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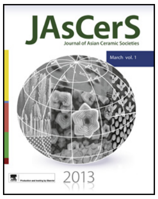

Biomimetic mineralization of calcium phosphates in polymeric hydrogels containing carboxyl groups

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ABSTRACT

Biomimetic processing is an attractive method for the fabrication of inorganic crystalline materials with designed morphology under ambient conditions. Precipitation of inorganic solids in a hydrogel matrix could be regarded as mimicking the process of biomineralization and be called gel-mediated processing. The importance of the functional groups in reaction media and templates has been pointed out for such gel-mediated processing. In the present study, the role of carboxyl groups in hydrogel matrices on precipitation of calcium phosphate was investigated. Carboxyl groups in the hydrogel matrices provided pH buffering action, chelate formation with calcium ions and decreased the diffusion rate of calcium ions. The crystalline phase of the calcium phosphate changed from octacalcium phosphate to hydroxyapatite with increasing carboxyl group concentration in the hydrogels. The crystalline phase change is attributed to a decrease in the activation energy for calcium phosphate formation via a chelate structure involving calcium ions and carboxyl groups.

1. Introduction

Ceramic biomaterials are now widely used as bone substitutes in orthopedic applications. Sintered hydroxyapatite (HAp; $Ca_{10}(PO_4)_6(OH)_2$) is a typical example of bioactive ceramics, providing direct bonding to living bone. Because of its biological properties, synthesis of HAp and its related calcium phosphate compounds has become attractive. We have focused on synthetic routes of calcium phosphate compounds for the fabrication of designed microstructures, including compositions, phases, and the morphology of inorganic compounds. Development of biomimetic processing is an active research area for the fabrication of designed calcium phosphate crystals to produce well-designed materials through environmentally friendly processing. Organisms produce materials and structures with particular mechanical, optical and structural properties under standard temperature and pressure conditions [1]. We aim to develop novel materials and synthetic procedures using the materials synthesis mechanisms found in nature.

Calcium phosphate, especially HAp, can be formed on substrates by using a simulated body fluid (SBF) and its modified solutions [2–4]. SBF has been used to estimate the potential for the osteoconduction of materials [5]. The SBF is supersaturated with respect to HAp and is a metastable solution. Specific functional groups, for example COOH [6,7], SiOH [8] and TiOH [9], on material surfaces induce heterogeneous nucleation of calcium phosphate in the SBF and its modified solutions. These functional groups are negatively charged in the SBF and attract calcium ions. A type of chelate structure, composed of calcium ions and functional groups, induces heterogeneous nucleation. Calcium phosphate precipitates spontaneously in SBF, thus SBF can be used for biomimetic HAp coating on substrates. A technique mimicking biomineralization is generally conducted under conditions with a low degree of supersaturation, and hence the application of functional groups to control nucleation on material surfaces is useful for biomimetic HAp coating.

For further development of biomimetic fabrication, controlled precipitation of calcium phosphates in 3-dimensional network structures is expected to be important. Crystallization of calcium phosphates in a hydrogel matrix is regarded as a process that mimics biomimeralization. Formation of poorly soluble compounds in hydrogel media is regarded as one crystal growth method.

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Controlled formation of precipitates in hydrogels is called as gel-mediated processing, which is inspired from biomineralization. Calcium phosphate [10–17] and calcium carbonate [18–20] were reported as typical precipitates synthesized through gel-mediated processing. Formation of inorganic compounds by a mechanism that is similar to gel-mediated processing can be observed in nature. Biomineralization, such as the calcification in bones and shells, takes place in hydrogel-like environments. From the viewpoint of physical chemistry, calcification of bones can be regarded as the formation of HAp crystals in a collagen hydrogel. Hence, gel-mediated processing is categorized as a biomimetic process.

In biomimetic mineralization, the importance of functional groups in the hydrogel for calcium phosphate mineralization has been pointed out. Crystalline phases are affected by the degree of supersaturation in the calcium phosphate system [21]. The crystalline phases of calcium phosphate could be changed, depending on the functional groups present in the hydrogel matrix, because functional groups might change the degree of supersaturation required for nucleation. Crystalline phase control is an important issue in materials fabrication. To cite a specific case, synthesis of calcium phosphate-polymer composites through gel-mediated processing was studied to fabricate novel bone-repair materials [10,12]. Control of the crystalline phases of calcium phosphate is important in these materials, because they influence the biological and physicochemical properties of the composites. Understanding the roles of functional groups in a polymeric hydrogel matrix for calcium phosphate formation results in the fabrication of well-designed calcium phosphate-polymer composites. However the precise roles of the functional groups remain unclear. In this paper, the roles of functional groups, in particular COOH groups, on calcium phosphates formed in polymeric hydrogels were investigated. We focused on the COOH group, because this was expected to affect calcium phosphate formation, based on the results of SBF studies [22].

