

Surface-layer Modification of Hydroxyapatite Ceramic with Acid and Heat Treatments

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The purpose of this study was to examine or characterize the surface layer of a calcium phosphate ceramic with a gradual compositional change from α -tricalcium phosphate (α -TCP) on the surface to hydroxyapatite (HAP) on the inside. The surface of a dense HAP ceramic was acid-treated for 1 hour with orthophosphoric acid (H_3PO_4) solutions of several concentrations (0.5, 1.0 and 5.0 mol/L) or a buffered solution (pH 4.0) consisting of phosphate solutions. After acid treatment, specimens were heat-treated at 1,250°C for 1 hour. X-ray photoelectron spectroscopy revealed that the compositional gradient layer could be modified on the surface of the HAP ceramic with all acid and heat treatments, and that 5.0 mol/L H_3PO_4 solution and heat treatments had a maximal thickness of approximately 2 μm for the surface-modified layer. It was confirmed that the outermost layer of HAP ceramics modified with the treatments, except 5.0 mol/L H_3PO_4 solution, showed a compound such α -TCP.

Key words: Apatite ceramic, Surface treatment, α -Tricalcium phosphate

INTRODUCTION

Bioceramics consisting of calcium phosphates are known to possess excellent tissue responses. In particular, hydroxyapatite (HAP ; $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) ceramics have been widely employed as bone-replacing materials in the dental and medical fields because of their biocompatibility and bioactivity^{1,2)}. The HAP materials excellently bond directly with bone in contrast to titanium, which only has osteointegration³⁾. One of the calcium phosphates, α -tricalcium phosphate (α -TCP; α - $\text{Ca}_3(\text{PO}_4)_2$), has not been applied for use as a biomaterial because its solubility is markedly higher than those of HAP and β -tricalcium phosphate (β -TCP). However, α -TCP powder sets to form calcium-deficient HAP when mixed with water, and thus is used in dental and medical clinics as a root sealer and bone-filling cement, respectively³⁻⁷⁾. Moreover, α -TCP was re-evaluated recently, and the use of an α -TCP-HAP mixture has become a topic of interest. It was reported that a mixture consisting of α -TCP and HAP bond with bone faster than HAP alone⁸⁾. This suggests that a small quantity of α -TCP in HAP is better than a large quantity or no α -TCP. One of the reasons for the faster bone formation may be the dissolution of α -TCP around the HAP. Although the detailed

mechanism of bone formation or bone bonding with HAP has not been clarified, it appears reasonable that bone formation needs original materials, that is, calcium and phosphate. However, it appears that the inside layer of the HAP material is unnecessary because the bioactivity and biocompatibility of materials are governed by the characteristics of the surface layer. We, therefore, tried to develop a functionally gradient material (FGM) made of a calcium phosphate ceramic whose surface of sintered HAP material was only α -TCP, and the content of α -TCP gradually decreased with increasing depth from the surface⁹. α -TCP was contained to a depth of 200 μ m from the surface. This functionally gradient ceramic calcium phosphate (FG-CCP) was produced by using a diamond powder and firing at 1,280°C under reduced pressure and atmospheric conditions. The FG-CCP consisting of HAP and α -TCP may have potential value as a bone-replacing and bone-substituting material since α -TCP gradually decreased from the surface to the interior. However, the preparation of this material has a bad influence on the furnace and its alumina furnace tube because there is the possibility that the furnace tube can be damaged by the change at 1,280°C from the reduced pressure to atmospheric conditions. Moreover, small quantities of calcium oxide (CaO) and tetracalcium phosphate (TTCP; $\text{Ca}_4(\text{PO}_4)_2\text{O}$) are produced in FG-CCP by heat decomposition of HAP in the diamond method.

