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Hydroxyapatite nanosheet-assembled microspheres: Hemoglobin-templated synthesis and adsorption for heavy metal ions

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A B S T R A C T

Hydroxyapatite (HAP) nanostructures have wide potential applications in many fields such as drug delivery, tissue engineering, bone repair, gas sensing, catalysis and water treatment. Inspired with the fact that HAP has a high affinity with proteins, we have designed and developed a new synthetic strategy for three-dimensional (3-D) HAP nanosheet-assembled microspheres (HAP-NMSs) by employing hemoglobin as a soft template. The as-prepared products are characterized by X-ray powder diffraction (XRD) and field-emission scanning electron microscopy (SEM). The experimental results show that 3-D HAP microspheres are constructed by the self-assembly of HAP nanosheets as the building blocks. The influences of hemoglobin concentration, hydrothermal temperature and time on the morphology and crystal phase of the product are investigated. Based on the systematic investigation, a possible formation mechanism of HAP-NMSs is proposed. The as-prepared HAP-NMSs are explored for the potential application in water treatment. The experimental results indicate that the HAP-NMSs have a high adsorption capacity for heavy metal ions and selective adsorption activity for Pb^{2+} ions in acidic solution, thus are promising for the application in wastewater treatment.

1. Introduction

Hydroxyapatite (HAP) is the main inorganic constituent of vertebrate hard tissues such as bones and teeth. The presence of HAP gives bones and teeth high stability, hardness and functions [1]. Synthetic HAP materials have many advantages such as high biocompatibility, bioactivity, non-toxicity and high stability. Thus, HAP nanostructures are considered as ideal biomaterials in many biomedical fields including bone repair [2], tissue engineering [3], drug and gene delivery [4–7]. Moreover, HAP nanostructured materials are of great significance in many other fields such as catalysis [8], gas sensing [9] and water treatment [10]. Hence, tremendous efforts have been made to synthesize various HAP nanostructures with different morphologies such as nanorods, nanowires, nanotubes and 3-D structures [11–17]. Among these nanostructures, 3-D nanostructure-assembled HAP structures have attracted much attention because of their promising applications. Up to now, various synthetic strategies have been developed to fabricate 3-D nanostructure-assembled HAP structures. These strategies include solvothermal method [12], microwave-assisted hydrothermal method [14,15], soft template method [16,17], and hard template method [18].

Compared with the hard template method, the soft template method usually does not need to remove the template. The soft template existing in the obtained product can usually increase the cargo loading capacity or be functionalized in the subsequent steps. Thus, the soft template method for the synthesis of 3-D nanostructure-assembled HAP structures has been studied, and different biocompatible soft templates including block copolymer [16], lipidosome [19], DNA [17] and phosphorus-containing organic biomolecule [20,21] have been used. However, the proteins, as biocompatible biomolecules and essential substances widely existing in cells, tissues and bones, have been rarely used to prepare 3-D nanostructure-assembled HAP structures [22,23].

HAP usually exists in the hexagonal structure and has two different binding sites (positively charged C sites and negatively charged P sites) on its surface [24]. The surface property makes HAP have a high affinity for many substances such as protein and DNA molecules. Thus, HAP has been studied as the adsorbent for blood purification [25,26] and the carrier for protein drugs [15]. The primary tissue of bone is a relatively hard and lightweight composite material made up of the inorganic mineral hydroxylapatite and an organic elastic protein collagen. Although bone is essentially brittle, it has a significant degree of elasticity, which is mainly contributed by collagen. The collagen is synthesized intracellularly as tropocollagen and then exported, forming fibrils. The organic part is also composed of various growth factors. Moreover, there is the electrostatic interaction between the carboxyl groups of protein and calcium ions in aqueous solution, and this interaction can also be used to regulate the growth of HAP. Hence, we speculate that the protein will be a good soft template for the

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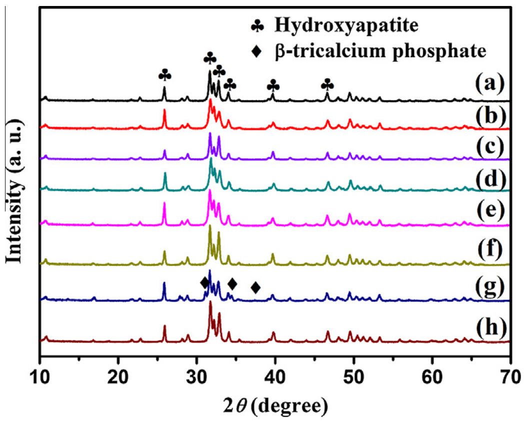

Fig. 1. (a–e) XRD patterns of the samples that were prepared by using 0.025 g Hb under different hydrothermal conditions: (a) 120 °C, 10 h; (b) 150 °C, 10 h; (c) 180 °C, 10 h; (d) 180 °C, 5 h; (e) 180 °C, 8 h. (f–h) XRD patterns of the samples that were prepared at 180 °C for 10 h by using different amounts of Hb: (f) 0.0125 g Hb; (g) 0.05 g Hb; (h) 0 g Hb.

synthesis of 3-D nanostructure-assembled HAP structures and is suitable to regulate the morphology of the HAP product.

