Fabrication of porous hydroxyapatite having nanometer/micrometer-size pores

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Hydroxyapatite having nanometer/micrometer-size pores was formed from the olive oil/amorphous calcium phosphate (ACP)-poly(vinyl alcohol) (PVA) gel emulsion. An ACP-PVA gel was prepared by a freeze-thaw process to form an interconnected nanoporous structure. An emulsion templating method was used to form micrometer-size pores. Nanometer/micrometer-size pores were formed from the olive oil/ACP-PVA gel emulsion. The blending of oils (olive oil/squalane) resulted in the formation of bimodal nanometer/micrometer-size pores.

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Porous ceramics with bimodal pore structure are expected to be utilized in a wide range of applications.¹,² We successfully fabricated an interconnected nanoporous hydroxyapatite [HAp; Ca₁₀(PO₄)₆(OH)₂].³ Amorphous calcium phosphate (ACP) nanoparticles, which are the precursor of HAp, were assembled by the addition of poly(vinyl alcohol) (PVA) aqueous solution and a subsequent freeze-thaw (F-T) process. The obtained HAp had an interconnected nanoporous structure having an average pore diameter of approximately 100 nm with a narrow distribution and a high open porosity (approximately 65%).³ We have developed our approach³ to fabricate porous HAp having nanometer/micrometer-size pores. We focused on an emulsion templating method to form micrometer-size pores.⁴ An emulsion consisting of ACP-PVA gel prepared by the F-T process and oil (an oil-in-ACP-PVA gel emulsion) is prepared, in which the state is similar to that of an emulgel.⁵ The ACP-PVA gel network structure stabilizes the oil droplets in the emulsion. Furthermore, PVA with both a hydrophobic hydrocarbon main chain and hydrophilic hydroxyl groups acts as a co-stabilizer for the oil in the water-emulsion system.⁶ Dispersed oil droplets in the ACP-PVA gel act as a micrometer-size pore template since the oil droplets in the emulsion are thermally decomposed during the calcination process. The ACP-PVA gel network matrix is converted into a HAp skeleton with interconnected nanopenes. The formation of nanometer-size pores by the F-T process (the formation of the ACP-PVA gel)³ and micrometer-size pores by the emulsion templating process are independently controlled, resulting in the formation of bimodal nanometer/micrometer-size pores in HAp bulk. In this study, we fabricated porous HAp having nanometer/micrometer-size pores. The effects of the type of oil and the ACP-PVA gel/oil ratio on the morphology of the fabricated porous HAp were investigated.

Calcium nitrate tetrahydrate [Ca(NO₃)₂·4H₂O, 99.9%, Kanto Chemical Co., Inc., Japan], diammonium hydrogen phosphate [(NH₄)₂HPO₄, 99.0%, Wako Pure Chemical Industries, Ltd., Japan], 10% ammonia solution (Wako), and acetone (99.5%, Wako) were used as received. PVA powder was supplied by Kuraray Co., Ltd., Japan, for which the degrees of polymerization and hydrolysis were 1700 and 98.6 mol %, respectively. Olive oil (Kanto Chemical) and squalane (2, 6, 10, 15, 19, 23-hexamethyltetrasosan), >98.0%, Kanto Chemical) were used as oils. Deionized water was used in all experiments. The preparation of an ACP-PVA gel was performed in accordance with our previous report.³ The blend solution of the Ca(NO₃)₂ aqueous solution and the (NH₄)₂HPO₄ aqueous solution was stirred at 30°C for 24 h to prepare ACP. The ACP-PVA gel was prepared by the F-T process. The ACP, 1 wt % PVA aqueous solution [PVA/HAp (theoretical yield) = 5 wt %], and acetone (acetone/PVA aqueous solution = 100 wt %) were mixed by stirring at room temperature (RT) for 1 h, which was followed by freezing at −18°C for 24 h and thawing at 4°C for 24 h. The obtained
ACP-PVA gel and oil (oil/PVA aqueous solution = 0–50 wt%) were mixed by ultrasonic irradiation using a Hielscher UP100H ultrasonic processor (100 W, 30 kHz) in an ice water bath for 1 h to obtain an emulsion gel. The obtained emulsion gel was cast into an aluminum die (φ13.5 mm) and dried at RT for one week to form a cylindrical block. The obtained cylindrical block was placed in an alumina boat and calcined at 800°C for 1 h in air at a heating rate of 10 °C/min. Scanning electron microscopy (SEM) observations of the morphologies of the products were conducted with a Hitachi S-4100 field-emission scanning electron microscope operated at 15.0 kV. The samples were coated with Pt-Pd before the observations. The pore size distribution, porosity, and specific surface area of the products were measured by mercury intrusion porosimetry (Quantachrome PoreMaster-60 GT).

