The controlled resorption of porous α-tricalcium phosphate using a hydroxypropylcellulose coating

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Tricalcium phosphate (TCP) ceramic is known in orthopedics to be a bioresorbable bone substitute. A porous TCP ceramic body also has high potential as a drug delivery system in bony defects. Porous α-TCP ceramic can be easily fabricated using conventional sintering of β-TCP, since α-TCP is the thermodynamically stable phase at temperatures above 1 100 °C. However, the solubility of α-TCP is much higher than that of β-TCP. Therefore, the dissolution of porous α-TCP progresses at a higher rate than bone repair. In the present study, we attempted to reduce the dissolution rate of porous α-TCP by employing an organic polymer coating. We fabricated porous α-TCP ceramic with a continuous 10–50 μm diameter pore structure by sintering a body made from a β-TCP and potato starch slurry. The porous body obtained was coated with hydroxypropylcellulose (HPC), and then subjected to heat treatment. The chemical durability and mechanical properties of the body were examined before and after coating with the HPC. The dissolution of porous α-TCP in buffered solutions was reduced by coating with HPC and drying at 60 °C. The compressive strength of the porous α-TCP was also improved by coating with HPC. The results of *in vivo* experiments showed that some parts of the porous α-TCP ceramic coated with HPC remained in the canal of the tibia of a rabbit four weeks after implantation, whereas no residual was observed in a non-coated α-TCP ceramic. Coating with HPC was found to be effective for controlling bioresorption and improving the workability of porous α-TCP ceramic. The prepared porous α-TCP ceramic is expected to be useful as a novel material for bone fillers by incorporating it with drugs or osteoinductive factors.

1. Introduction

Tricalcium phosphate (TCP) is a popular bioresorbable ceramic [1–4]. The porous body of TCP is gradually dissolved away from bony defects during bone repair, leading to its substitution by regenerated bone, and is already used clinically as a bone filler. Furthermore, the porous body of TCP allows several other uses [5–7] when either drugs or osteoinductive factors are incorporated in it. The porous body of α-TCP can be easily fabricated by conventional sintering β-TCP, since α-TCP is its thermodynamically stable phase at temperatures above 1100 °C. However, the solubility of α-TCP is higher than that of β-TCP [8]. It is liable to be completely resorbed in the body before a bony defect can be repaired by bone regeneration. Therefore, effective control of the dissolution rate of α-TCP is desirable.

In the present study, we attempted to reduce the dissolution rate of porous α-TCP ceramic by coating it with the organic polymer. Hydroxypropylcellulose (HPC), with a chemical structure as shown in Fig. 1, was the polymer selected, since it has attractive characteristics such as ease of film formation, biodegradability, and low toxicity [9, 10]. We investigated the chemical durability of porous α-TCP in various buffered solutions, and the effect of an HPC coating on the bioresorption of porous α-TCP ceramic was also evaluated *in vivo* through implantation in a rabbit tibia.

2. Materials and Methods

2.1. Preparation of porous α-TCP ceramic coated with hydroxypropylcellulose

Commercial β-TCP powder (Nacalai Tesque, Inc., Japan) was pulverized to obtain a powder with a mean particle size below 45 μm. The resulting β-TCP powder was mixed with an equivalent mass of potato starch (Nacalai

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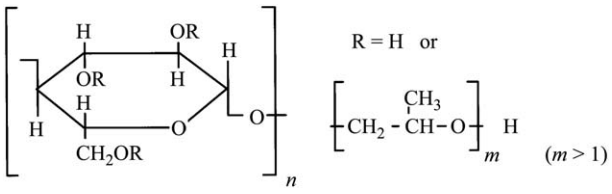

Figure 1 Structural formula of hydroxypropylcellulose (HPC).

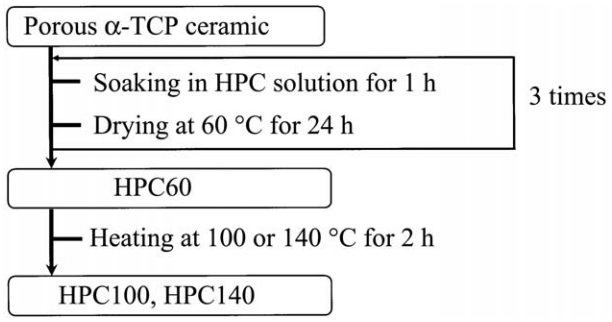

Figure 2 Procedure used for coating HPC onto porous α-TCP ceramic.

