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Hydrolytic conversion of amorphous calcium phosphate into apatite accompanied by sustained calcium and orthophosphate ions release

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ABSTRACT

The aim of this study is to investigate the calcium and orthophosphate ions release during the transformation of amorphous calcium phosphate (ACP) to hydroxyapatite (HA) in aqueous solution. The ACP is prepared by a wet chemical method and further immersed in the distilled water for various time points till 14 d. The release of calcium and orthophosphate ions is measured with calcium and phosphate colorimetric assay kits, respectively. The transition of ACP towards HA is detected by x-ray diffraction (XRD), transmission electron microscopy (TEM), and fourier transform infrared spectroscopy (FTIR). The results indicate that the morphological conversion of ACP to HA occurs within the first 9 h, whereas the calcium and orthophosphate ions releases last for over 7 d. Such sustained calcium and orthophosphate ions release is very useful for ACP as a candidate material for hard tissue regeneration.

1. Introduction

Hydroxyapatite (HA) is a well-known essential ingredient in bone and tooth-related hard tissues [1–4], which can be synthesized by soluble calcium and orthophosphate salts [5–7]. As the most important component of HA, the calcium and orthophosphate ions may play important role during the process of hard tissue regeneration [8–10]. According to the present knowledge, these ions can facilitate osteointegration through the formation of a thin calcium phosphate layer at the graft-host interface [11–15]. In cell level, the recent in vitro studies also suggest that the presence of these ions in cell culture medium can stimulate cell differentiation and mineralization [16–20], which reveals their importance in regulating cell function.

During the process of preparing HA in vitro through soluble calcium and orthophosphate salts, the initial formed precipitate is amorphous calcium phosphate (ACP), which further converts into HA in aqueous medium [5]. As a result, many recent researches focus on the possibility of using ACP as a candidate of bone and dental material [21–25]. The purpose of this paper is to investigate the calcium and orthophosphate ions release during the conversion of ACP to HA in aqueous solution. The ACP is immersed in distilled water for various time points up to 14 d. The release of calcium and orthophosphate ions is tested by calcium and phosphate colorimetric assay kits, respectively. The transition of precipitation process is detected by x-ray diffraction (XRD), transmission electron microscopy (TEM), and fourier transform infrared spectroscopy (FTIR).

2. Materials and methods

2.1. Preparation of ACP

ACP was synthesized by a wet chemical method [5]. Two kinds of solutions, $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ were dissolved individually in distilled water at Ca/P mole ratio of 1.67, and then calcium ion solution was gradually added into orthophosphate solution. Aqua ammonia was used to adjust pH to 7.4 during reaction process. The mixture was centrifuged immediately to get the precipitate and the distilled water was used to wash the precipitate to remove the dissolved ions in the reaction. The precipitate was collected for freeze-drying using SP Scientific VirTis Advantage XL-70 freeze dryers (USA) and then stored in drier for use.

2.2. The transformation of ACP

To investigate the conversion of ACP towards HA, the obtained ACP was immersed in distilled water for 0.25, 1, 4 and 14 d, and then lyophilized. The obtained HA was characterized by XRD, TEM and FTIR.

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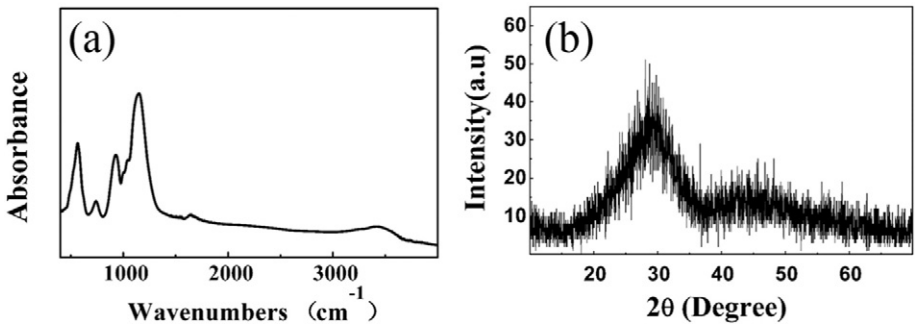

Fig. 1. FTIR spectrum (a) and XRD pattern (b) of the ACP sample. The FTIR shows a broad absorbance of orthophosphate and hydroxyl. The broad diffraction of XRD pattern is consistent with amorphous structure of ACP particles.

