

Fig. 5 FURUZONO *et al.*

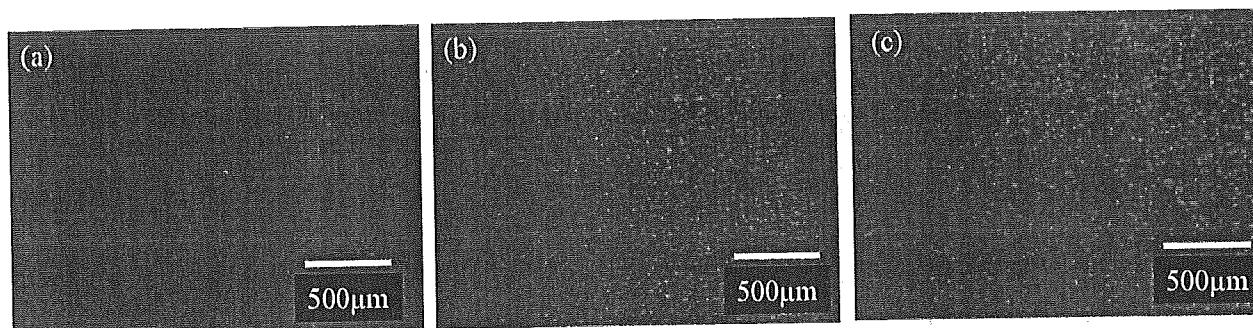


Fig. 6 FURUZONO *et al.*

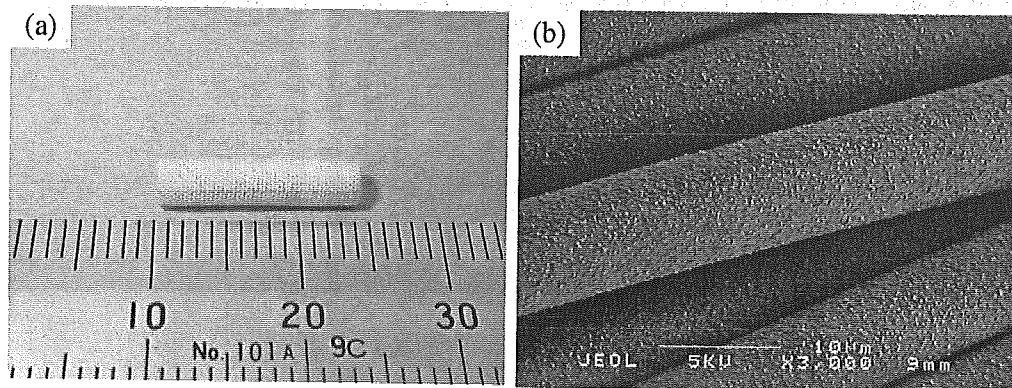


Fig. 7 Furuzono *et al.*

**Nano-sized ceramic particles of hydroxyapatite
calcined with an anti-sintering agent**

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Abstract

Nano-sized crystals of calcined hydroxyapatite (HAp) having spherical morphologies were fabricated by calcination at 800°C for 1 h with an anti-sintering agent surrounding the original HAp particles and the agent was subsequently removed by washing after calcination. The original HAp particles were prepared by a modified emulsion system, and surrounded with poly(acrylic acid, calcium salt) (PAA-Ca) by utilizing a precipitation reaction between calcium hydroxide and poly(acrylic acid) adsorbed on the HAp particle surfaces in an aqueous medium. In the case of calcination without PAA-Ca, micron-sized particles consisting of sintered polycrystals were mainly observed by scanning electron microscopy, indicating the calcination-induced sintering among the crystals. On the other hand, most of the crystals calcined with the anti-sintering agent were observed as isolated particles, and the mean size of the HAp crystals was around 80 nm. This result indicates that PAA-Ca and its thermally decomposed product, CaO, surrounding the HAp crystals could protect them against calcination-induced sintering during calcination at 800°C. The HAp crystals calcined with PAA-Ca showed high crystallinity, and no other calcium phosphate phases could be detected.