Tesque, Inc., Japan), and ultra-pure water was added to form a slurry with a viscosity determined at 550–600 mPa-s using a viscometer (LVF, Brookfield, USA). The slurry was trapped in a $15 \times 15 \times 15\,mm^3$ polyurethane sponge, with 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 kept at 1000 °C for 3 h in air to burn off the sponge. After cooling to room temperature, the sample was heated once more to 1400 °C at a rate of 5 °C/min, and kept at 1400 °C for 12 h, before being allowed to cool to room temperature at the natural cooling rate of the furnace.

The procedure used to coat HPC onto the porous α-TCP ceramic is shown schematically in Fig. 2. The porous α-TCP body was coated with HPC using a 5 mass % aqueous solution. The HPC solution was prepared by dissolving reagent-grade HPC (Wako Pure Chemical Industries Co. Ltd., Japan) in ultra-pure water at 70 °C. The porous α-TCP ceramic was soaked in the aqueous HPC solution under vacuum, and then dried at 60 °C for 24 h. This process was repeated three times. Some samples were selected for further heat treatment at a given temperature, e.g. 100 or 140 °C for 2 h. The sample abbreviations used are given in Table I.

2.2. Evaluation of the porous ceramic

The chemical durability of the samples was evaluated by immersing 1 g of a sample in $30\,cm^3$ of the buffered solutions listed in Table II. The solutions containing the porous sample were then placed under vacuum for 1 h, to allow the solutions to permeate inside the ceramic. The solutions were then kept at 36.5 °C for predetermined intervals of up to seven days. The concentration of Ca^{2+} in the buffered solutions after soaking the ceramic samples was measured using a Ca^{2+} electrode (6583-10C, Horiba Ltd., Japan). The change in pH of the solutions was also measured.

A preliminary investigation into the resorbability of the porous α-TCP ceramics before and after coating with HPC (NoHPC and HPC60) was carried out *in vivo* by implantation into the tibia of mature male Japanese white rabbits weighing 1.7–2.0 kg. The rabbits were reared at Japan SLC Inc., Japan, which was also the location in which the animal experiments were carried out. The company guidelines for animal experimentation were observed carefully; the rabbits were anesthetized using an intravenous injection of 5% pentobarbital sodium solution and local administration of 1% lidocaine. A longitudinal skin incision of about 2 cm in length was made along the medial aspect of the proximal metaphysis of the tibia using surgical techniques. The fascia and periosteum were also incised and retracted to expose the tibial cortex. A 4 mm diameter hole was cut at the medial aspect of the proximal metaphysis of the rabbit tibiae. A cylindrical sample, 4 mm in diameter and 10 mm in length, was then inserted into the hole to fill the defect. Another hole in the rabbit tibia that was allowed to self-heal served as a control. Six legs from three rabbits were used in the experiments. After four weeks, the rabbits were euthanased with extreme dosages of pentobarbital sodium solution, and the implants and surrounding bony tissues were extracted. After fixing in 10% formaldehyde solution, the implants and the surrounding bony tissues were examined using X-ray computed tomography (X-CT; MCT-CB130MF, Hitachi Medico Technology Corporation, Japan). The samples were further dehydrated using graded ethanol/water solutions, then potted in epoxy resin. The epoxy-embedded samples were stained with hematoxylin–eosin (HE) and thin slices were then cut for examination under an optical microscope.

2.3. Characterization of the samples

The HPC as received was analyzed using thermogravimetry and differential thermal analysis (TG-DTA; 2000S, MAC Science, Japan) at a heating rate of 5 °C/min in air. The crystalline phase of the samples was

TABLE I Abbreviations for the synthesized porous samples used

Sample	HPC coating followed by heat treatment at 60 °C for 24 h	Temperature on heat treatment for 2 h
NoHPC	No	No
HPC60	Three times	No
HPC100	Three times	100 °C
HPC140	Three times	140 °C

TABLE II Buffer solutions used to examine dissolution of porous α-TCP ceramic

Solution	Concentration (mol/m^3)		pH	Buffer
	Na^+	Cl^-		
BS(pH4)	142.0	142.0	4.0	A
BS(pH6)	142.0	142.0	6.0	B
BS(pH7)	142.0	142.0	7.25	C

A: Potassium hydrogen phthalate and sodium hydroxide.
B: Morpholinoethanesulfonic acid monohydrate (MES) and sodium hydroxide.
C: Tris(hydroxyethyl)aminomethane (Tris) and hydrochloric acid.