We propose that FG-CCP consisting of HAP and α -TCP can be produced by acid and heat treatments of a dense sintered HAP material. The surface of the HAP material was changed to a dicalcium phosphate dihydrate (DCPD; $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) or monocalcium phosphate monohydrate (MCPM; $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$) by treatment with a solution of an acid such as orthophosphoric acid. Moreover, the surface may be changed from DCPD or MCPM to α -TCP or β -TCP by heat treatment because of the diffusion of its elements from the surface to interior. If this hypothesis is correct, FG-CCP may provide better circumstances for bone formation. Its surface is covered with α -TCP and the composition changes gradually to HAP with the depth from the surface. Thus, upon implantation of this material, its surface will face interstitial fluid and the α -TCP will dissolve to supply calcium and phosphate. After the dissolution of α -TCP from the surface of FG-CCP, fresh HAP appears very close to the highly concentrated calcium phosphate solution. As an example of surface treatment on HAP material, Ioku *et al.* reported that a porous apatite ceramic coated with β -TCP could be prepared by soaking in a diammonium hydrogen phosphate ($(\text{NH}_4)_2\text{HPO}_4$) solution (no washing) and then heating at 900°C for 3 hours¹⁰. However, this method is difficult to construct the compositional gradient layer because it is not an acid treatment.

In this investigation, we prepared a calcium phosphate ceramic consisting of HAP and α -TCP such as FG-CCP produced by the diamond method and using phosphoric acid solutions. The aim of this investigation was to characterize the properties of the HAP ceramic surface-modified with acid and heat treatments. The surface-modified layers of the HAP ceramic prepared with various phosphoric acid solutions were measured and evaluated using X-ray photoelectron spectroscopy, and the effects

of various phosphoric acid solutions on the modificatory compounds and depth of the surface-modified ceramic are discussed.

MATERIALS AND METHODS

Preparation of the specimens

As a starting material, the HAP ceramic was prepared using commercial dental implant HAP 7 mm in diameter (APACERAM; 1-piece type, Asahi Optical, Tokyo, Japan). This commercial HAP ceramic was an excellent sintered material with low porosity and uniform grain size. The sintered HAP for the implant material had a density of 3.14 g/cm³ (HAP; 3.16 g/cm³), and a mean grain size of approximately 0.5 μm. This dense HAP ceramic was cut into about 3 mm thickness for a circular plate specimen using a low-speed diamond cutter (Minitom; Struers, Copenhagen, Denmark). The edge of the specimen was made flush using SiC paper (#800). However, the cutting surface of each specimen was not ground or polished. The microstructures (SEM) of specimen surfaces sliced with the diamond cutter and etched with an acetic acid solution (1.0 mol/L) are shown in Fig. 1.

For acid treatments of the HAP ceramic, four solutions were prepared using 0.5, 1.0 and 5.0 mol/L orthophosphoric acid (H₃PO₄), and a buffered solution (pH 4.0) consisting of 1.0 mol/L H₃PO₄ and 1.0 mol/L diammonium hydrogen phosphate ((NH₄)₂HPO₄). The pH values of the 0.5, 1.0 and 5.0 mol/L H₃PO₄ solutions were 1.71, 1.45 and 0.8, respectively. Ten specimens of HAP ceramic in the circular plate form (size; 7 mm φ × 3 mm) were immersed in each acid solution for 1 hour. After immersion, the HAP specimens were first rinsed in distilled water, and then ultra-