Introduction

Hydroxyapatite (HAp, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) ceramic is an important biomaterial and has been widely used by taking into account its biocompatibility, because its constituent is chemically similar to those of the mineral of bones and teeth. HAp also has been extensively used in a variety of applications, such as adsorbents, carrier for drug delivery system, ion exchangers, and catalysts, by exploiting its adsorbability of many compounds and biocompatibility. However, owing to its mechanical weakness and brittleness [1-3], applications of HAp have been confined to those with low mechanical stress. We recently developed a novel inorganic/organic composite [4, 5]: a flexible polymer substrate, whose surface was modified with calcined HAp nanoparticles through covalent bonding. The novel composite retained the flexibility of the polymer substrate and showed surface properties of HAp such as tissue adhesion in a living body.

Throughout these studies, the HAp particles were prepared by a modified emulsion system [6, 7] and used after calcination at 800°C to reduce sorbability *in vivo*. When low-crystallinity HAp nanoparticles are calcined to increase thermal and chemical stability, the particles sinter randomly into large agglomerates consisting of polycrystals [8-12]. Therefore, calcined HAp crystals on a nanoscale have been difficult to obtain. Because the sintered polycrystals has poor dispersibility in liquid media and a large size distribution, it was difficult to control the surface morphology (covering ratio, surface roughness) of the above-mentioned composite. Hydrothermal treatment of HAp particles in an aqueous medium under high pressure is known to enable the preparation

of agglomerate-free HAp crystals [13-15]. However, this treatment generally leads to an increase in crystal size due to Ostwald ripening [16, 17], and is restricted to laboratory-scale products as it is a high-pressure process.

In this article, nano-sized and calcined HAp crystals protected against calcination-induced sintering will be fabricated by using an anti-sintering agent surrounding the HAp particles. Thus, there was no contact between the particles during calcination. Calcium hydroxide [$\text{Ca}(\text{OH})_2$] was selected as a component of the anti-sintering agent because it would not melt at the calcination temperature (800°C), presumably not dissolve the HAp, and could be removed by washing with water after calcination. In order to surrounding the HAp particles efficiently with the anti-sintering agent, a precipitation reaction between $\text{Ca}(\text{OH})_2$ and poly(acrylic acid) (PAA) adsorbed on the HAp particles in an aqueous medium was utilized. The obtained crystals were evaluated by means of X-ray diffraction (XRD) measurement, Fourier-transform infrared (FT-IR) spectroscopy, and scanning electron microscope (SEM) observation.

Experimental

Materials

$\text{Ca}(\text{OH})_2$ was prepared by the hydrolysis of calcium oxide, which was obtained by calcination of alkaline-analysis-grade calcium carbonate (CaCO_3 ; Wako Pure Chemical Industries, Ltd., Osaka, Japan) at 1050°C for 3 h. PAA (weight-average molecular weight: 15,000) used as a polymeric stabilizer was purchased from Sigma-Aldrich Co., MO, USA. Other materials were reagent grade and purchased from Nacalai Tesque Inc.,

Kyoto, Japan. Milli-Q water with a specific resistance of $18.2 \times 10^6 \Omega \cdot \text{cm}$ was used (Millipore Corp., MA, USA).

Calcination of HAp particles

Starting HAp particles with low crystallinity were prepared by a modified emulsion system at 25°C using Ca(OH)_2 and potassium dihydrogen phosphate, according to the previous articles [6, 7]. The particles prepared was centrifugally washed with water and redispersed in water (solid content: 5 wt%). The calcination procedure of HAp particles with the anti-sintering agent is sketched in Fig. 1. An amount of PAA, approximately equal in weight to the HAp particles, was dissolved in water at 1.0 wt%, and the pH of the PAA *aq.* was adjusted to 10.0. The PAA *aq.* was added in the HAp dispersion, and the dispersion was ultrasonicated for 5 min. An excess amount of calcium ions was added to the dispersion in the form of a saturated Ca(OH)_2 *aq.* ($\text{Ca(OH)}_2/\text{COOH}$ in PAA = 1/1 molar ratio) to precipitate poly(acrylic acid, calcium salt) (PAA-Ca) onto the HAp particles. The resultant HAp/PAA-Ca mixture was filtered, dried at 80°C for 2 h under reduced pressure, and calcined at 800°C for 1 h at a heating rate of 10°C/min. Calcination was carried out in a horizontal furnace with an alumina tube in air. In order to remove the thermal decomposed product of PAA-Ca, CaO, after calcination, the mixture was centrifugally washed with 100 mM NH_4NO_3 *aq.* under N_2 to reduce the formation of CaCO_3 by the reaction between calcium ion and carbon dioxide in air, until the pH of the aqueous dispersion decreased to almost 7.0, and then washed further with water three times. To investigate the effect of the anti-sintering agent, the HAp particles

were calcined with the same procedure, but without adding PAA and $\text{Ca}(\text{OH})_2$.