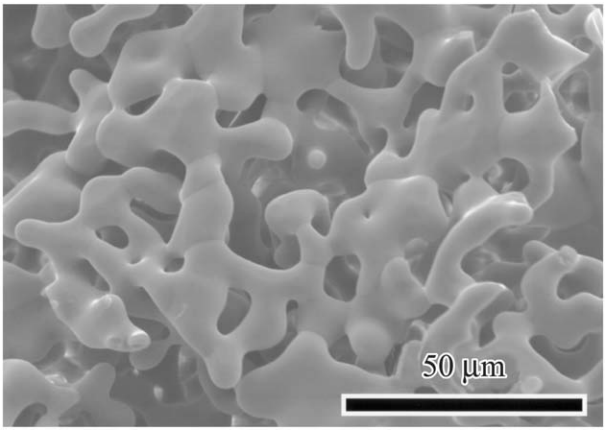

Figure 3 SEM image of porous ceramics synthesized using the present method.

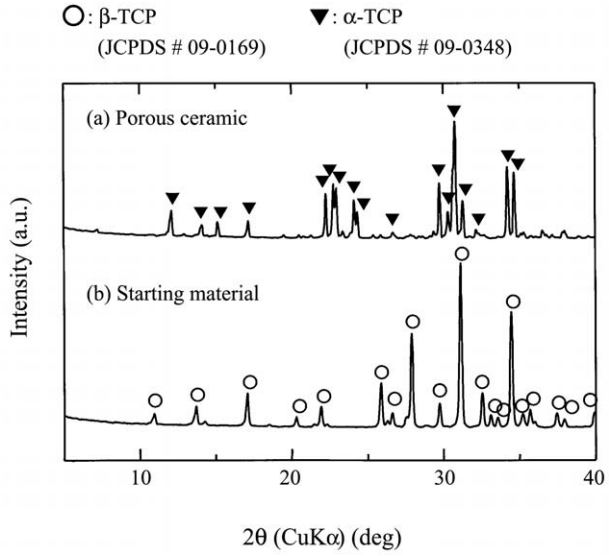

Figure 4 Powder X-ray diffraction pattern in the porous ceramic synthesized using the present method and starting material consisting of β-TCP.

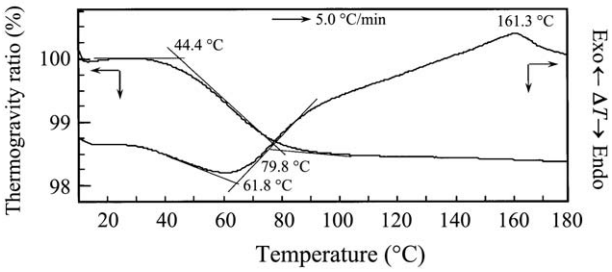

Figure 5 TG-DTA curve of the HPC powder as received.

determined using powder X-ray diffraction (XRD; M18XHF^{22}-SRA, MAC Science, Japan), under CuKα radiation. The morphology of the samples was examined using a scanning electron microscope (SEM; S-3500N, Hitachi Co. Ltd., Japan). The porosity and pore size were evaluated using a mercury intrusion porosimetry (Autopore 9220, Shimadzu Co., Japan). The compressive strength of the samples was measured using an Instron Type 5566 material testing machine (Instron Co., USA). The maximum load was defined as the compressive strength when a $10 \times 10 \times 10\,\text{mm}^3$ cubic sample was loaded using a constant crosshead speed of 20 mm/min.

3. Results

Fig. 3 shows an SEM image of a porous ceramic synthesized using the present method. The ceramic body had a continuous pore structure, with a pore diameter of approximately 10–50 μm, with a sponge-like 10 μm diameter ceramic scaffold. The result of mercury intrusion porosimetry indicated that the pore size of the porous ceramic distributed in the range from 10 to 50 μm, and its porosity was 72.9%. Fig. 4 shows powder X-ray diffraction patterns of the porous ceramic as well as the starting material of TCP. The porous ceramic consisted

