Formation of Octacalcium Phosphate from an α-TCP Porous Body

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Tricalcium phosphate (TCP) ceramics are bioresorbable materials that are used in several applications, such as filling for bone defects. A porous TCP body also shows promise as a drug delivery system. Recently, we developed an α-TCP porous ceramic with a continuous pore structure ranging between 10 and 50 μm. The higher surface area of the α-TCP porous body confers higher drug carrier performance. In the present study, the formation of mesopores on the surface of a scaffold consisting of an α-TCP porous ceramic has been attempted by treatment with a buffer solution. Porous bodies of α-TCP ceramic having continuous pores ranging between 100 and 200 μm and between 10 and 50 μm in size were fabricated by sintering a slurry composed of β-TCP and potato starch that was impregnated into a urethane sponge. The porous α-TCP ceramic body was exposed to a buffer solution at pH = 4 at 36.5°C. The formation of octacalcium phosphate (OCP) and OCP incorporated with dicarboxylate (d-OCP) was observed on the surface and on the inside the porous body after soaking for 3 d. The OCP and d-OCP formed spherical particles of about 100 μm in diameter, which consisted of agglomerated finer, plate-like crystals. A peak in the pore-size distribution, occurring between 10 and 100 nm, appeared after soaking in the buffer solution. The formation of OCP and d-OCP brought about a remarkable increase in surface area of about 300X the value before soaking (43 and 0.13 m²/g, respectively). On the other hand, the porosity of samples soaked in the buffer solutions was lower than the porosity before soaking (60% and 73%, respectively). This decrease was due to the degradation of the α-TCP framework, and the formation of coarse OCP spherical particles. The formation of OCP and d-OCP was initiated by a slight dissolution of α-TCP on soaking, followed by precipitation of OCP and d-OCP on the porous body by the consumption of the calcium and phosphate ions from the surrounding solution. Consequently, treatment of an α-TCP porous body with a buffer solution can easily fabricate a calcium phosphate body that has a higher surface area through the formation of OCP with finer pores ranging from 10 to 100 nm, including mesopores.

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1. Introduction

Tricalcium phosphate (TCP; Ca₃(PO₄)₂) is a popular bioresorbable ceramic. Biodegradable ceramics can substitute for bone tissue during the regeneration of bone, as they gradually dissolve after being implanted into bony defects, and TCP has already been used clinically as a bone filler. Recently, we reported that an α-TCP porous body with continuous pores could be easily fabricated using a conventional sintering process that utilized a slurry composed of β-TCP and potato starch. The synthesized porous body exhibited two pore-size distribution peaks at 10–50 μm and 100–200 μm. A porous body of calcium phosphate containing mesopores is expected to be useful as a drug delivery system as it could release antibiotic or osteoinductive factors. Mesopores provide a high surface area that would adsorb a large volume of these factors. In this work, we attempted to form mesopores on the surface of an α-TCP porous body scaffold by post-treatment with buffer solution at pH = 4.

Monma et al. reported that the hydrolysis of α-TCP produces brushite (DCPD; CaHPO₄·2H₂O), octacalcium phosphate (OCP; Ca₁₀(PO₄)₆·5H₂O), and hydroxyapatite (HAP; Ca₁₀PO₄·(OH)₂), depending on the temperature and the pH. OCP mainly forms after the hydrolysis of α-TCP at a temperature of T = 40°C and at pH = 5.8–6.8. Therefore, a porous α-TCP body can be gradually converted to OCP after exposure to a solution at pH = 6.0. OCP is expected to have similar characteristics to HAP in regard to the adsorption of proteins on the basis of previous studies having reported that an OCP body in combination with a transforming growth factor can enhance bone regeneration. Therefore, we focused on OCP as a means to form mesopores on an α-TCP body.

2. Experimental

2.1 Materials

Commercial β-TCP powder (Nacalai Tesque, Inc., Japan) was mixed with an equivalent mass of potato starch (Nacalai Tesque, Inc., Japan). Ultra-pure water was mixed with this powder mixture to form a slurry using a fixed water: powder weight ratio = 0.875. The viscosity of the slurry was set at around 680 mPa s, which was measured using an LVF viscometer (LVF, Brookfield, USA). The slurry was trapped in a 15 × 15 × 15 mm³ polyurethane sponge, which had a continuous 1000 μm diameter pore structure, by dipping the sponge in the slurry, followed by drying at 60°C for 1 h. The sample was then heated to 1000°C at a rate of 5°C/min, and held at this temperature for 3 h, in air, to burn off the sponge. After cooling to room temperature, the sample was heated to 1400°C at a rate of 5°C/min, and held at this temperature for 12 h, followed by cooling at the natural rate of the furnace down to room temperature. The obtained porous ceramic samples were denoted using the label SP50.