Homogeneous and stable emulsion gel (the oil-in-ACP-PVA gel emulsion) was prepared using olive oil. Furthermore, homogeneous porous structure consisting of micrometer-size pores derived from the template oil droplets in the emulsion gel was observed for the product prepared from the olive oil/ACP-PVA gel emulsion. Thus, olive oil was selected as the template oil in this study. The effect of the ACP-PVA gel/oil ratio on the morphology of the HAp was clarified. The preparation conditions of the emulsion gel are summarized in Table 1. Figure 1 shows SEM images of the products (samples S1–S4) prepared with different amounts of olive oil added (0–50 wt%). X-ray diffraction measurements revealed that the products were single-phase HAp regardless of the preparation conditions (not shown). A uniform nanoporous structure in the HAp skeleton was observed for all products regardless of the addition of olive oil. Moreover, micrometer-size structure was observed for the products prepared with olive oil added (samples S2–S4). These findings indicate that this approach, the combination of the F-T process and emulsion templating process, achieves the formation of HAp having nanometer/micrometer-size pores. The micrometer-size pores became clear with increasing amount of added

| Table 1. Preparation conditions of emulsion gel |
| Sample code | Oil/PVA aqueous solution (wt%) | Composition of oil |
| Sample code | Olive oil (wt%) | Squalane (wt%) |
| S1 | 0 | — | — |
| S2 | 10 | 100 | 0 |
| S3 | 30 | 100 | 0 |
| S4 | 50 | 100 | 0 |
| S5 | 30 | 50 | 50 |
| S6 | 30 | 22 | 78 |

Fig. 1. SEM images of samples (a–c) S1, (d–f) S2, (g–i) S3, and (j–l) S4.
olive oil owing to the increase in the template oil droplet size. However, the micrometer-size pores collapsed when excess olive oil was added (50 wt%, sample S4) owing to the formation of a continuous oil phase. Clear micrometer-size pores were formed from the emulsion gel including 30 wt% olive oil [sample S3, Figs. 1(g)–1(i)]. These results demonstrate that the combination of the F-T process and emulsion templating process is favorable for the formation of HAp having nanometer/micrometer-size pores.

To improve the homogeneity of the micrometer-size pores, we considered the oil/water interfacial tension. The reduction of the oil/water interfacial tension is closely related to the formation and stability of an emulsion. Kudo et al.\(^7\) reported that the combination of nonpolar squalane and polar oleic acid reduces the interfacial tension. On the basis of this report, the effect of the blending of squalane in olive oil on the micrometer-size pores was investigated. Uniform oil droplets with little flocculation and coalescence were observed for the emulsion gel prepared with squalane added (not shown), indicating that the blending of squalane in olive oil forms the stable emulsion with the ACP-PVA gel. Figure 2 shows SEM images of the products (samples S5 and S6) prepared with different amounts of squalane blended in the olive oil. The micrometer-size pores became uniform with the blending of squalane in the olive oil compared with that obtained without the blending [sample S3, Figs. 1(g)–1(i)], reflecting the formation of uniform oil droplets in the emulsion gel. This indicates that the combination of olive oil and squalane is suitable for the formation of HAp having uniform nanometer/micrometer-size pores from an ACP-PVA gel. The pore size distributions of samples S1 (without oil), S3 (with olive oil), and S6 (with olive oil/squalane) are shown in Fig. 3. Nanopores having a diameter of approximately 80 nm with a narrow distribution were observed for all products regardless of the preparation conditions. Samples S3 and S6 (prepared with oil added) had a bimodal pore structure with nanometer-size pores (approximately 80 nm) and micrometer-size pores (~3 μm). The bimodality of the pore size distribution became more noticeable for sample S6 prepared with the blending of squalane in olive oil. The open porosities of samples S1, S3, and S6 were 57, 86, and 87%, respectively, and the specific surface areas were 24, 46, and 43 m\(^2\)/g, respectively. An approach to produce a bimodal porous structure by the sintering with a pore-forming agent is difficult to form nanopores with a narrow distribution and to achieve high open porosity.\(^1\),\(^8\) The interconnected nanoporous structure in skeleton induces a high open porosity and a high specific surface area. These results demonstrate that porous HAp having an interconnected nanoporous skeleton and micrometer-size pores with a high open porosity and a high specific surface area can be fabricated by the combination of a F-T process and an emulsion templating process.

In conclusion, we fabricated HAp having nanometer/micrometer-size pores by the combination of a F-T process and an emulsion templating process. Clear nanometer/micrometer-size pores were formed from the emulsion gel including 30 wt% olive oil. Furthermore, the blending of squalane in olive oil resulted in uniform micrometer-size pore formation. The obtained HAp had a bimodal pore structure having nanometer-size pores (approximately 80 nm) and micrometer-size pores (~3 μm).
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