Measurements

Identification of the product was conducted by XRD measurement (RAD-X; Rigaku International Co., Tokyo, Japan) with CuK_α radiation, and diffuse reflectance FT-IR spectroscopy (Spectrum One; Perkin-Elmer Inc., MA. USA). The size and morphology of the HAp crystals were observed by SEM (JSM-6301F; JEOL Ltd., Tokyo, Japan). The Ca/P atomic ratio of each HAp was measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES: SPS4000; Seiko Instrument Inc., Chiba, Japan). Calcium and phosphorus standard solutions for ICP-AES were purchased from Kanto Chemical Co., Inc., Tokyo, Japan.

Results and discussion

In order to surround the particles with the anti-sintering agent, PAA was adsorbed on the surfaces of the HAp particles in an aqueous medium. PAA can adsorb on HAp surfaces [18-19], and thus act as a polymeric dispersant to prevent flocculation of HAp particles in an aqueous medium. An addition of calcium ions into an aqueous PAA solution under alkaline condition induces precipitation of PAA-Ca. Accordingly, when $\text{Ca}(\text{OH})_2$ aq. is added to the PAA-stabilized HAp dispersion, PAA-Ca would precipitate onto the surfaces of the HAp particles. The PAA-Ca presumably acts as an anti-sintering agent, because there is no contact among the HAp particles by surrounding them with PAA-Ca and its thermally decomposed product, CaO, during

calcination.

First, the influence of PAA-Ca on the crystal phase and composition of HAp were investigated. Fig. 2 (a) shows the XRD profile of the HAp particles after calcination with PAA-Ca. From the profile, highly crystallized HAp was detected, and no other calcium phosphate phases such as tricalcium phosphate could be detected. The Ca/P ratio of the HAp calcined with PAA-Ca was 1.58 (1.56 in the case of the HAp calcined without additives), indicating the formation of calcium-deficient HAp with high crystallinity after calcination with PAA-Ca.

In the FT-IR spectrum shown in Fig. 2 (b), the absorption bands at 603/572 and 474 cm^{-1} are respectively attributed to $\nu_4\text{PO}_4^{3-}$ and $\nu_2\text{PO}_4^{3-}$ in crystalline HAp. Absorptions at 1092/1045 and 963 cm^{-1} are respectively attributed to $\nu_3\text{PO}_4^{3-}$ and $\nu_1\text{PO}_4^{3-}$. The sharp absorptions of OH stretching and deformation vibrations at 3573 and 632 cm^{-1} indicate that the material exhibits high crystallinity after calcination. Bands at 1456/1413 and 877 cm^{-1} , attributed to CO_3^{2-} substituting phosphate positions in the HAp lattice [20]. The carbonate ion should be from atmospheric carbon dioxide during the preparation of the original HAp particles and from carbon dioxide generated by thermal decomposition of PAA-Ca during calcination. It should be noted that peak at 3644 cm^{-1} due to the stretching of OH in $\text{Ca}(\text{OH})_2$ and the peaks at 1453/1415 cm^{-1} corresponding to the CH_2 and CH bending mode of the PAA chain were not found, suggesting complete removal of the anti-sintering agent by the thermal decomposition and the centrifugal washing with water. A small peak at 3544 cm^{-1} , which was not observed in the case of calcination without additives, was additionally observed after calcination with PAA-Ca.

The origin of the additional peak is under investigation, and will be reported in the near future.

Fig. 3 shows the morphologies of the HAp crystals calcined with or without PAA-Ca. Crystals with spherical or irregular morphologies were observed, irrespective of the presence or absence of PAA-Ca as shown in Fig. 3 (b) and (d). In the case of calcination without additives shown in Fig. 3 (a) with lower magnification, micron-sized particles consisting of sintered polycrystals were mainly observed, indicating the calcination-induced sintering among the crystals. On the other hand, in the case of the HAp crystals calcined with the anti-sintering agent shown in Fig. 3 (c), most of the crystals were observed as isolated particles, and the mean size of the particles was around 80 nm. This indicates that sintering among the HAp nanocrystals could be mostly prevented by surrounding them with PAA-Ca prior to calcination.

