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In vitro study of calcium phosphate layers on hydroxyapatite ceramics surface mineralized in different solutions

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Abstract

Calcium phosphate layers, which are widely applied on the surface of biomaterials, are important for improving tissue regeneration performance. The composition of layers formed on the surface of biomaterials differs depending on the soaking fluid. Herein, revised simulated blood fluid and fast calcification solution were investigated as immersing solutions for the mineralization of hydroxyapatite ceramics. The morphology of the hydroxyapatite particles and precipitate formation on the hydroxyapatite ceramics surface were characterized by transmission electron microscopy. Furthermore, scanning electron microscopy was used to examine the morphology of the bioactive ceramics surface. The structure of the layers was determined by selected-area electron diffraction. The prepared hydroxyapatite powders were characterized by powder X-ray diffraction. The results showed that hydroxyapatite was preferentially formed on the hydroxyapatite ceramics surface in revised simulated blood fluid and octacalcium phosphate precipitated in fast calcification solution. Additionally, the content of HCO_3^- in the mineralization fluid strongly influenced the crystal phase composition and morphology of the calcium phosphate layers on the bio-ceramics materials studied herein.

Keywords: B. Surfaces; D. Apatite; E. Biomedical applications; Mineralization

1. Introduction

Hydroxyapatite (HA) and octacalcium phosphate (OCP) are widely employed as bones or dental substitutions in clinical orthopedic and dentistry because of their high bioactivity and biocompatibility. HA is the major inorganic component of human hard bone tissue, and is the most thermodynamically stable phase of calcium phosphate. OCP is mainly distributed in enamel and early bone [1].

In the growth and development of human bone, calcium phosphate ions and other inorganic ions are first deposited into the amorphous calcium phosphate (ACP), then gradually transformed into the metastable phase OCP, and finally converted into a thermodynamically stable phase HA. Currently, the shift mechanism is not clear, and pending inquiry [2–4].

The bone mineralization in vitro and in vivo is similar. The composition and structure of mineralization layers are different under different mineralization stages and environmental conditions. Revised simulated body fluid (r-SBF) is widely used as an immersion solution for biomaterials, because the concentration of the ions constituting SBF is consistent with that in the blood [5–7]. In comparison with SBF, the concentration of calcium phosphate ions of fast calcification solution (FCS) is more higher. Therefore, FCS is commonly employed as immersion solution for difficult mineralized medical materials [8]. To this date, understanding the crystal growth process and structure of layers mineralized in different solutions is good for us in understanding the mechanism of transition between different sedimentary facies and explaining the formation mechanism of bone.

Until now, three main conclusions were reached to illustrate the formation mechanism of layers mineralized in vitro solutions. Lu et al. believe that OCP forms in mineralization solutions rather than transforming into HA with the immersing time increasing [9–13]. However, this view did not take the effect of ions concentration on the composition and structure

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into consideration. Iijima et al. argue that OCP can form in calcium phosphate buffer with continuous addition of Ca, and can subsequently transform into HA when Ca addition is discontinued [14–16]. Even though the effect of calcium ions concentration on phase composition of calcium phosphate was studied systematically, the effect of PO_4^{3-}, HCO_3^- concentration was not considered. Regardless, Feng et al. believe that OCP crystals first grow on the surface, followed by HA preferential orientation on OCP. They believe that OCP is the intermediate phase, and OCP can transform into HA during mineralization process [17,18]. However, they did not pay attention to the condition for OCP transforming to HA.

The aim of this study is to investigate the phase structure and composition of crystals formed in FCS and SBF. The effect of ions concentration, solution saturation on the composition and structure of crystals mineralized in different solutions was comparatively studied. Classical thermodynamic and kinetic models of crystal heterogeneous nucleation and growth theories were additionally applied to determine differences in the formation behaviors. Moreover, transmission electron microscopy (TEM) and selected area electron diffraction (SAED) methods were employed to characterize the morphology and structure of layer growing on the surface.

2. Experimental

2.1. Samples preparation

Nano HA powders were prepared by a hydrothermal method, as previously described[19]. All reagents were of analytical grade with a purity of $\geq 99.0\%$.