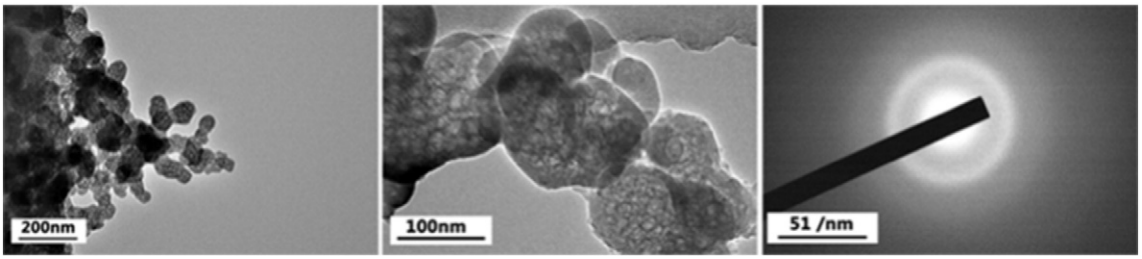

Fig. 2. TEM images of the ACP sample. The images show spherical precursor, which can be defined as ACP, since this unstable status of calcium orthophosphate has a typical diffraction pattern of amorphous halo ring.

2.3. Characterization

XRD patterns were obtained from a Rigaku D/Max X-ray diffractometer (Japan) using Cu Kα radiation at 40 kV, 40 mA and a scanning rate of 10°/min from 10° to 70°. TEM (JEOLJEM-2100, Japan) was used to evaluate surface morphology of crystal that changed with soaking time. The sample was dispersed in alcohol and ultrasonic for about 0.5 h for better dispersion and copper net films were used for the observation of surface morphology. Infrared spectra were recorded on a FTIR spectroscope with the wave length ranging from 400 to 4000 cm^{-1}.

2.4. Ion release

Ion release system was prepared by dispersing 0.1 g ACP powder with 10 mL distilled water and the temperature was maintained with constant temperature oscillator (37 °C, 160 rpm). For ions release determination, the solution was centrifuged in order to get the supernatant at specific time point and the precipitate was further soaked with distilled water until the next time point. The supernatant obtained from centrifugation was collected for calcium and orthophosphate ions concentration test by ion assay kits.

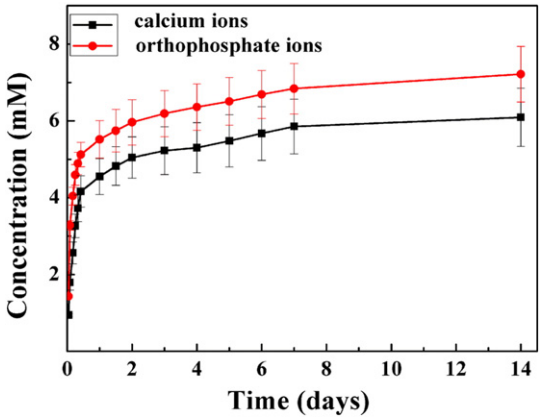

Fig. 3. Kinetic releases of calcium and orthophosphate ions.

The calcium colorimetric assay kit (obtained from Sigma-Aldrich) was used to evaluate the release of calcium ion. In this assay, the calcium ion concentration was determined by the chromogenic complex formed by calcium ion and *o*-cresolphthalein, which was measured at 575 nm and the value of OD_{575} was proportional to the concentration of calcium ion. A total of 90 μL chromogenic reagent and 60 μL calcium assay buffer were added to 96 well plates containing 50 μL supernatant, then mixed gently. The reaction system was incubated and protected from light at room temperature for 5–10 min before absorbance measurement.

The phosphate colorimetric assay kit (obtained from Sigma-Aldrich) was used to evaluate the release of orthophosphate ion. Orthophosphate reacted with a chromogenic complex and the produced colorimetric product was proportional to the amount of orthophosphate. A total of 30 μL phosphate reagent was added to 96 well plates containing 200 μL supernatant, then mixed gently. The reaction system was incubated and protected against light at room temperature for 30 min and the absorbance was measured at 650 nm.

2.5. Statistical analysis

Values are presented as mean ± standard deviation (SD) ($n \geq 3$) and the data were analyzed using origin 8.0 (Origin Lab Corporation, Northampton, MA, USA).

3. Results

3.1. Characterization of ACP

Fig. 1 shows the FTIR spectra and XRD pattern of ACP sample. The broad absorbance at 1040 (ν_3) and 580 cm^{-1} (ν_4) were associated