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Rapid Communication

Controlled hydrothermal synthesis of strontium-substituted hydroxyapatite nanorods and their application as a drug carrier for proteins

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ABSTRACT

Without the addition of any organic additives, both undoped hydroxyapatite (HAp) and different strontium (Sr)-substituted hydroxyapatite (SrHAp) samples with amounts from 1 to 20 mol.% were synthesized via hydrothermal route. The crystal texture, chemical functional group and microstructure were well characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and inductively coupled plasma (ICP), respectively. And the protein adsorption and release properties of bovine serum albumin (BSA) and lysozyme (LYS) were investigated on the samples to search for a well protein drug delivery vehicle. The results revealed that the pure HAp consisted of uniform nanobelts and all SrHAp samples had a rod-like morphology. The lattice parameters of SrHAp were larger than HAp, while they had similar crystal structure. The protein adsorption and release tests indicated that the incorporation of Sr into HAp lattice could increase the amounts of BSA and LYS adsorption. The BSA adsorption capacity of 1SrHAp was found to be 55.52 mg/g, which was about 14.64% higher than that of HAp. The 15SrHAp possessed the highest LYS loading amount of 90.54 mg/g, which was about three times that of HAp. And SrHAp exhibited sustained protein release capability.

1. Introduction

Due to its nontoxicity, excellent biocompatibility and bioactivity, the HAp has been widely applied to biomedical fields [1]. In addition, HAp exhibit strong surface adsorption ability because of its surface function groups and special chemical constitution. Thus, it is of great significance in the field of drug carrier [2–4].

Up to now, various preparation methods such as sol–gel synthesis [5], hydrothermal method [6], microemulsion [7], microwave synthesis [8], spray pyrolysis [9], chemical precipitation [10] and template-directed methods [11] have been used to synthesize HAp nanomaterials. Furthermore, HAp with different morphologies such as fibers, rods, needles, microspheres and plates has been fabricated and investigated by many researchers for drug release [12–15]. However, the structure of synthetic pure HAp is stable since it has greater crystallinity than the HAp in natural bone. That is to say, synthetic pure HAp is not easily biodegradable in vivo and difficult to reach the requirements of clinical medicine.

According to many reports [16,17], the incorporation of a certain amount of other functional elements into biological materials leads to advantageous effects on biomaterial properties, such as the stability of structure, solubility in chemical solvents, surface charge and dissolution rate under simulated physiological conditions. The Ca^{2+} ions in HAp structure can substituted by Ag^{+}, Mg^{2+}, Zn^{2+}, Al^{3+}, Ce^{3+}, La^{3+}, Bi^{3+}, Co^{2+}, Eu^{3+} and other ions [18–25], which affects the crystal parameters and morphology of pure HAp. Because the physicochemical and biologic properties of HAp are related to its structure and composition, so ionic substitutions is an appropriate method to influence bioactivity of HAp.

Sr is a bone trace element, which could enhance the bioactivity of biomaterials, especially in accelerating bone formation. In addition, researches suggest that the biological properties of Ca–P materials are improved by incorporation of Sr [26,27]. Thus, compared with pure HAp, SrHAp might have greater potential in biomaterial, such as protein drug carrier.

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Researchers always use surfactants or organic additives to prepare porous or hollow HAp nanomaterials, and their adsorption and release of protein or drug performances have been fully studied [4,14]. However, the HAp nanomaterials obtained by these preparation processes will inevitably affect the interaction between HAp and human tissue. The biosafety is difficult to be ensured, and the preparation process is cumbersome and costly. In contrast, the preparation of the HAp with nanorods structure don't have to use organic agents. It means that the materials have high biological safety, and the preparation method is simple. Meanwhile, the adsorption and release behaviors of proteins on HAp nanobelts and SrHAp nanorods haven't been studied. Therefore, it is necessary to assess the storage and release characteristics of proteins on them to find out a good protein drug carrier. There are many investigations in using BSA and LYS as model protein medicines such as interferon to establish biodegradable nanoparticle drug delivery system [14,28,29]. At the same time, BSA and LYS are acidic and basic proteins, respectively, and they have good biological stability and are suitable protein drug models.

We demonstrate a simple method to synthetise both undoped HAp and SrHAp with variable amounts of Sr via a hydrothermal route without any surfactants or organic additives. XRD, SEM, ICP and FTIR were employed to characterize the samples. Additionally, BSA and LYS were used as model proteins to investigate their storage and release properties.

2. Materials and methods

2.1. Chemicals and materials

BSA and LYS were purchased from Huashun Biotechnology Co., Ltd. (Wuhan, China) and Bestchrom Biosciences Co., Ltd. (Shanghai, China), respectively. Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$), strontium nitrate ($Sr(NO_3)_2$), sodium hydroxide (NaOH) and ethanol absolute (C_2H_6O) were all purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the initial chemicals in this work were all chemical pure. And deionized (DI) water was used for rinsing and preparing solutions in the study.

