

# Formation of hydroxyapatite on ceramics consisting of tricalcium phosphate in a simulated body fluid

Tomohiro UCHINO, Kohei YAMAGUCHI, Giichiro KAWACHI, Koichi KIKUTA, Masanobu KAMITAKAHARA\* and Chikara OHTSUKI

Graduate school of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603

\*Graduate School of Environmental Studies, Tohoku University, 6-6-20, Aoba, Aramaki, Aoba-ku, Sendai 980-8579

Tricalcium phosphate (TCP) is used as biodegradable bone substitutes. When TCP is implanted in a bony defect, it is gradually decomposed in living body, and finally substituted by living bone tissue. Solubility of  $\alpha$ -phase of TCP ( $\alpha$ -TCP) is higher than that of  $\beta$ -phase ( $\beta$ -TCP), and hence the degradation of  $\alpha$ -TCP is faster than that of  $\beta$ -TCP. Recently, we fabricated  $\alpha$ -TCP porous ceramic with continuous pores in the range of 10–50  $\mu\text{m}$  as highly bioabsorbable bone fillers. The rate of bioabsorption was controlled by combination of  $\beta$ -TCP with  $\alpha$ -TCP. Although the TCP ceramic is suggested to form hydroxyapatite in a simulated body fluid (SBF), the ability of the apatite formation is affected not only by the phase contents, but also by the porosity of the ceramics. In this study, we fundamentally investigated behavior of the hydroxyapatite formation on  $\alpha$ -TCP ceramics with different surface and biphasic TCP ceramic in SBF proposed by Kokubo et al. Hydroxyapatite formation was observed on the surface of the biphasic TCP consisting of  $\alpha$ -TCP and  $\beta$ -TCP, after soaking in SBF. The surface of the polished  $\alpha$ -TCP ceramic showed the hydroxyapatite formation, but the surface of the as-sintered  $\alpha$ -TCP ceramic did not. The polished surface of the  $\alpha$ -TCP ceramic acted as a significant parameter on the hydroxyapatite formation in body environment. Thus, the polishing of the surface allows the osteoconductivity due to the potential of the hydroxyapatite formation on their surfaces in body environment.

Key-words: Tricalcium phosphate, Simulated body fluid, Hydroxyapatite, Surface morphology, Bioactivity

[Received October 12, 2007; Accepted November 15, 2007] ©2008 The Ceramic Society of Japan

## 1. Introduction

Tricalcium phosphate (TCP) is used as biodegradable bone substitutes.<sup>1)–4)</sup> When TCP is implanted in a bony defect, it is gradually decomposed in living body, and finally substituted by living bone tissue.  $\beta$ -TCP has been commercialized as bone substitutes with bioabsorbability.  $\alpha$ -TCP is the thermodynamically stable phase at temperatures above 1100°C, and shows higher solubility than  $\beta$ -TCP.  $\alpha$ -TCP is used as a major component of bioactive paste for fillers of bone defects, since it is easily transformed to form hydroxyapatite through hydrolysis in aqueous condition. We have recently developed  $\alpha$ -TCP porous ceramic with continuous pores ranging 10–50  $\mu\text{m}$  and with 80% of porosity.<sup>5)</sup> Such a  $\alpha$ -TCP porous ceramic with high porosity can be useful as a scaffold and drug carrier system for bone regeneration. The behaviors of the dissolution and adsorption in body environments are significantly influenced by the surface morphology and porosity of the ceramics. In addition, the absorption of the TCP ceramics is controlled by the combination of  $\alpha$ - and  $\beta$ -phases.<sup>6)</sup>

For evaluation on surface changes of bioactive materials, Kokubo and his colleagues proposed a simulated body fluid (SBF).<sup>7),8)</sup> They reported that the bioactive materials achieve direct bonding to living bone through a hydroxyapatite layer formed on their surfaces after implantation in bony defects. Thus, essential condition for artificial materials to show direct bonding to living bone is a biologically active apatite layer on their surfaces.<sup>9),10)</sup> The formation of the hydroxyapatite layer can be observed in the SBF with ion concentration to those of human blood plasma. The behavior of the hydroxyapatite formation on the material in SBF is

worth to be investigated on bioactivity and bioabsorbability of the developed ceramics. However, behavior of TCP ceramics in SBF is not clarified yet. Therefore, we have still argumentation on the surface reaction of TCP ceramics, although we understand the HAp formation in the body environment is essential for the ceramic materials to achieve direct bonding to living bone, i.e. to show bioactivity. In the present study, we investigated hydroxyapatite formation on the TCP ceramics in SBF. TCP ceramics with different surface treatment were examined, as well as biphasic TCP containing  $\alpha$ - and  $\beta$ -phases due to exposure to SBF.