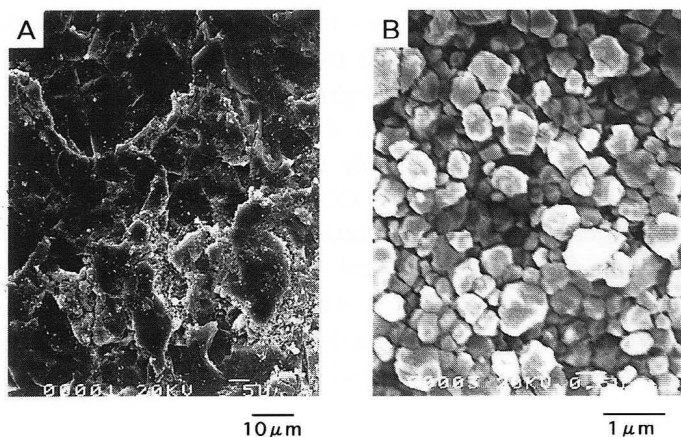


Fig. 1 Surface microstructures of commercial hydroxyapatite ceramics sliced and etched with a diamond cutter and an acetic acid solution, observed using a scanning electron microscope (SEM). A; sliced surface, B; etched surface. Scale bars are 10 μm (A) and 1 μm (B).

sonically washed in distilled water for 15 min. The HAP specimens treated with several acid solutions were heat-treated to 1,250°C at a heating rate of 5°C/min, and kept at 1,250°C for 1 hour in a furnace under atmospheric conditions.

Analysis of the specimens

The HAP specimens were characterized using X-ray photoelectron spectroscopy (XPS; Quantum 2000, Perkin-Elmer Co., Wellesley, MA, USA) with an Al K α line and 100 μ m beam diameter, after acid-only treatment or acid and heat treatments. The XPS measurements were performed with argon (Ar) ion sputtering at a rate of approximately 50 nm/min. The sputtering rate was calculated by measuring the sputtered depth with SEM observation. The specimen was sputtered to a maximum depth of approximately 2 μ m. The calcium/phosphorus (Ca/P) ratios of specimens from the surface to maximal depth of 2 μ m were determined from the relative concentrations of elements measured using XPS. The chemical compositions of calcium phosphates were decided with the binding energies of Ca 2p and P 2p XPS spectra. The specimen of each condition was tried by XPS measurements of above three times in different spots. The crystal phase in the treated specimens was analyzed with a powder X-ray diffractometer system (XRD; ADG-301, Toshiba Co., Tokyo, Japan). The specimen for XRD analysis was prepared by acid and heat treatments of the broken fragments (approximately 1 mm length) of the HAP ceramic, to confirm the reactions caused by treatments. Moreover, the confirmation of reactions was performed with a Fourier transform infrared spectrometer (FTIR; FTS-40, Bio-Rad, Hercules, CA, USA). The specimen for FTIR was prepared by scraping the surface and inside layers of treated HAP ceramics. A scanning electron microscope (SEM; S-700, Hitachi Co., Tokyo, Japan) was used to observe the microstructure of the surface of the HAP specimens. For SEM observation, the specimen was sputtered with gold.

RESULTS

Fig. 2 shows the powder XRD patterns of a HAP ceramic specimen (A) before treatment and a specimen (B) after acid and heat treatments. Specimen (A), a commercial HAP implant material, had only a HAP crystal phase and no peaks were confirmed for the other crystals. After the HAP specimen was acid-treated with 5.0 mol/L H₃PO₄ solution, XRD confirmed that the specimen contained no new peaks. The crystal phase of specimen (B) heat-treated at 1,250°C after acid treatment was similar to the non-treated HAP ceramic, that is, the crystal phase was almost all HAP. However, an estimated broad bump (α -TCP peak) was fractionally detected at 30.7 degree (2θ). In the FTIR measurements for the specimens prepared with acid (5.0 mol/L H₃PO₄) and heat treatments, the IR spectra of the surface and inside layers showed similar (Fig. 3). The inside of the specimen was confirmed by a typical IR spectrum of HAP. The IR spectrum on the surface layer had a small OH peak compared with that of the inside.