Herein, we report a new and facile hydrothermal synthesis of 3-D nanosheet-assembled HAP microspheres (HAP-NMSs) by using the protein hemoglobin (Hb) as a soft template. The as-obtained microspheres are constructed by self-assembly of HAP nanosheets with the thickness of dozens of nanometers. The Hb plays a significant role in the formation of HAP nanosheet-assembled microspheres. In the absence of Hb, only disperse HAP nanosheets can be obtained. The effects of Hb concentration and hydrothermal temperature and time on the morphology and crystal phase of the product are investigated. And a possible formation mechanism of HAP-NMSs is proposed on the basis of the experimental results. The as-prepared HAP-NMSs are explored for the potential application in water treatment. The experimental results exhibit that HAP-NMSs have a high adsorption ability for heavy metal ions and selective adsorption activity for Pb^{2+} ions, thus are promising for the application in wastewater treatment.

2. Experimental section

Hemoglobin (Hb, >98.0%) was purchased from Sangon Biotech (Shanghai) Co., Ltd. Other chemicals were purchased from Sinopharm Chemical Reagent Co. All chemicals were analytical grade and used as received without further purification.

In a typical experiment for the preparation of HAP-NMSs, an aqueous solution of $Ca(CH_3COO)_2$ (0.10 M, 10 mL) and an aqueous solution of Hb (1 mL, 0.025 g mL^{-1}) were mixed together under magnetic stirring. Then, the resulting solution was added into an aqueous solution of NaH_2PO_4 (20 mL, 0.03 M), and the pH value of the resulting solution was adjusted to 4.5 by using 1 M HCl aqueous solution. Then, the resulting precursor solution was transferred to a Teflon-lined stainless steel autoclave (50 mL) and heated to 180 °C and kept at this temperature for 10 h. The product was collected by centrifugation, washed with deionized water twice and absolute ethanol twice, and dried at 60 °C in air. Dispersed HAP nanosheets were prepared under the same experimental conditions in the absence of Hb.

Solutions containing heavy metal ions were prepared by dissolving $Pb(NO_3)_2$, $Cu(NO_3)_2{\cdot}3H_2O$ and $CdCl_2{\cdot}2.5H_2O$ in deionized water, respectively. The adsorption kinetic experiments were conducted as follows: the HAP-NMSs powder (10 mg each) was immersed into 20 mL aqueous solution containing heavy metal ions. The initial concentration of heavy metal ion solution was 100 mg L^{-1} for Pb^{2+}, Cu^{2+} and Cd^{2+}, respectively. The pH of each solution was adjusted to 5.5 by using dilute $NH_3{\cdot}H_2O$. The suspensions were stirred at room temperature for 2 h. The supernatant (1 mL) of the suspension was collected by centrifugation at given time intervals, and the concentration of heavy metal ions in the supernatant was analyzed by an inductively coupled plasma optical emission spectrometer (ICP-OES). In order to study the selective adsorption activity of HAP-NMSs for different heavy metal ions, the HAP-NMSs powder (5 mg) was immersed into 20 mL aqueous solution containing heavy metal ions of 10 mg L^{-1} Pb^{2+}, 10 mg L^{-1} Cu^{2+} and 10 mg L^{-1} Cd^{2+}. The effect of pH value on the adsorption ability of HAP-NMSs was also studied. The pH value of an aqueous solution containing three heavy metal ions of Pb^{2+}, Cu^{2+} and Cd^{2+} was adjusted to be 4.0, 5.5, 7.0 and 8.5, respectively, by using dilute

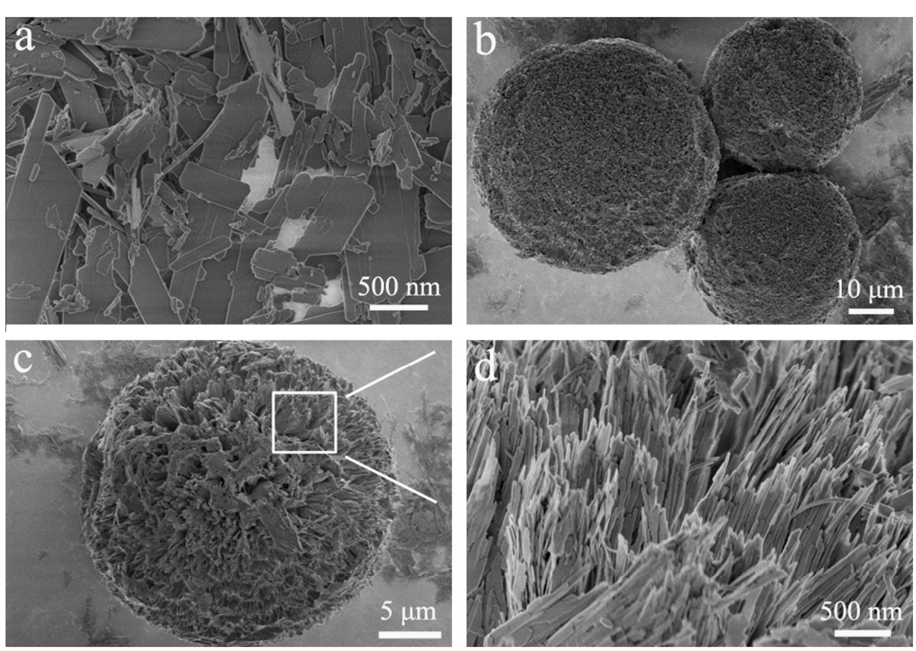

Fig. 2. SEM micrographs of the samples that were prepared without Hb (a) and with 0.025 g Hb (b–d) by hydrothermal treatment at 180 °C for 10 h.