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Synthesis and characterization of hydroxyapatite nanoparticles prepared by a high-gravity precipitation method

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Abstract

Hydroxyapatite nanoparticles (nHAP) were successfully synthesized using a high-gravity precipitation method. Crystal structure and morphology of the nHAP were confirmed by X-ray diffraction (XRD) and transmission electron microscopy (TEM). Chemical nature of the nHAP was analyzed by Fourier transform infrared spectroscopy (FTIR) and inductively coupled plasma atomic emission spectrometry (ICP-AES). The characterization results demonstrated formation of nHAP with diameters of 1.9–14.2 nm and lengths of 4.0–36.9 nm and carbonated apatite structures, which were comparable to that of natural bone apatite. Further, biocompatibility of the nHAP was determined by MTT assays on MCF-7 cells. Moreover, potential applications of the nHAP in preparing Calcium Phosphate Cements (CPC) and Gelatin/HAP composite hydrogels were explored. The high-gravity precipitation method provides a new approach in fabrication of nHAP and opens possibilities to realizing great potential of nHAP in various biomedical applications.

Keywords: Hydroxyapatite; Nanoparticles; Calcium phosphate cements (CPC); Gelatin/HAP composite hydrogels

1. Introduction

Calcium phosphate apatite is a group of appealing compounds for fabricating bone-repair scaffolds because of their intrinsic similarity to the mineral constituent of natural hard tissues and their exceptional biocompatibility [1]. The main inorganic component of human hard tissues is hydroxyapatite (HAP), with a chemical formula $Ca_{10}(PO_4)_6(OH)_2$ [2]. Hence, synthetic HAP has attracted extensive interest in biomedical fields and clinical applications, serving as bone graft substitute [3] and bone defect filler [4] materials. To meet increasing demand for these materials, various methods to synthesize HAP nanoparticles (nHAP) are being developed, including solid-state reactions, chemical precipitation, hydrothermal treatment, sol-gel process, microemulsion, hydrolysis of other calcium phosphates, electro-spinning, surfactant-assisted approaches and bone-mimetic strategies [5–19]. Despite the variety of nHAP synthesis methods, nHAP fabrication remains complex and challenging. Herein, we aimed to develop a new method for fabricating nHAP through utilizing a high-gravity precipitation technology.

The principle component of the high-gravity precipitation process is a rotating packed bed (RPB). The RPB generates a high-gravity environment via a centrifugal force, leading to formation of tiny liquid droplets to strongly intensify substances

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mixing [20,21]. The better mixing derived from the RPB enables better contact of the reagents and more thorough reaction between the reagents. Thus, the high-gravity precipitation technology could give better performance in precipitation reaction than conventional precipitation method in stirred tank reactor (STR). Therefore, utilization of the high-gravity precipitation technology would benefit fabricating nHAP. The concept of producing nHAP by the high-gravity precipitation technology is not new per se. A combined route of high-gravity reactive precipitation and hydrothermal method was employed to produce nHAP with a mean size of 55–110 nm [21]. However, in the previous study, although the hydrothermal method was beneficial for the prompt formation of nHAP, the high temperature process may also make the resultant nHAP with larger dimensions and well-crystallized structures with little or no bioactivity. Those results were in contrast to bio-crystal apatite, which is non-stoichiometric and exhibits high bioactivity [22]. In addition, potential applications of nHAP prepared by the high-gravity precipitation method have not been addressed as yet, and still deserve attention.

In this study, we took the unique advantage of the high-gravity precipitation technology to develop a new method for fabricating nHAP (HAP_{RPB}). The object of this study was to synthesize nHAP and to characterize their physicochemical properties in terms of morphologies, chemical compositions and crystalline structures. We further evaluated biocompatible performance of the resultant nHAP using MCF-7 cells. Moreover, a comparison was made with respect to nHAP synthesized by conventional precipitation method in STR (HAP_{STR}). Beside the utilization of the high-gravity precipitation method to synthesize nHAP, one additional particularity of this work is to explore the potential of the HAP_{RPB} in preparing Calcium Phosphate Cements (CPC) and Gelatin/HAP composite hydrogels, which are two of the most common materials in biomedical applications.