## 2. Experimental procedure

Procedure for preparing TCP ceramics was schematically shown on Fig. 1.  $\alpha$ -TCP ceramics was prepared through conventional sintering processing.  $\alpha$ -TCP powder was kindly supplied from Taihei Chemical Industrial Co., Ltd., Osaka. The  $\alpha$ -TCP powder was compacted to be rectangular specimen by cold isostatic pressing at 40 MPa. The specimen was heated in SiC furnace to 1400°C at a rate of 5°C·min<sup>-1</sup>, and kept at 1400°C for 12 h, followed by cooling to room temperature at the natural cooling rate in the furnace. Rectangular specimens 6 × 10 × 1 mm<sup>3</sup> in size were cut from the obtained specimen. The surfaces of the obtained rectangular TCP specimens were then polished by a #4000 abrasive paper. The polished specimen was denoted as RP-TCP. RP-TCP specimens were heated again at 1400°C for 12 h to obtain as-sintered surface of  $\alpha$ -TCP specimen. The as-sintered specimen was denoted as RA-TCP. Relative density of RP-TCP and RA-TCP was approximately 95%, due to the geometric measurement and density of 2.86 g·cm<sup>-3</sup>, which is assumed

to be completely  $\alpha$ -TCP phase.

Biphasic TCP consisting of  $\alpha$ - and  $\beta$ -phases was prepared from commercial  $\beta$ -TCP powder (Nacalai Tesque, Inc., Kyoto). The  $\beta$ -TCP was compacted to be circular pellets by uniaxial pressing at 160 MPa. The specimens were heated in SiC furnace to 1400°C at a rate of 5°C·min<sup>-1</sup>, and kept at 1400°C for 12 h, followed by cooling to room temperature at the natural cooling rate in the furnace. The surface of obtained circular TCP sample (14 mm in diameter) was polished by a #4000 SiC abrasive paper. This circular polished sample from commercial  $\beta$ -TCP powders was denoted as CP-TCP. Relative density of CP-TCP was approximately 80%, due to the geometric measurement and density of 2.97 g·cm<sup>-3</sup>, which is assumed to be 50% of  $\alpha$ -TCP phase and 50% of  $\beta$ -TCP.

Three types of the prepared TCP ceramics, RP-TCP, RA-TCP and CP-TCP, were soaked in SBF (Na<sup>+</sup> 142.0, K<sup>+</sup> 5.0, Mg<sup>2+</sup> 1.5, Ca<sup>2+</sup> 2.5, Cl<sup>-</sup> 147.8, HCO<sub>3</sub><sup>-</sup> 4.2, HPO<sub>4</sub><sup>2-</sup> 1.0, and SO<sub>4</sub><sup>2-</sup> 0.5 mol·m<sup>-3</sup>) at pH 7.25 at 36.5°C up to 7 days (d). CP-TCP was soaked in 30 cm<sup>3</sup> of SBF, while RP-TCP and RA-TCP was soaked in 15 cm<sup>3</sup> of SBF to be almost equal to the ratio of volume of SBF against apparent surface of a specimen, that is 0.1 cm<sup>3</sup>·mm<sup>-2</sup>. After soaking

for predetermined periods, the samples were taken out from the fluid, gently rinsed with ultra-pure water, followed by drying at room temperature. The surfaces of the samples before and after soaking in SBF were characterized by X-ray diffraction (XRD) with CuK $\alpha$  radiation, and scanning electron microscopic (SEM) observation. Changes in calcium (Ca) and phosphorus (P) concentrations of SBF after immersions of the specimens were measured by inductively coupled plasma emission spectroscopy. pH of the SBF also was measured with pH meter with glass-electrode.

### 3. Results and discussion

Figure 2 shows XRD patterns of RP-TCP, RA-TCP and CP-TCP before and after soaking in SBF for various periods. Before soaking in SBF, all the peaks on samples RP-TCP and RA-TCP were assigned to  $\alpha$ -TCP phase, while CP-TCP pattern shows  $\beta$ -TCP phase, characterized by peaks at 25.8° and 27.7° in addition to  $\alpha$ -TCP phase. Therefore, CP-TCP ceramic consisted both of  $\alpha$ - and  $\beta$ -phases. After soaking in SBF, peaks at about 26° and 32°, that are assigned to hydroxyapatite, were detected on CP-TCP for 3 and 7 d, and on RP-TCP for 7 d, but not on RA-TCP within 7 d. Figure 3 shows SEM images of RP-TCP, RA-TCP and CP-TCP before and after soaking in SBF for 7 d. Some pores were observed on CP-TCP and RP-TCP samples before soaking in SBF. These specimens had polished surface by #4000 SiC abrasive papers. On the other hand, it was hard to observe such pores on the RA-TCP before soaking in SBF. RA-TCP had smooth surface. After soaking in SBF for 7 d, fine particles were observed on the surfaces of RP-TCP and CP-TCP, whereas not on the surface of RA-TCP. From the results of the detection of hydroxyapatite on XRD patterns, the fine particles were assigned to formed hydroxyapatite in SBF. RA-TCP showed smooth surface even after soaking in SBF for 7 d.