Fig. 4 shows the SEM microstructures on the surfaces with and without heat-

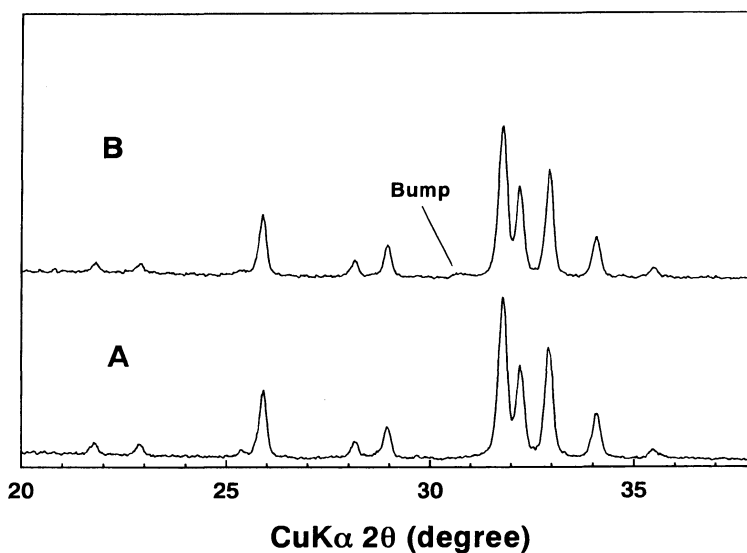


Fig. 2 Powder X-ray diffraction patterns of hydroxyapatite specimens before (A) and after treatments (B). The specimen was acid-treated and heat-treated with 5.0 mol/L orthophosphoric acid solution and at 1,250°C, respectively.

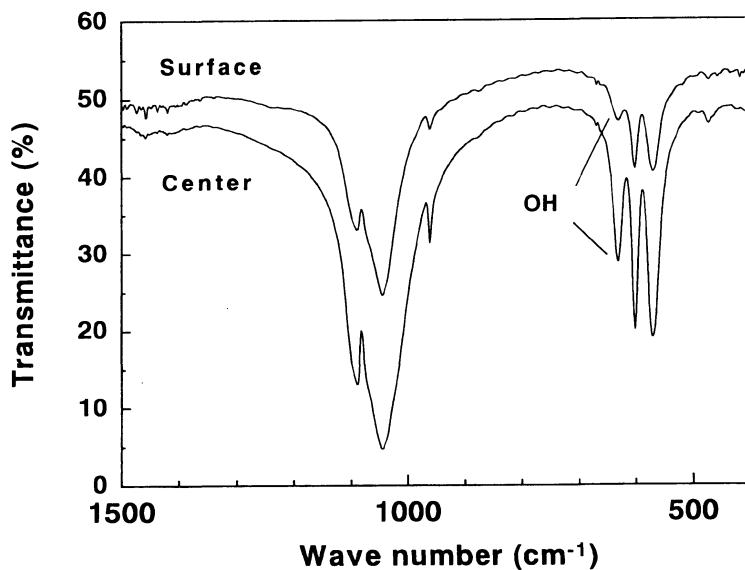


Fig. 3 FTIR spectra for surface and inside layers of hydroxyapatite specimens prepared with 5.0 mol/L orthophosphoric acid treatment and heat treatment at 1,250°C.

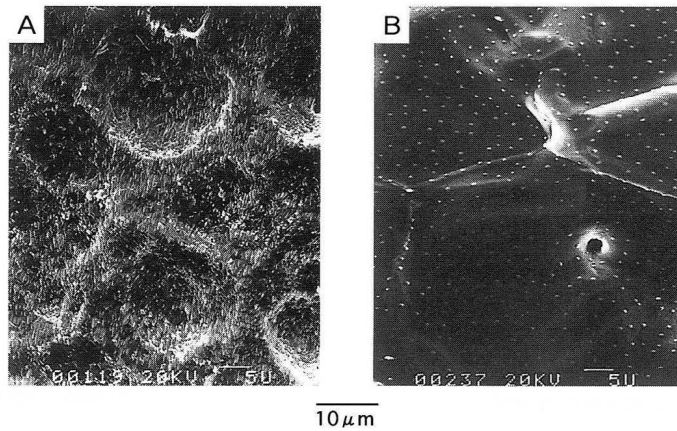


Fig. 4 Scanning electron micrographs (SEM) showing the surface microstructures of hydroxyapatite specimens with and without heat treatment at 1,250°C after immersion in 1.0 mol/L orthophosphoric acid solution. A; surface with acid only treatment, B; surface with acid and heat treatments. Scale bar=10 μ m.