2. Materials and methods

2.1. Materials

Analytical reagent grade calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$), sodium phosphate (Na_3PO_4), sodium hydroxide (NaOH), absolute ethyl alcohol (CH_3CH_2OH) and glutaraldehyde (25% v/v solution) were purchased from Sinopharm Chemical Reagent Beijing Co. Ltd., Gelatin (powder from cold water fish) from Sigma–Aldrich, and used without further purification.

Experimental setup for the high-gravity precipitation process has been described previously [21]. The key component is a rotating packed bed (RPB), which mainly consists of a stainless wire mesh packing, two liquid inlets and a liquid outlet. The rotator is installed inside fixed casing, with inner and outer diameters of 35 and 105 mm, respectively, and an axial length of 35 mm. More details about the RPB can be found elsewhere [20].

2.2. Preparation of hydroxyapatite (HAP)

nHAP were prepared by the high-gravity precipitation method. In a typical reaction, aqueous solutions of $Ca(NO_3)_2 \cdot 4H_2O$ (0.1 M, pH = 10) and Na_3PO_4 (0.1 M, pH = 10) were prepared. The two solutions were simultaneously pumped into the RPB from their reservoirs to different slotted pipe distributors in the RPB at a high-gravity level of 1098 m/s^2 (a rotating rate of 1600 rpm (RPB1600)). The flow rates of the two reactant solutions were calculated and set to give a Ca/P molar ratio at 1.67 under the idealized stoichiometric equation: $10Ca(NO_3)_2 + 6Na_3PO_4 + 2NaOH \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20NaNO_3$ (Equation 1). After entering the RPB through the liquid distributor, the two solutions vigorously mixed and reacted in the packing under a high-gravity circumstance to form HAP precursor. The HAP precursor was collected and aged at room temperature overnight. After aging, the suspension was filtered and washed several times with deionized water. The product was dried at 80 °C for 12 h and then ground into powder to obtain HAP ($HAP_{RPB1600}$). Different high-gravity levels could be achieved through adjusting the rotating rates. Due to the limit of the setup, three other rotating rates were set at 800 rpm (RPB800), 1200 rpm (RPB1200) and 2000 rpm (RPB2000), respectively.

For comparison purposes, nHAP were also prepared by conventional chemical precipitation method in a stirred tank reactor (STR). Aqueous solutions of $Ca(NO_3)_2 \cdot 4H_2O$ and Na_3PO_4 were prepared in the same way as that in the high-gravity precipitation method. Then the Na_3PO_4 solution was added into the $Ca(NO_3)_2$ solution (4 ml/min) under vigorously stirring. The volumes of $Ca(NO_3)_2$ and Na_3PO_4 solutions were calculated to give a Ca/P molar ratio at 1.67. After completing the addition of Na_3PO_4 solution, the suspension was continually stirred for another 2 h to form HAP precursor. The HAP precursor was aged at room temperature overnight. After aging, the suspension was filtered and washed with deionized water, followed by drying at 80 °C for 12 h and then grounding into powder to obtain HAP (HAP_{STR}).

2.3. Preparation of calcium phosphate Cements (CPC)

The HAP powder was shaped into cylindrical specimens of ~13 mm in diameter and ~10 mm in height under compression pressure of 5 MP for 5 min using a powder tablet machine (FW-4A, TIAN JIN TUO PU INSTRUMENTS CO., LTD), which was kindly provided by Prof. Zhiping Liu (Beijing University of Chemical Technology). The cylindrical samples were removed from the cylindrical mold and tested in mechanical measurements.

2.4. Preparation of gelatin/HAP composite hydrogels

Gelatin was dissolved in deionized water (to a final concentration of 10% w/v). Subsequently, the HAP powder was added (to a final concentration of 0.5% w/v). After continuous stirring for 2 h, glutaraldehyde solution was added (to a final concentration of 0.15% v/v) under vigorously stirring to serve as