In summary, calcined HAp nanocrystals were successfully fabricated by calcination with an anti-sintering agent surrounding the particles, followed by removal of the agent. The HAp nanocrystals fabricated here should be suitable for the various applications such as biomaterials, ion exchangers, adsorbents, and catalysts owing to their high dispersibility in liquid media and high thermal and chemical stability. The calcination with an anti-sintering agent should be applicable to other nanoceramic powders, such as alumina, titania, and magnesia, and have significant benefits over existing technologies, because the method is facile, inexpensive, and amenable to scale-up and processing.

Acknowledgments

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Figure captions

Fig. 1 A schematic model for the fabrication of nano-sized crystals of hydroxyapatite by calcination with an anti-sintering agent surrounding the crystals and subsequent removal of the agent

Fig. 2 X-ray diffraction pattern (a) and FT-IR spectrum (b) of HAp crystals calcined at 800°C for 1 h with PAA-Ca surrounding the particles. The thermal decomposed product of PAA-Ca, CaO, was centrifugally washed with an aqueous solution after calcination.

Fig. 3 Lower magnified (a, c) and higher magnified (b, d) SEM photographs of HAp crystals calcined without additives (a, b) and with PAA-Ca (c, d)

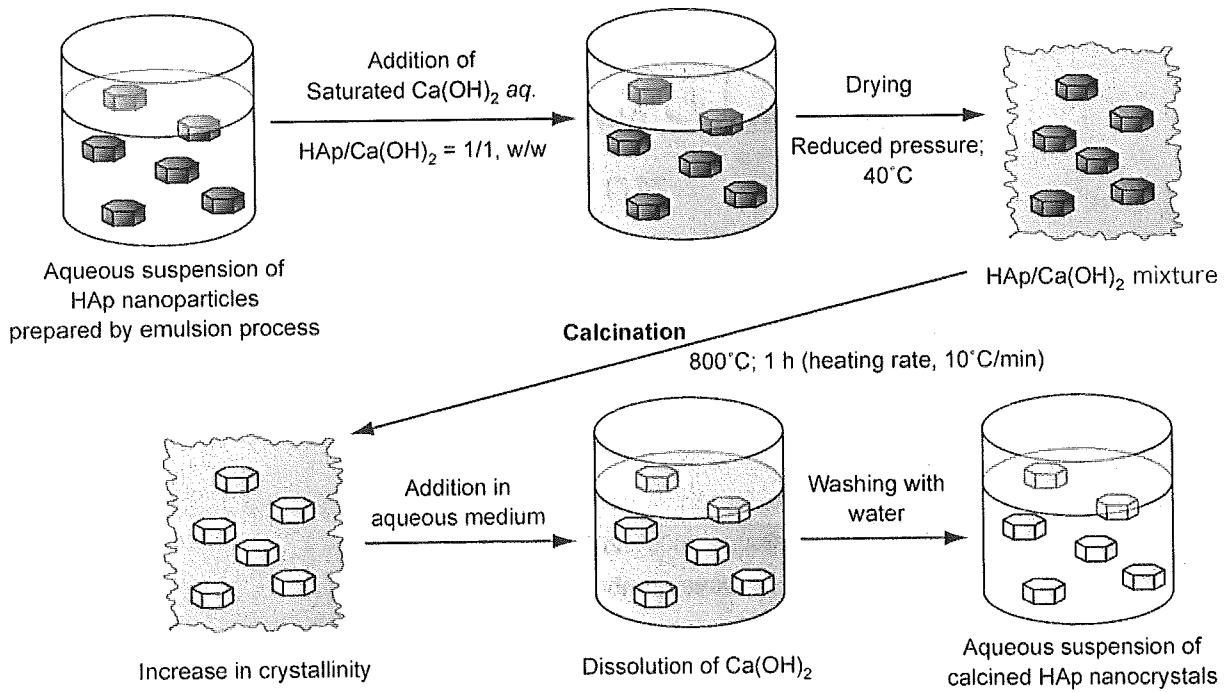


Fig. 1 Okada *et al.*

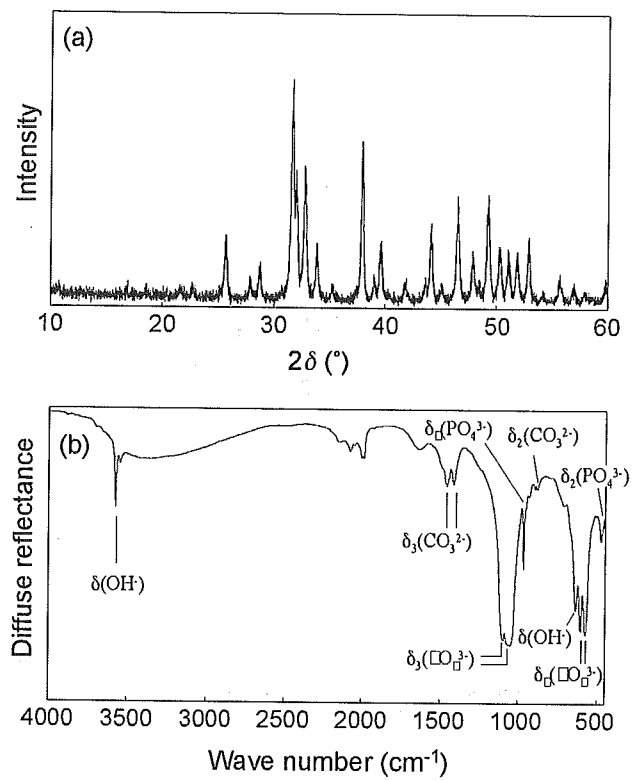


Fig. 2 Okada *et al.*

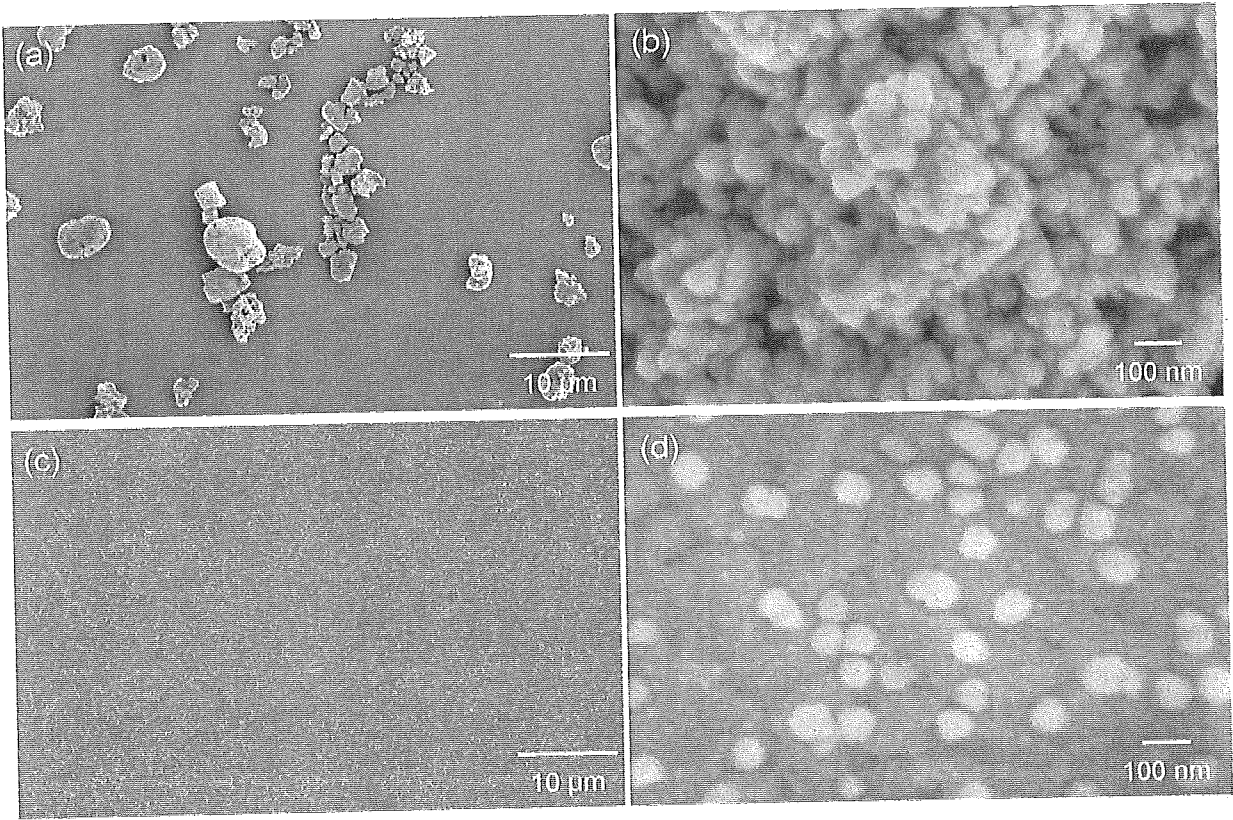


Fig. 3 Okada *et al.*

Fabrication of high-dispersibility nanocrystals of calcined hydroxyapatite