Preliminary post-treatment trials using a pH = 6 buffer solution resulted in no observable changes in the surface morphology of the SP50 samples. To accelerate the reaction of the SP50 samples with the surrounding solution, the post-treatment used in this study was to expose the SP50 samples to a buffer solution at pH = 4, as shown in Table 1. The buffer solution was prepared using potassium hydrogen phthalate, and was denoted as buffer solution BS4. The post-treatment
Table 1. The Buffer Solutions Used for the Post-Treatment of Sample SP50

<table>
<thead>
<tr>
<th>Solution</th>
<th>Concentration (mol/l)</th>
<th>pH</th>
<th>Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS4</td>
<td>142.0</td>
<td>4.0</td>
<td>Potassium hydrogen phthalate and NaOH</td>
</tr>
</tbody>
</table>

Fig. 1. The pore-size distribution of the SP50 samples before and after soaking in buffer solution BS4 for various periods.

was applied to 1 g of the SP50 samples, which were immersed in 30 mL of buffer solution BS4. The porous samples immersed in buffer solution BS4 were then placed under vacuum for 1 h, so that the solution permeated the SP50 samples. The samples were kept in buffer solution BS4 at 36.5°C for predetermined intervals of up to 7 d. After soaking, the SP50 samples were then removed from the BS4 solution, and gently washed with ultra pure water. These samples were then denoted by the label SP50(nd), where n denotes the soaking period in days.

2.2 Characterization

The pore-size distribution, porosity, and total surface area of the samples were measured using a mercury intrusion porosimeter (Autopore9220, Shimadzu Co., Japan). The SP50 samples, after soaking in buffer solution BS4, were observed using a scanning electron microscope (SEM; S-3500N, Hitachi Ltd., Japan). The crystalline phase of the samples was determined using powder X-ray diffraction (XRD; MXP3V, MAC Science Co., Ltd., Japan) employing CuKα radiation. The concentration of calcium and phosphorus ions in buffer solution BS4, after the soaking of the SP50 samples, was measured using an inductively coupled plasma (ICP) emission spectrometer (Optima 2000DV, Perkin Elmer, UK). The change in pH of the solutions was measured using a pH electrode (6366-10D, Horiba Ltd., Japan).

3. Results

Figure 1 shows the pore-size distribution of the SP50 samples before and after soaking in buffer solution BS4 for various periods. The SP50 samples before soaking had a continuous pore structure exhibiting two peaks in the pore-size distribution, with the macropores distributed in the ranges 10–50 μm and 100–200 μm. After soaking in buffer solution BS4, finer pores in the range 10–100 nm were observed. According to an IUPAC definition, pores ranging from 2 to 50 nm are mesopores, and those > 50 nm are macropores. Therefore, the post-treatment of the SP50 samples, with buffer solution BS4, formed finer pores that included mesopores ranging from 10 to 50 nm in the macroporous body of the α-TCP ceramic. The volume of macropores > 10 μm in the porous ceramic decreased as the soaking period increased. Figure 2 shows the total pore surface area and the porosity of the SP50 samples after soaking in buffer solution BS4 for various periods. The total pore surface area of the SP50 samples increased marked-
Fig. 2. The total area of the pore surface and the porosity of the SP50 samples after soaking in buffer solution BS4 for various periods.

![Image](image1.png)

Fig. 3. SEM images of a fractured surface of the SP50 samples after soaking in buffer solution BS4 for various periods.

![Image](image2.png)

Fig. 4. Powder XRD patterns of the SP50 samples after soaking in buffer solution BS4 for various periods.

![Image](image3.png)

Fig. 5. Changes in the concentration of Ca and P ions in buffer solution BS4 due to the exposure of the SP50 samples.

![Image](image4.png)

likely from 0.13 to 43 m²/g because of soaking in buffer solution BS4. A large increase in the total pore surface area was observable after a soaking period of 2–3 d. The porosity of the SP50 samples gradually decreased from 72.9% to 61.3% after soaking in buffer solution BS4. Figure 3 shows SEM images of the fracture surfaces of the SP50 samples soaked in buffer solution BS4 for various periods. A continuous pore structure ranging between 10 and 50 μm and a 50 μm diameter framework were observed in the SP50 samples before soaking. Sample SP50(1d) showed a distinct degradation in its framework. Coarse spheres consisting of aggregates of finer, plate-like particles appeared in Samples SP50(3d) and SP50(7d) after soaking for 3 and 7 d, respectively. These coarse spherical particles had diameters larger than 100 μm, and were consisted of a large number of fine, plate-like crystals. The coarse spherical particles were observed in both inside of the samples, and on their surfaces, after soaking in buffer solution BS4 for 3 d or more, and appeared to cover all the surface of the SP50 samples after soaking in buffer solution BS4 for periods > 3 d.

Figure 4 shows powder XRD patterns of the SP50 samples before and after soaking in buffer solution BS4 for various periods. There was a mono α-TCP phase in the SP50 samples before soaking. Some other phases, including OCP, d-OCP, and DCPD were identified in the XRD patterns after soaking for 3 d. The d-OCP peaks can be assigned to OCP that had incorporated dicarboxylates, according to a previous report by Momma et al. The peak intensity of DCPD decreased as the soaking period was increased from 3 to 7 d, while the peak intensity of OCP increased as the soaking period was increased from 3 to 7 d. The peak intensity of α-TCP gradually decreased as the soaking period was increased. These observations imply that OCP was formed on and in the porous α-TCP body, along with formation of DCPD as an intermediate phase.