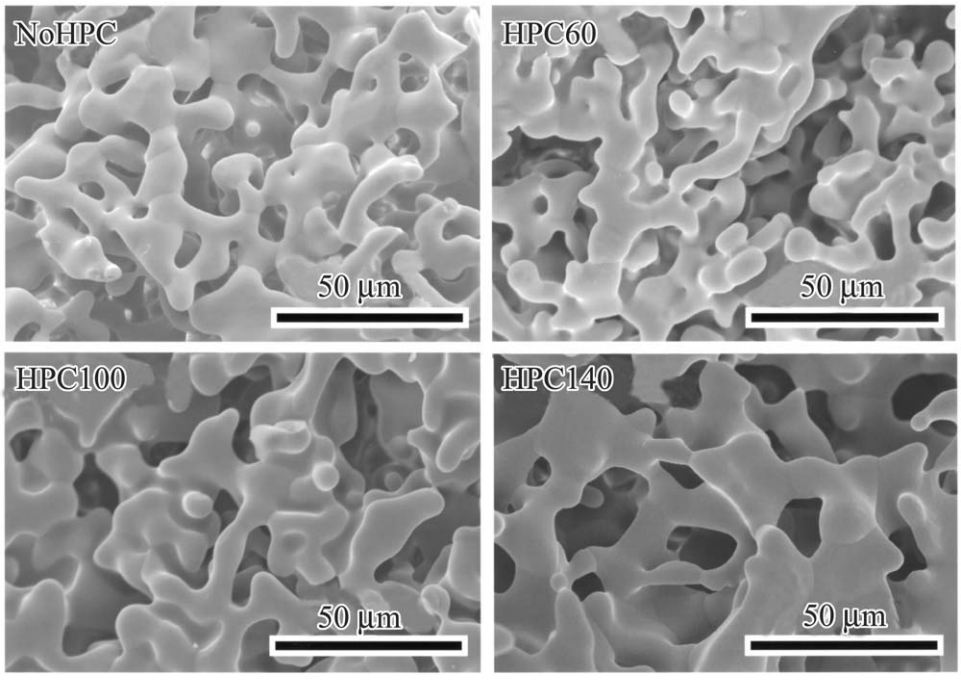

Figure 6 SEM images of the porous α-TCP samples (NoHPC, HPC60, HPC100, and HPC140).

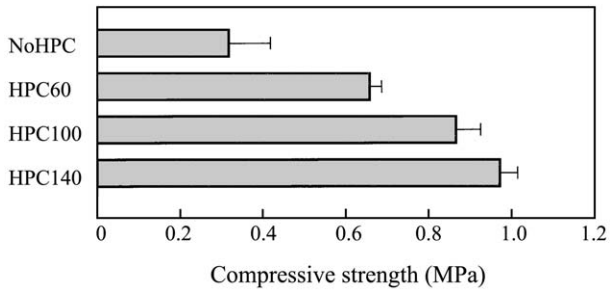

Figure 7 Compressive strength of the samples with and without HPC coating ($n = 5$). The bars indicate standard deviation.

solely of α-TCP phase (JCPDS#09-0348), while the starting material of β-TCP phase (JCPDS#09-0169). It is distinct that β-TCP phase of starting material completely transforms to α-TCP phase during the heat-treatment process. These results indicate that the present synthetic process is an easy method for producing porous α-TCP ceramic using conventional sintering processes.

Fig. 5 shows a TG-DTA curve of the HPC powder as received. A broad endothermic peak was observed at around 60 °C, and an exothermic peak appeared at 160 °C. Fig. 6 shows SEM images of samples HPC60, HPC100, and HPC140. The morphology of the samples was similar to that before coating. Continuous pores with diameters of 10–50 μm still remain, even after the HPC coating. There appeared to be no morphological difference between the samples. Fig. 7 shows the compressive strength of samples NoHPC, HPC60, HPC100, and HPC140. The HPC coating resulted in a distinct increase in the compressive strength of the porous α-TCP ceramic. The compressive strength increased with increasing heat treatment temperature.