Table 1 Ca/P ratios on the surface layer of HAP ceramics with acid (5.0 or 1.0 mol/L H₃PO₄ solution) and heat (1,250°C) treatments, measured by XPS

Depth from surface (nm)	Acid and heat treatments	
	5.0 mol/L H ₃ PO ₄	1.0 mol/L H ₃ PO ₄
0	1.03	1.47
50	1.31	1.56
100	1.42	1.55
200	1.44	1.69
300	1.49	1.67
500	1.54	1.75
1,000	1.49	1.69
1,500	1.60	—
2,000	1.62	—

treatment after immersion in 1.0 mol/L H₃PO₄ solution. Observation of the microstructure of specimen (A) with only acid treatment revealed that the H₃PO₄ solution attacked the surface layer and ate away the HAP ceramic. In the treatments using the 3 other phosphoric acid solutions, the surface microstructures depended on the concentration (*i.e.* pH) of the phosphoric acid solution. The microstructure of specimen (B) heat-treated at 1,250°C after acid treatment revealed that the surface attacked with acid treatment changed to a dense surface with sintering.

The Ca/P ratios in the surface layer for the HAP ceramic with acid and heat treatments were measured by XPS, as shown in Table 1. The HAP ceramic with no treatments had a Ca/P ratio of 1.68 (XPS). When the HAP ceramic was treated

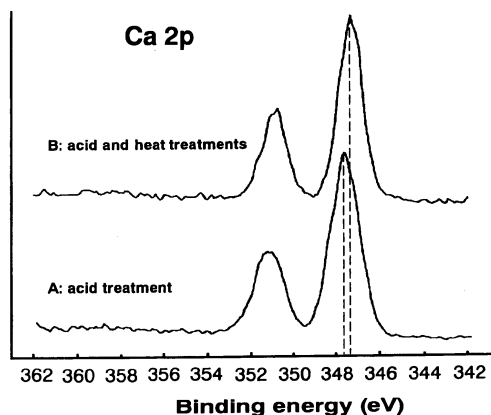


Fig. 5 Ca 2p XPS spectra obtained from the outermost layers of hydroxyapatite specimens treated with 5.0 mol/L orthophosphoric acid solution. A; outermost layer with acid only treatment, B; outermost layer with acid and heat (at 1,250 °C) treatments.

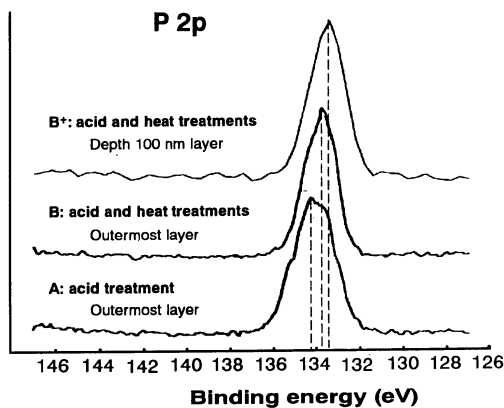


Fig. 6 P 2p XPS spectra obtained from the outermost layers of hydroxyapatite specimens treated with 5.0 mol/L orthophosphoric acid solution. A; outermost layer with acid only treatment, B; outermost layer with acid and heat (at 1,250 °C) treatments, B⁺; layer at a depth of approximately 100 nm with acid and heat (at 1,250 °C) treatments.