Figure 5 shows the change in calcium and phosphorus ion concentration in buffer solution BS4 after exposure to the SP50 samples. An increase in the concentration of calcium and phosphorus was observed after soaking for 1 d. The increase in concentration of both ions is attributed to dissolution of the α-TCP body. The calcium concentration gradually increased as the soaking period was increased up to 5 d, while the phosphorus concentration decreased. The decrease in phosphorus concentration may be related to the formation of calcium phosphates, including DCPD, OCP, and d-OCP. In addition, the calcium ion concentration decreased slightly from 5 to 7 d. The Ca/P molar ratio of the released calcium and phosphorus ion concentration was 1.5 after 1 d, as shown in Figure 6. The Ca/P molar ratio increased as the soaking
period was increased, to a value of 4.5 after 7 d. The pH of buffer solution BS4 gradually increased after soaking the SP50 samples, and reached pH = 5.8.

4. Discussion

The SP50 α-TCP porous ceramics synthesized had two types of macropore: those with diameters of 10–50 μm, and those with diameters of 100–200 μm. These were derived from the thermal decomposition of the potato starch and urethane sponge, respectively. Post-treatment with the buffer solution at pH = 4 produced finer pores with diameters ranging between 10 and 100 nm, which includes the mesopores. These finer pores result from the formation of aggregates of fine, plate-like particles. From the results of the XRD patterns of samples soaked in buffer solution BS4, the fine, plate-like particles are composed of OCP and d-OCP. The plate-like morphology of OCP is similar to that reported in other papers. The initial dissolution of the α-TCP framework is uniform in buffer solution BS4, since the Ca/P molar ratio is 1.5. The formation of DCPD, OCP, and d-OCP arises from an increase in the local concentration of calcium and phosphate ions around the α-TCP ceramic. A decrease in phosphorus concentration in buffer solution BS4, after soaking for 3 d, is related to the formation of DCPD, OCP, and d-OCP, because they induce a reduction in the calcium and phosphate ions from the surrounding fluid, as they have Ca/P molar ratios of 1.0, 1.3, and 1.3, respectively. Initial dissolution of the α-TCP ceramic increases the pH of buffer solution BS4 to pH = 5.0–5.8. This facilitates the precipitation of OCP from the solution, because, according to previous reports, crystalline OCP is stable at pH = 5.8–6.8. Therefore, the initial formation of OCP and d-OCP in buffer solution BS4 is governed by a dissolution-precipitation process. Monna et al. reported that the formation of OCP occurs through the hydrolysis of α-TCP, summarized by the following reaction:

$$3\text{Ca}_4\text{P}_2\text{O}_{7}\cdot 7\text{H}_2\text{O} \rightarrow \text{Ca}_8\text{H}_2\text{(PO}_4\text{)}_6\cdot 5\text{H}_2\text{O} + \text{Ca}(\text{OH})_2.$$  

This is consistent with an increase in the Ca/P molar ratio and pH, as shown in Fig. 6. The increase in the Ca/P molar ratio and pH, therefore, relates to the increase in concentrations of Ca²⁺ and OH⁻ that accompany the formation of OCP and d-OCP after soaking the α-TCP ceramics in buffer solution BS4 for > 3 d.

The formation of fine OCP and d-OCP particles in buffer solution BS4 creates finer pores with diameters ranging between 10 and 100 nm, which includes mesopores, and this increases the total surface area of the pores, although a slight decrease in porosity of about 15% occurs in macropores with diameters > 10 μm. This decrease can be attributed to the formation of coarse spherical particles with diameters > 100 μm. Even so, macropores with diameters > 10 μm remain even after soaking in buffer solution BS4 for 7 d, but these are also effective as spaces for the permeation of body fluids. Consequently, such a bimodal structure with fine pores ranging from 10 to 100 nm, which includes mesopores and macropores with diameters > 10 μm, can adsorb a larger volume of drugs than non-treated porous α-TCP. Therefore, post-treated porous α-TCP may be useful as a biodegradable drug carrier.

5. Conclusions

A post-treatment using a buffer solution at pH = 4 was applied to α-TCP porous ceramics with macropores having diameters in the ranges 10–50 μm and 100–200 μm. Finer pores with diameters in the range 10–100 nm, which includes mesopores formed in the α-TCP porous ceramic, arose from the formation of octacalcium phosphate and octacalcium phosphate incorporating dicarboxylates particles having a large number of plate like crystals. These finer pores led to an increase in the total surface area of up to 43 m²/g, compared with the 0.13 m²/g of an untreated α-TCP porous ceramic. The formation of octacalcium phosphate progressed via a dissolution-precipitation process in the buffer solution. This process is expected to be an easy way to fabricate calcium phosphate bodies, such as drug carriers, that would have higher surface areas through OCP formation, and finer pores in the range 10–100 nm, including mesopores.

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