Fig. 8 shows the change in concentration of Ca^{2+} in the buffered solutions due to soaking of the samples. The increase in Ca^{2+} concentration is attributed to the dissolution of porous α-TCP ceramic. A buffered solution with a given pH value of x was denoted as BS(pH x). In solution BS (pH 4), the release of Ca^{2+} ions was reduced by the HPC coating. The effect of this reduction on the release of Ca^{2+} ions through HPC coating was dependent on the temperature of the heat treatment, and occurred in the following order: HPC100 > HPC60 > HPC140. In solution BS(pH 6), sample HPC140 released more Ca^{2+} ions than sample NoHPC (the porous α-TCP ceramic with no HPC coating). By contrast, samples HPC60 and HPC100 showed a marked reduction in the number of Ca^{2+} ions released from the α-TCP ceramic. This suppression of the dissolution of Ca^{2+} ions by the HPC coating was much higher at a heat treatment of 100 °C than at a heat treatment of 60 °C. Sample BS(pH 6) showed a similar tendency in terms of the dissolution properties of the porous α-TCP ceramic to that of sample BS(pH 7). Fig. 9 shows the change in pH of the solutions after exposure to ceramic samples. Irrespective of which sample was used, the pH of solution BS (pH 4) increased with increasing soaking period. In solution BS (pH 6), the pH of the solution decreased after exposure to sample HPC140 for

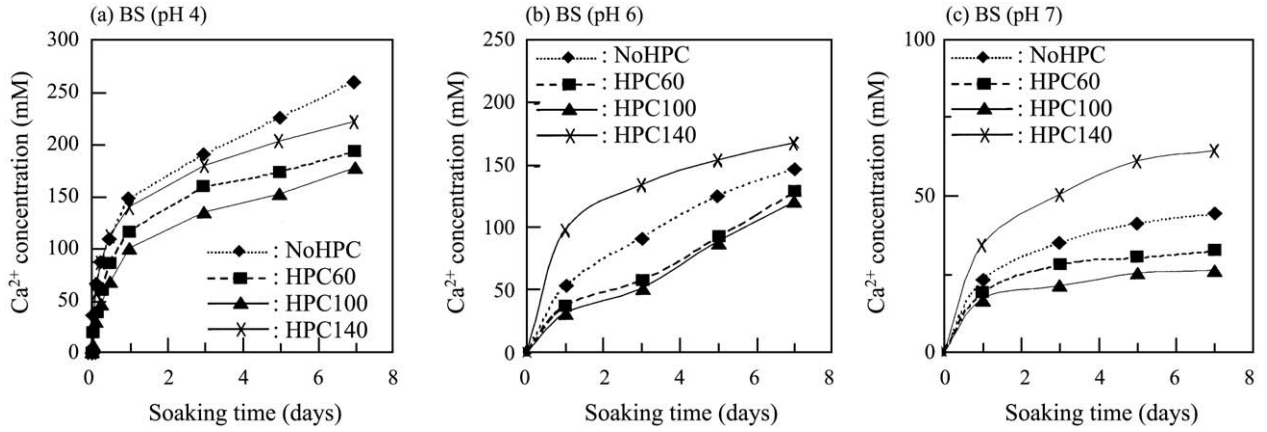

Figure 8 Changes in concentration of Ca^{2+} in the buffered solutions due to soaking samples of: (a) BS (pH 4); (b) BS (pH 6); and (c) BS (pH 7).

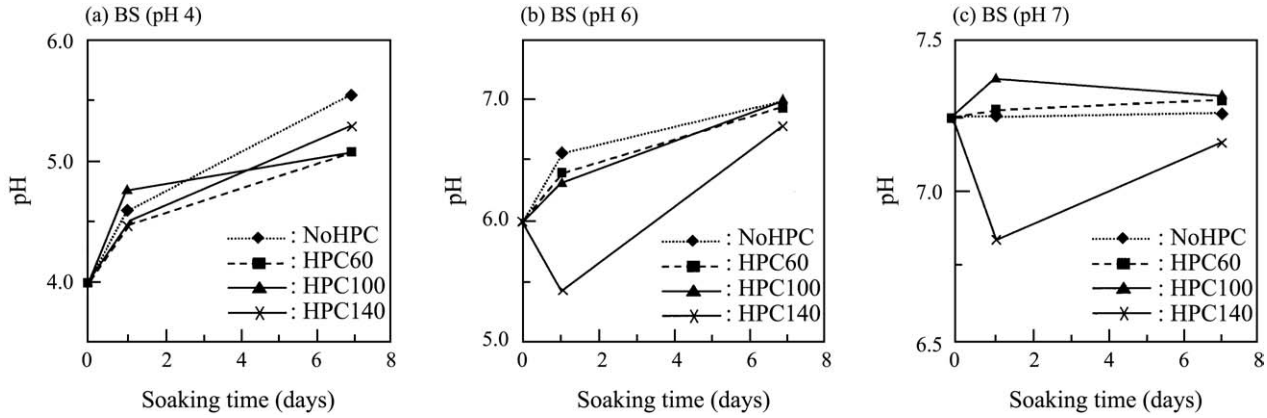

Figure 9 pH of buffered solutions after soaking the samples.