Figure 4 shows the changes in Ca and P concentration of SBF due to immersion of the specimens. Remarkable decreases in calcium and phosphorus were observed on RP-TCP and CP-TCP. In contrast, decrease in calcium ion seems to be less for RA-TCP. Furthermore, phosphorus concentration of SBF after exposure of RA-TCP did not show

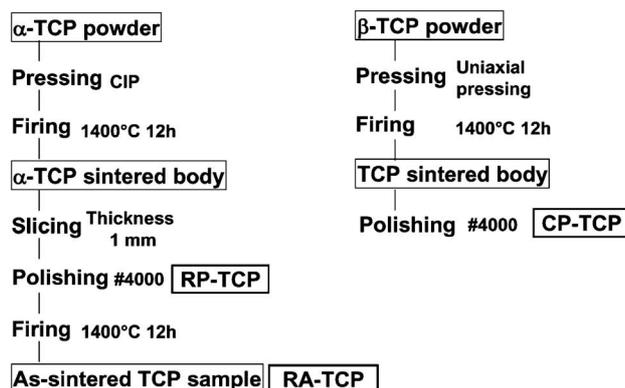


Fig. 1. Procedure of preparing TCP ceramics.

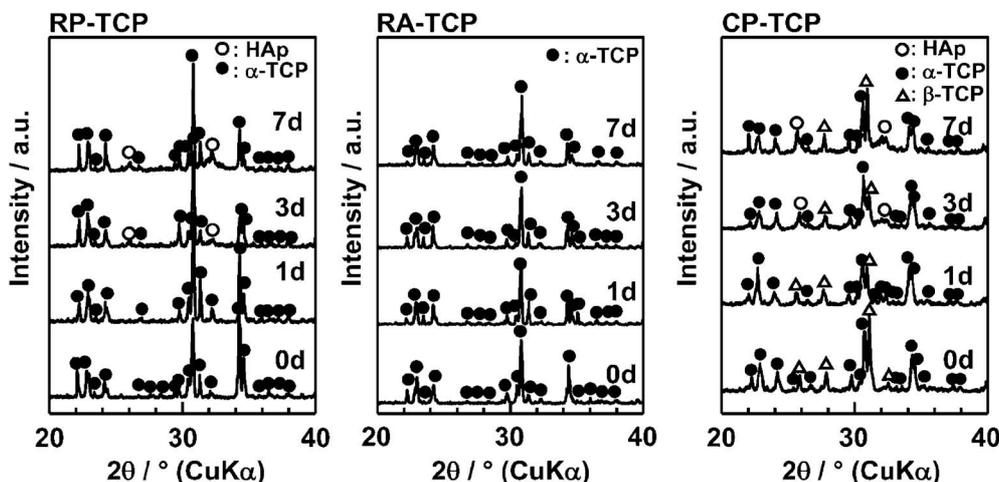


Fig. 2. XRD patterns of RA-TCP, RP-TCP and CP-TCP before and after soaking in SBF for various periods. 0d: before soaking, HAp: hydroxyapatite.