2.2. Synthesis of HAp and SrHAp

The samples with theoretic $Sr^{2+}/(Ca^{2+}+Sr^{2+})$ molar ratios of 0, 0.01, 0.03, 0.05, 0.07, 0.09, 0.10, 0.15 and 0.20 were prepared by a hydrothermal process. The obtained products were labeled as HAp, 1SrHAp, 3SrHAp, 5SrHAp, 7SrHAp, 9SrHAp, 10SrHAp, 15SrHAp and 20SrHAp, respectively. The Ca/P and (Ca + Sr)/P molar ratios were adjusted to 1.67. Typical procedures for preparing samples were described as follows. First of all, 6 mmol of $Na_2HPO_4{\cdot}12H_2O$ was dissolved in 50 mL DI water to form solution (labeled as solution-1). And 1 mol/L NaOH solution was added to adjust the pH value of the solution-1 to 11.5. Then the aqueous solution (labeled as solution-2) was prepared by dissolving $Ca(NO_3)_2{\cdot}4H_2O$ and $Sr(NO_3)_2$ in 30 mL DI water, which contained 10 mmol of $(Ca^{2+}+Sr^{2+})$, and slowly added into the solution-1 under magnetic stirring. After that, the obtained white milky suspension was transferred into a Teflon-lined stainless-steel autoclave (100 mL capacity), sealed, and maintained at 200 °C for 8 h. Finally, the precipitate was separated and washed by DI water and ethanol in sequence when autoclave cooled naturally. The obtained powders were dried at 100 °C for 6 h.

2.3. Characterization

The crystal structures of the samples were identified by an X-ray diffractometer (D8 Advance, Bruker, Germany) using Cu Kα (λ = 1.5418 Å) radiation. The constituents of samples were examined with a FTIR spectrometer (Vertex 70, Bruker, Germany) using the KBr pellets. The morphologies of products were determined using a scanning electron microscopy (Nova 400, FEI, America). The contents of Ca, P and Sr elements were determined by the ICP method using an ICP spectrometer (IRIS Advantage ER/S, Thermo Elemental, America).

2.4. Protein adsorption and release on HAp and SrHAp

The methods of protein adsorption and release in vitro were carried out according to the previous report [30]. BSA and LYS were selected as model proteins in the experiments. Typically, 0.2 g of product was added into 50 mL of 0.01 mol/L phosphate buffer solutions (PBS, pH 7.4) with protein concentration of 1.0 mg/mL. Then the above solution was shaked at 37 °C for 24 h. The protein-loaded sample was collected by centrifuging the solution and dried in vacuum. The absorbance of protein in the filtrate was measured by UV-visible spectrophotometer at a wavelength of 280 nm and the content of protein was determined by calibration curve shown in Fig. 1. Finally, the amount of protein adsorption was calculated according to the following formula:

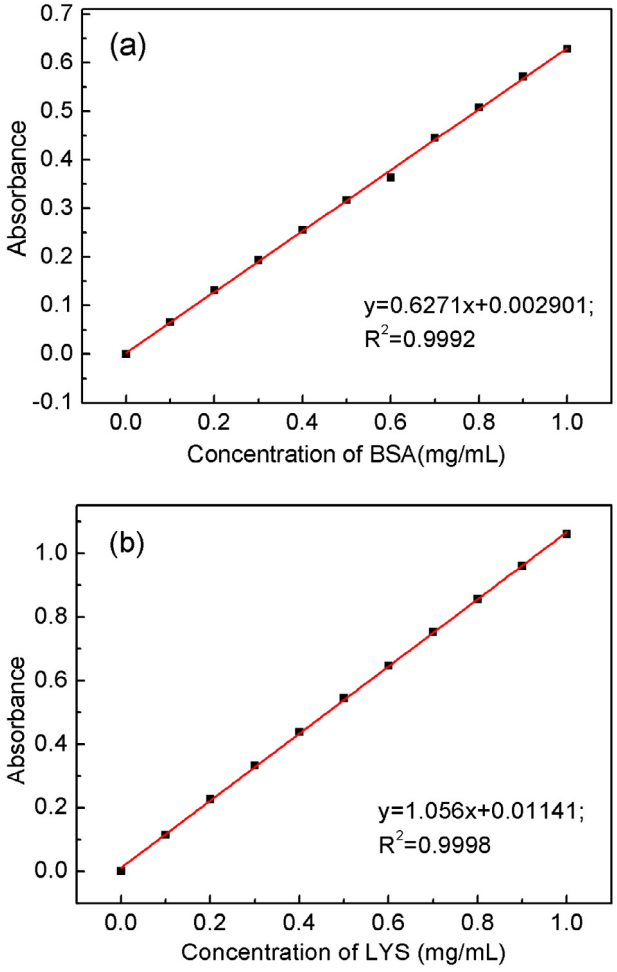

Fig. 1. The standard curves of BSA (a) and LYS (b).

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