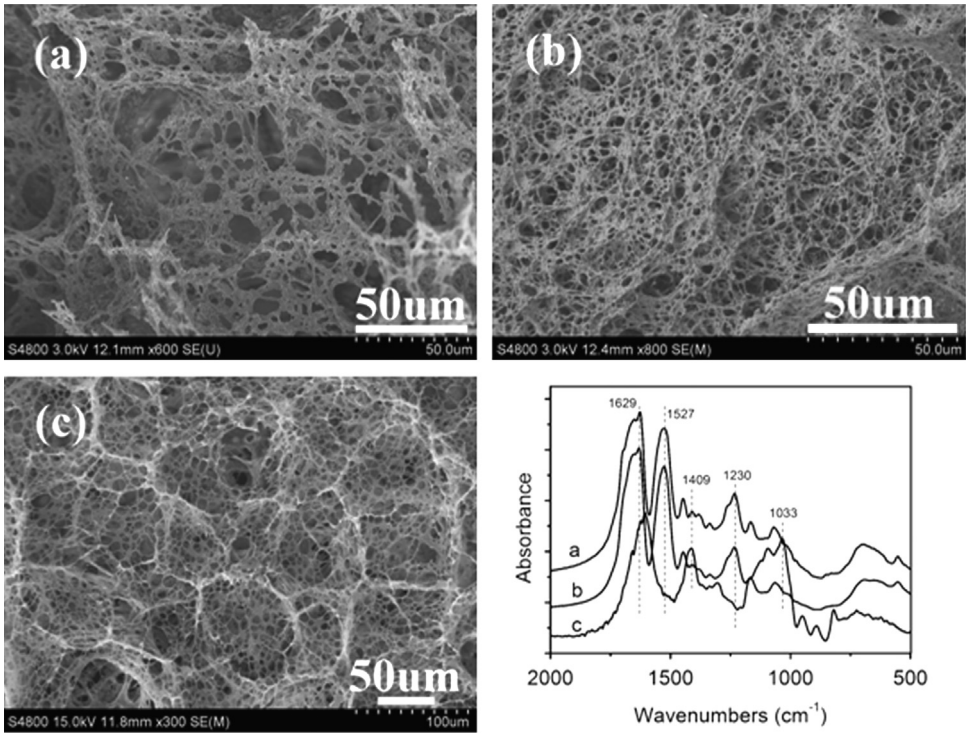

Fig. 1. SEM images of nanofiber hydrogels and its FTIR results: (a) SF hydrogel, (b) SF/SA hydrogel, and (c) SA hydrogel.