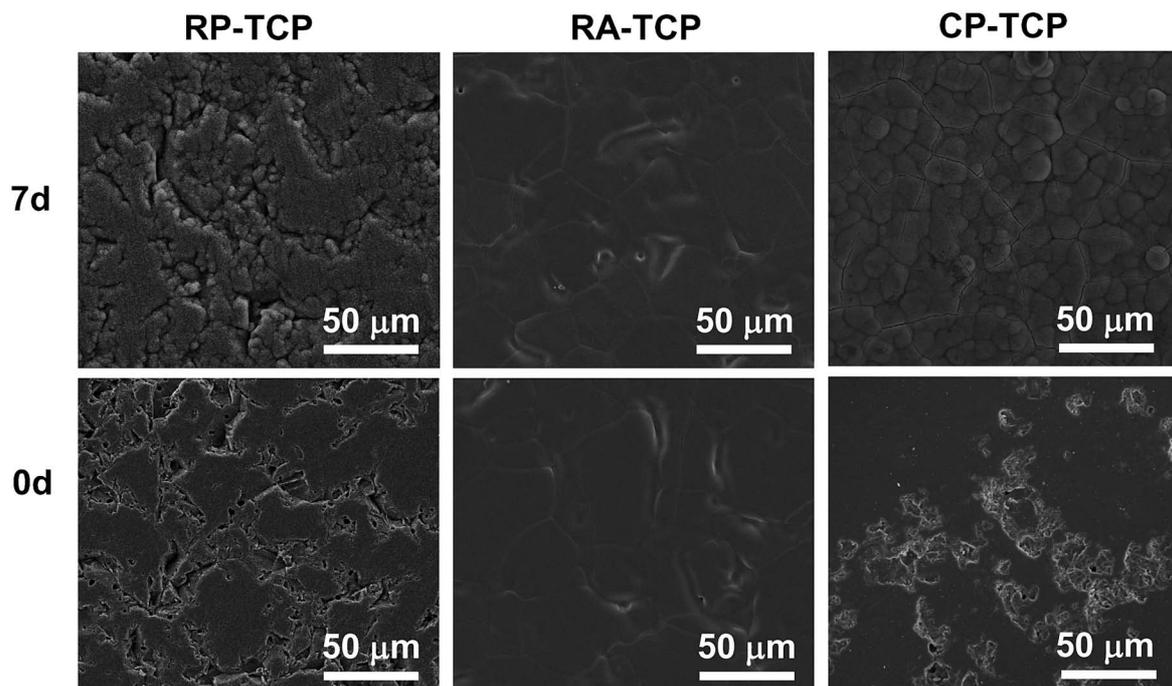


Fig. 3. SEM images of RP-TCP, RA-TCP and CP-TCP before and after soaking in SBF. 0d: before soaking.

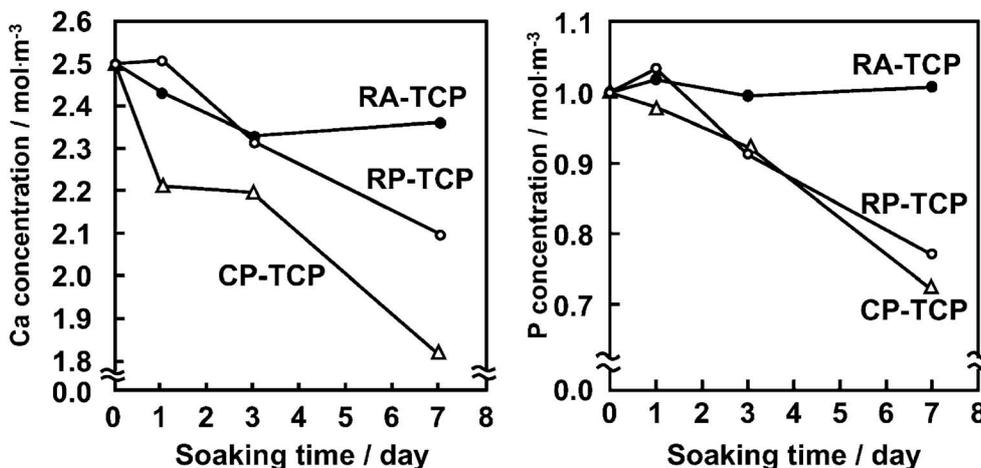


Fig. 4. Ca and P concentrations of SBF due to immersion of TCP specimens. 0d: before soaking.

any changes compared to the others, RP-TCP and CP-TCP. These phenomena imply that hydroxyapatite was formed by consumption of calcium and phosphate ions from surrounding fluid. Differences in changes in pH of the SBF were not observed among the specimens after the immersion. The dissolution of the TCP ceramics and formation of hydroxyapatite on the TCP ceramics did not remarkably affect pH in the surrounding solution. Based on the data given on Figs. 4 and 5, significant decrease in both of the calcium and phosphate ions was observed for RP-TCP and CP-TCP, to be attributed to formation of hydroxyapatite by consumption of calcium and phosphate ions from surrounding fluid. Degree of decrease in the ionic activity product for CP-TCP was larger than for RP-TCP. This suggests that the ability of hydroxyapatite formation on CP-TCP is higher than that on

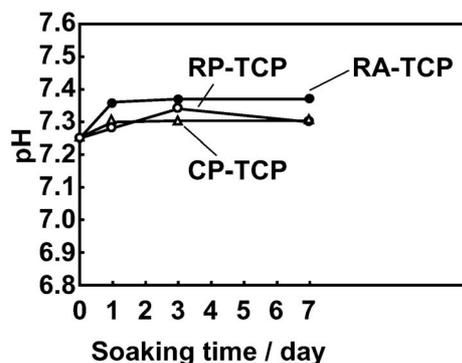


Fig. 5. pH of SBF due to immersion of TCP specimens. 0d: before soaking.