The obtained powders were shaped using a mold with a diameter of 20 mm to prepare 20 mm × 2 mm bio-ceramic specimens using uniaxial compression. Then, all disks were placed into latex sets and pressed using a cold isostatic pressing method at 200 MPa for 2 min. Lastly, the compact disks were heated in a muffle furnace to 1200 °C, with a ramp of 10 °C min^{-1}, after which the temperature was maintained for 10 h. After sintering, the samples were cooled to room temperature at a ramp of 4 °C min^{-1} to produce the final HA ceramics.

2.2. Characterization of samples

The phase composition of the obtained nano HA powders was characterized by powder X-ray diffraction (XRD, D8 Advance, Bruker, Germany) with monochromatic Cu Kα radiation. Data were collected over a 2θ range of 20–60° with a step size of 0.02°.

TEM was used to assess the morphology of the synthesized powders and crystals growing on the surface of the bio-ceramics on a transmission electron microscope (JEM-2010 h, Japan) operating at 200 kV. For sample preparation, ultrasonic vibration was used to achieve good dispersion of the precipitates that were growing on the surface of the HA bio-ceramics. All bio-ceramic samples were introduced into 10-mL centrifuge tubes containing 8 mL ethanol, and sonicated for 15 min. The precipitates that separated from HA were collected on copper meshes containing a carbon film coating. Each sample was prepared with five sets of parallel samples, and 5 precipitates extracted from the HA ceramics were examined.

The morphology of the sintered ceramics surface was investigated using scanning electron microscopy (SEM; Navo NanoSEM 430, FEI).

2.3. HA ceramics mineralization in vitro

The immersion test was performed in two types of mineralization solutions, i.e, r-SBF and FCS. The concentration of the ions in the solutions is listed in Table 1. The pH of the r-SBF solution was adjusted to 7.4 at 37 ± 0.5 °C with HEPES ($C_8H_{18}N_2O_4S$) (AR, Aladdin, Shanghai) and 1.0 mol L^{-1} NaOH solutions, whereas the pH of the FCS solution was adjusted to 7.4 at 37 ± 0.5 °C with Tris ($C_4H_{11}NO_3$) (AR, Aladdin, Shanghai) and 1.0 mol L^{-1} HCL solutions.

To assess the morphology and structure of the crystals growing on the surface of the HA bio-ceramics in different mineralization solutions, two types of experiments were conducted. A group of HA bio-ceramics was vertically placed in 30-mL transparent plastic vials containing 15 mL FCS fluid, and incubated under static condition in a carbon dioxide incubator (INC-108, MEMMET) for 5 days at 37 °C. Concurrently, a second group of HA bio-ceramics were immersed in r-SBF and incubated under the same conditions for 5 days. Following incubation, the samples were cleaned with distilled water and dried at 50 °C for 12 h. The resulting surface morphology was examined using a field-emission scanning electron microscopy (Nova NanoSEM 430, FEI).

3. Results

3.1. Sample microstructure

The phase compositions of the HA powders synthesized by hydrothermal method and HA bio-ceramics sintered at 1200 °C for 10 h are shown in Fig. 1. As observed,

the non-sintered HA powers (Fig. 1a) featured diffraction peaks that were indexed to HA (PDF Card No. 9-432). No other (impurity) phases were noted. Thus, the XRD results indicated that the HA powders synthesized by this method featured good crystallinity and stability. In contrast, the sintered samples (Fig. 1b and c) displayed dominant HA diffraction peaks and secondary β-TCP diffraction peaks. The crystallinity of the sintered samples

Table 1
Ion concentration of human blood plasma, r-SBF and FCS.

Ion concentration (mM)	Na^+	K^+	Ca^{2+}	Mg^{2+}	HCO_3^-	Cl^-	HPO_4^{2-}	HPO_4^{2-}
Blood plasma	142.0	5.0	2.5	1.5	27.0	103.0	1.0	0.5
r-SBF	142.0	5.0	2.5	1.5	27.0	103.0	1.0	0.5
FCS	137.0	3.71	3.10	–	–	103.0	1.86	–

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