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Hydroxyapatite (HAp) is a major inorganic component of bone and teeth. Artificially synthesized HAp has been extensively used in a variety of applications, such as biomaterials, ion exchangers, adsorbents, and catalysts, by exploiting its biocompatibility and adsorbability of many compounds. When low-crystallinity HAp nanoparticles are calcined to increase thermal and chemical stability, the particles typically sinter into large agglomerates [1-5]. Thus, calcined HAp crystals dispersed in liquid medium on a nanoscale have been difficult to obtain. This paper describes the preparation of HAp nanocrystals by calcination with an anti-sintering agent interspersed between the particles and the subsequent removal of the agent. The HAp nanocrystals obtained here should be suitable for the above applications owing to their high dispersibility in liquid media, high specific surface area, and high thermal and chemical stability.

We have recently developed a novel inorganic/organic nanocomposite for a soft-tissue-compatible material: a flexible silicone elastomer [6] or a silk fibroin [7], whose surface was modified with calcined HAp crystals through covalent bonding. The novel composite retained the flexibility of the polymer substrate and showed good tissue adhesion due to the HAp crystals on the surface [8]. Throughout these studies, the HAp crystals were used after calcination at 800°C to reduce *in vivo* sorbability. As mentioned above, the HAp nanoparticles mostly sintered into large agglomerates of polycrystals during calcination. This made it difficult to control the surface coverage of the composite by HAp crystals and the roughness of the composite surface.

Hydrothermal treatment of HAp particles in water medium under high pressure is known to enable the preparation of agglomerate-free HAp crystals [9-11]. However, this treatment generally leads to an increase in crystal size due to Ostwald ripening [12, 13],