2. Experimental procedures

2.1. Chemicals

Acrylamide (Am, $CH_2CHCONH_2$, 98%) was purchased from Sigma–Aldrich Japan (Tokyo, Japan). Acrylic acid (Ac, $CH_2CHCOOH$, 98%), *N,N′*-methylenebisacrylamide (MBAAM, 99%), *N,N,N′,N′*-tetramethylethylenediamine (TEMED, 98%), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$, 99%), ammonium peroxodisulfate ($(NH_4)_2S_2O_8$, 99%) and calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, 98.5%) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Tris(hydroxymethyl)aminomethane (Tris, 99%) was purchased from Nacalai Tesque Inc. (Kyoto, Japan).

2.2. Preparation of polymeric hydrogels containing phosphate ions

Copolymer hydrogels composed of various mole fractions of Am and Ac were used as crystal growth matrices. Appropriate amounts of Am, Ac, MBAAM, TEMED and $(NH_4)_2HPO_4$ were dissolved in ultra-pure water. The pH values of the monomer solutions were adjusted to 7.9 by the addition of aqueous ammonia or nitric acid. The volume of the solution was adjusted in a volumetric flask. The final concentrations of chemicals in the solution were as follows: the sum of the concentrations of Am and Ac, 1.5 mol dm^{-3} (M); $(NH_4)_2HPO_4$, 0.20 M; MBAAM, 1.5×10^{-2} M; and TEMED, 3.0×10^{-2} M. The molar ratios of Am:Ac were 100:0, 75:25, 50:50, 25:75 and 0:100. A 20 cm^3 aliquot of the monomer solution was transferred to a glass vessel (capacity 50 cm^3, 5 cm in diameter). 9.0×10^{-5} mol of $(NH_4)_2S_2O_8$ was dissolved in the aliquot solution under vigorous stirring. After dissolution of $(NH_4)_2S_2O_8$, the glass vessel was covered with a poly(vinylidene chloride) film and a polyethylene lid to prevent water evaporation. Gelation was then allowed to take place at 60 °C for 24 h.

The initial pH values of the prepared gels were measured with a glass electrode-type pH meter (InLab® Solid; Mettler Toledo, Switzerland). The chemical structures of the prepared gels were characterized using a Fourier-transform infrared (FTIR) spectrometer (FT/IR-610; JASCO Co., Tokyo, Japan) using the KBr pellet method. The mass ratio of sample:KBr was 1:50. Each sample was scanned between 2000 and 1000 cm^{-1} at a resolution of 4 cm^{-1}. The gels were sliced and soaked in ultra-pure water for 1 h to remove chemicals contained within the hydrogels. This soaking operation was repeated three times. The rinsed gels were dried and the gels were then used for FTIR characterization.

2.3. Mineralization of calcium phosphate

Appropriate quantities of $Ca(NO_3)_2{\cdot}4H_2O$ and Tris were dissolved in ultra-pure water. The pH of the mixed solutions was adjusted to 7.0 by the addition of 1.0 M HCl solution. The volume of the solution was adjusted using a volumetric flask. The final concentrations of $Ca(NO_3)_2$ and Tris were fixed at 0.50 and 0.10 M, respectively. A 20 cm^3 volume of the $Ca(NO_3)_2$ solution was poured onto a synthesized polymeric hydrogel in a glass vessel. The vessel was covered with a poly(vinylidene chloride) film and a polyethylene lid, then maintained at 40 °C. After 1, 3, 5 or 10 days, the supernatant $Ca(NO_3)_2$ solutions on the polymeric hydrogels were removed and the gels were extracted from the glass vessels. The gels were cut in a vertical direction and the cross-section of each gel was then observed by the naked eye. The pH values of the removed solutions were measured with a glass electrode-type pH meter (D-51; Horiba Ltd., Japan). The concentrations of calcium and phosphate ions in the solutions were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Optima 2000DV, PerkinElmer Japan Co., Ltd., Japan).

The gels with precipitates were sliced and soaked in ultra-pure water for 1 h. This soaking operation was repeated three times. After the soaking operations, the sliced gels with precipitates were dried at 40 °C for 24 h. The dried samples were coated with thin gold films and then examined using scanning electron microscopy (SEM, JSM5600; JEOL Ltd., Japan). In addition, the dried samples were ground, and then the powdered samples were placed on glass sample holders for powder X-ray diffraction (XRD). The crystalline phases of the precipitates were identified by powder XRD (RINT 2100V; Rigaku Co., Tokyo, Japan) in the range $3^\circ \leq 2\theta \leq 40^\circ$ with a scan rate 1.0° min^{-1}, using CuKα radiation.

2.4. Measurement of the calcium ion adsorption capacity of the polymeric hydrogels

The polymeric hydrogels, synthesized from 1.0 cm^3 of monomer solution, without phosphate ions, were soaked in a 15 cm^3 volume of a 0.10 M $Ca(NO_3)_2$ solution. The pH values of the monomer solutions were adjusted to 7.0 using appropriate amounts of ammonia solution or nitric acid. The pH value of the $Ca(NO_3)_2$ solution was adjusted to 7.0 by the addition of Tris and HCl solution. The concentration of Tris in the $Ca(NO_3)_2$ solution was 1.0 mM. After 5 days, calcium ion concentrations in the solutions were measured by ICP-AES. The amounts of calcium ions adsorbed by the polymeric hydrogels were calculated from the decrease in the calcium ion