with 5.0 mol/L H_3PO_4 solution and heating of 1,250 °C, the outermost layer had a Ca/P ratio of approximately 1.03. Moreover, the Ca/P ratio of the HAP ceramic was increased with increasing depth from the surface, to reach the Ca/P ratio of 1.62 at a depth of approximately 2 μm . The outermost layer with acid only (5.0 mol/L H_3PO_4) treatment had a Ca/P ratio of 0.46. In the case of treatment with 1.0 mol/L H_3PO_4 solution and heating of 1,250 °C, a Ca/P ratio of 1.47 was obtained for the outermost layer. The Ca/P ratio from the surface to depth was also increased with increasing depth, and at 200 nm deep the Ca/P ratio was 1.69. The Ca/P ratio of 1.03 in the outermost layer was obtained by acid treatment with 1.0 mol/L H_3PO_4 solution. On the other hand, the treatments with 0.5 mol/L H_3PO_4 and phosphate-buffered solutions were almost analogous to the case that used 1.0 mol/L H_3PO_4 solution. Ca 2p and P 2p XPS spectra of the HAP ceramic treated with 5.0 mol/L H_3PO_4 solution and heating of 1,250 °C are shown in Figs. 5 and 6. The peaks of Ca 2p spectra on the outermost layer without and with heat-treatment were 347.7 eV and 347.3 eV, respectively (Fig. 5). The binding energies of peaks of Ca 2p spectra with acid and heat treatments were not changed in the range of 347.3-347.4 eV from the surface to 2 μm deep. The P 2p spectrum on the outermost layer with only acid treatment had a binding energy 134.2 eV at the peak. In the case of acid and heat treatments, the P 2p peaks of the outermost layer and about 100 nm-depth layer were 133.7 eV and 133.4 eV, respectively (Fig. 6). Measurements of the binding energies for the specimen surfaces treated only with 0.5 mol/L H_3PO_4 , 1.0 mol/L H_3PO_4

and phosphate-buffered solutions, revealed that those of P 2p peaks on the outermost layers were in the range of 133.5-133.7 eV. However, the outermost layers after heat treatment had binding energies of 133.2-133.4 eV. The Ca 2p peaks of these layers ranged from 347.2 eV to 347.4 eV. No changes in binding energy in the Ca 2p peak were confirmed on the surface or in the inside except when 5.0 mol/L H_3PO_4 solution was used.

DISCUSSION

The results of SEM observations and XPS measurements suggested that the four kinds of phosphoric acid solutions ate away the surface of the HAP ceramic. HAP is known to be chemically stable in neutral pH compared with other calcium phosphates^{11,12)}. However, it is unstable in the low pH zone below pH 4.0 compared with DCPD and MCPM¹¹⁾. Therefore, the surface layer of the HAP ceramic may be exchanged to stable DCPD and MCPM because the phosphoric acid solutions used in the present study were below pH 4.0. Moreover, the result of measurements of the Ca/P ratios suggested that the depth of acid erosion in the HAP ceramic was increased with decreasing pH of the acid solutions. The surface layers of samples subjected only to acid treatment did not contain α -TCP because it was unable to precipitate at low temperatures.

Table 2 shows the binding energies of Ca 2p and P 2p XPS spectra for calcium phosphates, measured by Hanawa *et al.*¹³⁾. The surface layers of HAP ceramics treated with 1.0 and 5.0 mol/L H_3PO_4 solutions were DCPD and MCPM, respectively, confirmed by the chemical shifts of binding energies of Ca 2p and P 2p XPS spectra (Table 2, Figs. 5 and 6). Moreover, the DCPD and MCPM on the outermost layers were reconfirmed by the Ca/P ratios of 1.03 and 0.46, respectively. After heat treatment of these materials, these layers were changed to the Ca/P ratios of 1.47 and 1.03, respectively, because the DCPD and MCPM were decomposed and diffused by heating at 1,250°C. These calcium phosphates are unstable at a temperature of 1,250°C. Moreover, the constituents of the surface layer and second layer will be diffused by heat-treatment. The outermost layers of the HAP ceramic treated with heat treatment are the most likely to change from DCPD and MCPM to α -TCP and calcium pyrophosphate (CPP; $\text{Ca}_2\text{P}_2\text{O}_7$) by diffusion with the second layer, respectively²⁾. However, the chemical shifts of XPS spectra could not confirm the α -TCP