and is restricted to laboratory-scale products as it is a high-pressure process.

The present study reports the preparation of nano-sized, calcinated HAp single crystals protected against calcination-induced sintering using an anti-sintering agent interspersed between the HAp particles. Thus, there was no contact between the particles during calcination. Calcium hydroxide [Ca(OH)₂] was selected as an anti-sintering agent because it would not melt at the calcination temperature (800°C), presumably not dissolve the HAp, and could be removed by washing with water after calcination.

Starting HAp particles with low crystallinity were prepared by a modified emulsion system at 25°C [14]. The resulting product was centrifugally washed and redispersed in water (solid content: 5 wt%). In order to intersperse Ca(OH)₂ between the particles, the HAp aqueous dispersion was added into a saturated aqueous Ca(OH)₂ solution (0.17 wt%), and the mixture was dried under reduced pressure at 40°C. The resultant HAp/Ca(OH)₂ (1/1, w/w) mixture was calcined at 800°C for 1 h in air (heating rate: 10°C/min). After calcination, the mixture was centrifugally washed with water to remove the Ca(OH)₂. As a control, some HAp was calcined using the same procedure, but without adding Ca(OH)₂. The HAp crystals were observed by scanning electron microscopy (SEM; JSM-6301F, JEOL Ltd., Tokyo, Japan) and transmission electron microscopy (TEM; JEM-2000 EXII, JEOL Ltd.). Size distribution of the HAp crystals dispersed in ethanol was measured at 10-ppm concentration by dynamic light scattering (DLS) (ELS-8000, Otsuka Electronics Co., Ltd., Kyoto, Japan) at a light-scattering angle of 90°.

In order to examine the effect of Ca(OH)₂ on the crystal phase and composition of HAp, X-ray diffraction (XRD; RAD-X, Rigaku International Co., Tokyo, Japan) with