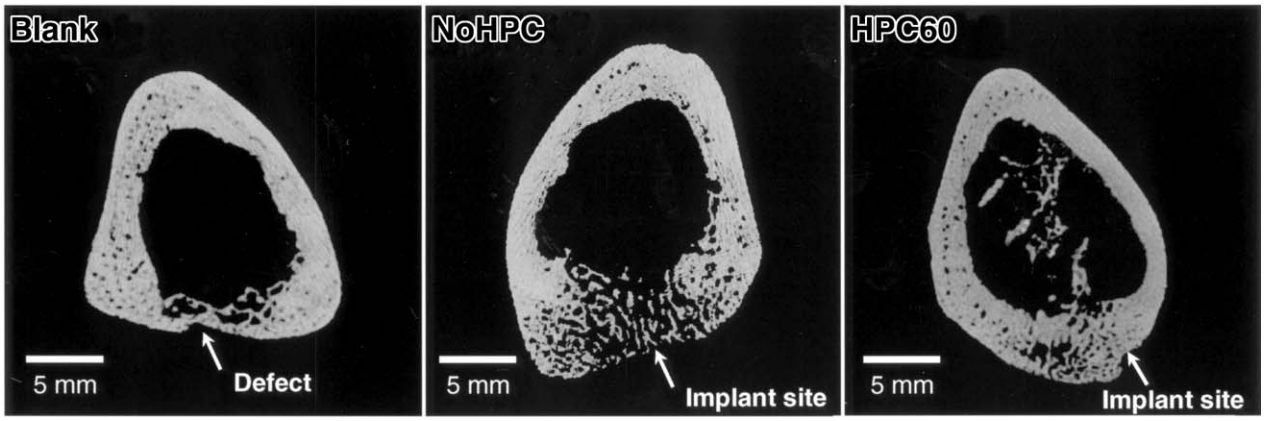

Figure 10 X-CT images of rabbit tibia after implantation of sample for four weeks. The arrow indicates the implant site.

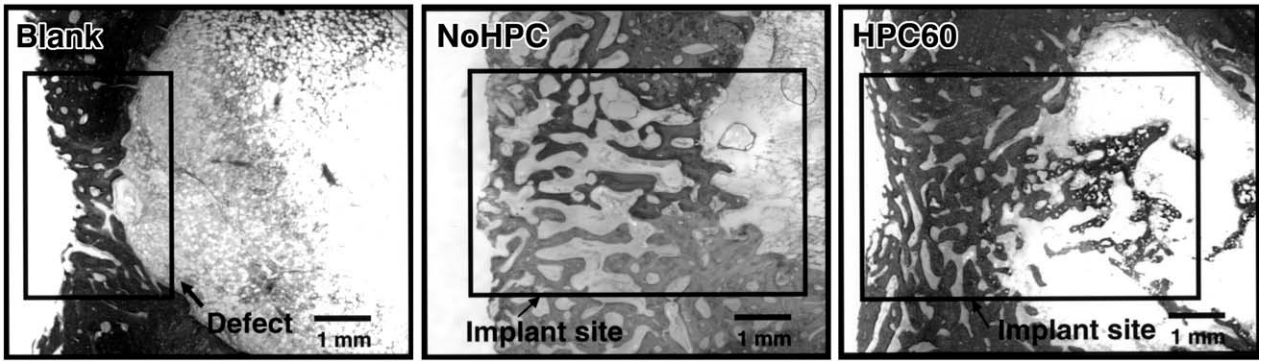

Figure 11 Optical microscope images (with HE staining) of rabbit tibia after implantation of sample for four weeks. Original magnification = × 25.

a period of one day, while the pH of the solution increased after exposure to the other samples. After seven days, the pH of solution BS(pH 6) showed almost the same value, irrespective of which sample was used. This phenomenon also took place in solution BS(pH 7). Thus, sample HPC140 decreased the pH of the solution in the initial soaking period. The pH of the solution subsequently increased through the reaction of sample HPC140 with the surrounding fluid.