RP-TCP, although the CP-TCP contains  $\beta$ -TCP phase that shows lower solubility than  $\alpha$ -TCP phase.

From the results described above, both RP-TCP and CP-TCP have ability of hydroxyapatite formation on their surfaces in SBF. From the results of changes in ion concentrations, it is said the hydroxyapatite formation was conducted with deposition of the particles through supply of calcium and phosphate ions from surrounding body fluid. This means the hydroxyapatite formation on the TCP ceramics is not simple transformation through its hydrolysis. This is supported by the results of SEM observation that the RA-TCP, which has smooth surface, did not show significant changes after exposure to SBF. Polished surface allows large surface area with high roughness to form a potential surface capable to release ions and/or accumulation of calcium and phosphate ions. CP-TCP has expected to be lower dissolution than RP-TCP, since CP-TCP contains  $\beta$ -TCP with lower solubility than  $\alpha$ -TCP. Nevertheless, CP-TCP seemed to have higher ability on formation of hydroxyapatite in SBF than RP-TCP. Previous study on hydroxyapatite formation on  $\beta$ -TCP ceramics reported that  $\beta$ -TCP ceramics hardly dissolve in SBF.<sup>11)</sup> It is therefore said that the high solubility is not required to form the hydroxyapatite layer, and that the induction of heterogeneous nucleation at the surface of the TCP ceramics governs the potential of hydroxyapatite formation in body environment. Consequently, the morphology of the surface after the polishing TCP ceramics containing  $\alpha$ -TCP governs the hydroxyapatite formation on their surfaces in the body environment. On the design of bone substitute based on TCP ceramics, we have to pay much attention on the morphological effects of the surface on bioactivity and bioabsorbability.

#### 4. Conclusion

Hydroxyapatite formation on  $\alpha$ -TCP ceramics with differ-

ent surface treatment was examined as well as biphasic TCP consisting of  $\alpha$ -TCP and  $\beta$ -TCP. The surface treatment with polishing by an abrasive paper allows higher potential to induce the hydroxyapatite nucleation on their surface of TCP ceramics in the simulated body fluid. Design of surface characteristics can effectively result in higher potential on induction of hydroxyapatite formation in the body environment, and is important not only to control bioabsorbability of TCP ceramics, but also to enhance bioactivity.

#### References

- 1) B. V. Rejda, J. G. Peelen and K. deGroot, *J. Bioeng.*, **1**, 93-97 (1977).
- 2) D. S. Metsger, T. D. Driskell and J. R. Paulsrud, *J. Am. Dent. Assoc.*, **105**, 1035-1038 (1982).
- 3) H. Oonishi and H. Oomamiuda, "Handbook of biomaterial properties," Ed. by J. Black and G. Hastings, Chapman & Hall, London (1998) pp. 406-419.
- 4) H. Yuan, J. D. deBruijn, Y. Li, J. Feng, Z. Yang, K. deGroot and X. Zhang, *J. Mater. Sci.: Mater. Med.*, **12**, 7-13 (2001).
- 5) M. Kitamura, C. Ohtsuki, S. Ogata, M. Kamitakahara and M. Tanihara, *Materials Transactions*, **45**, 983-988 (2004).
- 6) M. Oishi, C. Ohtsuki, M. Kitamura, M. Kamitakahara, S. Ogata, T. Miyazaki and M. Tanihara, *Phosphorus Research Bulletin*, **17**, 95-100 (2004).
- 7) T. Kokubo, H. Kushitani, S. Sakka, T. Kitsugi and T. Yamamuro, *J. Biomed. Mater. Res.*, **24**, 721-734 (1990).
- 8) T. Kokubo and H. Takadama, *Biomaterials*, **27**, 2907-2915 (2006).
- 9) T. Kokubo, *J. Ceram. Soc. Japan (Seramikusu Ronbunshi)*, **99**, 965-973 (1991).
- 10) H.-M. Kim, *J. Ceram. Soc. Japan*, **109**, S49-S57 (2001).
- 11) C. Ohtsuki, T. Kokubo, M. Neo, S. Kotani, T. Yamamuro, T. Nakamura and Y. Bando, *Phosphorus Research Bulletin*, **1**, 191-196 (1991).