Table 2 Binding energies of Ca 2p and P 2p XPS spectra for calcium phosphates¹³⁾

Calcium phosphate	Ca/P ratio	Binding energy (eV)	
		Ca 2p	P 2p
$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$; HAP	1.67	347.4	133.2
$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$; DCPD	1.00	347.4	133.7
$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$; MCPM	0.50	347.8	134.3
CaO	—	347.2	—

or CPP on the outermost layer after heat treatment. The binding energies of Ca 2p and P 2p for α -TCP were similar to those for HAP. The binding energies of Ca 2p and P 2p for CPP are 347.3 and 133.7 eV, respectively, and were similar to that for DCPD (Table 2). Therefore, in the case of acid treatment with 5.0 mol/L H_3PO_4 solution followed by heat treatment, there was no α -TCP on the outermost layer. However, the second layer at a depth of 100 nm was mostly α -TCP because the P 2p peak and Ca/P ratio had a binding energy of 133.4 eV and 1.42, respectively. On the other hand, the Ca/P ratio confirmed the existence of α -TCP on the outermost layer with the acid and heat treatments except when 5.0 mol/L H_3PO_4 solution was used.

The compositional gradient layer with acid (5.0 mol/L H_3PO_4) and heat treatments was at a depth of approximately $2\mu\text{m}$ from the surface. In the case of the other acid treatments, the depth for the compositional gradient layer was from the outermost layer to around 200 nm. Therefore, it was clear that the compositional gradient layer of the HAP ceramic prepared with the acid and heat treatments in this study was limited to approximately $2\mu\text{m}$. On the other hand, the FG-CCP prepared with the diamond method in our previous study had a compositional gradient layer from the surface to a depth of approximately $200\mu\text{m}$, that is, the layer containing α -TCP⁹). However, it is difficult to estimate the most appropriate thickness of the compositional gradient layer for bioactivity.

The bioactivity and biocompatibility of biomaterials are governed by the character of the surface layer. α -TCP possessing high solubility is not stable under environmental conditions around neutral pH. The α -TCP transforms to HAP in water, *in vitro* or *in vivo*^{3,14}). The α -TCP with high solubility is known to increase its pH when it dissolves in water or solution^{3,14}). This increase in pH in the α -TCP dissolution results in the presence of inflammatory cells in the surrounding biomaterial until several weeks after implantation^{4,5,7,15}). We confirmed that the α -TCP in the surface layer of HAP ceramics was more effective *in vitro* (simulated body fluid) reactions compared with an all HAP ceramic^{16,17}).

We demonstrated that the surface composition of the HAP ceramic could be changed gradually by acid and heat treatments. More rapid dissolution of calcium and phosphate from its surface may be expected than in the case of HAP alone when implanted in the body¹⁸). The application of this method is not limited to HAP ceramics because its method can also be applied to several biomaterials coated with or containing calcium phosphates.

CONCLUSIONS

A HAP ceramic was investigated by surface modification of the compositional gradient layer containing α -TCP using two-step treatments with immersion in H_3PO_4 solution and heating at $1,250^\circ\text{C}$ for 1 hour. The results suggested that the surfaces of specimens could be modified with a compound such as α -TCP. However, the surface-modified layer or compositional gradient layer was remarkably thin, with a maximal thickness of approximately $2\mu\text{m}$. It appears that the compositional gradient layer

containing α -TCP on the surface of HAP has more effective bioactivity than the non-treated HAP ceramic.

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