A higher chemical durability was expected for samples HPC60 and HPC100 as opposed to the porous α-TCP ceramic with no HPC coating (sample NoHPC). The bioresorbability was then initially determined by an *in vivo* experiment using rabbit tibia. Fig. 10 shows X-CT images of a rabbit tibia after implantation of samples NoHPC and HPC60 for a period of four weeks. The ''Blank'' denotes the control tibia with no implant to allow for natural healing. Although the similarity in the density of the porous TCP ceramics and natural bone makes it difficult to compare the differences between them quantitatively, we could note that ceramic body did not remained after four weeks in the canal of the bone after the implantation of NoHPC. In contrast, some trace of the ceramic body was observed inside the bone after implantation of HPC60. It looks that the bone regeneration with higher density at the defect where HPC60 were implanted than where NoHPC implanted. Fig. 11 shows optical microscopic images of the HE-stained samples after operation for four weeks. Optical larger amounts of bone formation toward to implantation site are distinctly observed for the specimen HPC60 than those for Blank and NoHPC. Higher degree of bone ingrowth seemed to occur in HPC60 than in NoHPC.

4. Discussion

The present process provides an easy method for producing a porous ceramic with a continuous pore structure with a diameter of about 10–50 μm. The ceramic body is made up of crystalline α-TCP due to phase transformation from β-TCP using starting materials. A structure such as this with continuous pores and high porosity more than 70% would be useful as a drug delivery system, if its degradation rate can be controlled. It is apparent from the results described above that an HPC coating is effective in reducing the degradation of porous α-TCP ceramic when the samples are heat treated at 60 and 100 °C, whereas an HPC coating followed by heat treatment at 140 °C results in a higher degradation rate than α-TCP ceramic alone. The TG-DTA curve of HPC indicate that a decrease in weight occurs during heat treatment at around 60 °C. This may be attributed to the curing of the HPC. The cured HPC may prevent α-TCP from reacting with the buffered solutions. Cured HPC also increases the compressive strength of porous α-TCP ceramic. A heat treatment at 140 °C gave the highest compressive strength among the samples prepared in this study. However, a heat treatment at 140 °C leads to a higher dissolution rate of α-TCP compared to porous α-TCP ceramic without any coating. These phenomena were clearly evident in samples after soaking in BS (pH 6) and BS (pH 7) solutions. The pH of the solution

decreased after the immersion of sample HPC140 during the initial stages after exposure to the porous α-TCP ceramic coated with HPC and subsequently heat-treated at 140 °C. The initial drop in the pH accelerated the dissolution of α-TCP in this sample, because α-TCP may have a higher dissolution rate in solutions with low pH values [11]. It is suggested that heat treatment at temperatures above 140 °C may form acidic functional groups in the HPC due to oxidation, although there is no distinct evidence for this in the TG-DTA curve of HPC at temperatures around 140 °C.

The *in vivo* evaluation showed that HPC coating is effective for controlled degradation rate on α-TCP implantation. The residual ceramic provides a surface to support bone ingrowth. The relationship between the structure of the porous α-TCP ceramic with HPC coating and its bioresorption needs to be investigated in detail in further research.

5. Conclusions

Using a conventional sintering process, we successfully fabricated a porous α-TCP body with a continuous 10–50 μm diameter pore structure. An HPC coating on the porous α-TCP ceramic increased its chemical durability. A subsequent heat treatment of the coated porous ceramic up to a temperature of 100 °C lead to an increase in its chemical durability, while a subsequent heat treatment at a temperature of 140 °C enhanced the dissolution properties of the α-TCP porous body. The compressive strength of the porous α-TCP was also improved by coating with HPC and subsequent heat treatment. Some porous α-TCP remained in the canal of the bone of rabbit tibia, even after a four week implanatation and showed bone ingrowth clearly, whereas no residual non-coated α-TCP ceramic was observed inside the bone. Coating with HPC was found to be effective in controlling bioresorption and in improving the workability of porous α-TCP. The prepared porous α-TCP ceramic is expected to be useful as a novel bone filler material by incorporating it with drugs or osteoinductive factors.

Acknowledgments

This work was conducted under the auspices of the research project, ''Technology Development for Medical Materials Merging Genome Information and Materials Science'', in the Kansai Science City Innovative Cluster Creation Project, supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan. This work was also partially supported by the Industrial Technology Research Grant Program in 2001 of the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

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Received 23 April 2003
and accepted 5 February 2004