

## Comparison of the Effects of Added $\alpha$ - and $\beta$ - Tricalcium Phosphate on the Basic Properties of Apatite Cement

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Effects of added  $\alpha$ -tricalcium phosphate ( $\alpha$ -TCP) and  $\beta$ -TCP were investigated to shed light on the setting reaction of apatite cement (AC) consisting of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA). Added  $\beta$ -TCP showed no reactivity, and thus resulted in extended setting time and decreased mechanical strength. In contrast,  $\alpha$ -TCP dissolved to supply calcium and phosphate ions after initial apatite crystal formation by the reaction of TTCP and DCPA. Although setting time was delayed because  $\alpha$ -TCP was involved only in the latter reaction of apatite cement, larger apatite crystals were formed due to its addition. As a result of larger apatite crystal formation, the mechanical strength of  $\alpha$ -TCP-added apatite cement increased by approximately 30%, as compared to  $\alpha$ -TCP-free apatite cement.

Keywords: Apatite cement, Tricalcium phosphate (TCP), Hydroxyapatite

### INTRODUCTION

Pertaining to the reconstruction of bone defects, apatite cement may be considered as one of the major breakthroughs in biomaterials.

In 1976, an initial finding of the setting reaction of  $\alpha$ -tricalcium phosphate ( $\alpha$ -TCP:  $\text{Ca}_3(\text{PO}_4)_2$ ) was reported by Monma and Kanazawa<sup>1</sup>. They found that when  $\alpha$ -TCP was hydrated in water at 60–100°C and at pH range of 8.1–11.4,  $\alpha$ -TCP set to form calcium-deficient apatite with a Ca/P molar ratio of 1.5. However, owing to its long setting time,  $\alpha$ -TCP was prevented from clinical use for a long time. To shorten the setting time and hence make  $\alpha$ -TCP applicable for clinical use, chelating agents have been studied for use in conjunction with  $\alpha$ -TCP-based cements<sup>2,3</sup>.

In 1986, Brown and Chow reported that a mixture of tetracalcium phosphate (TTCP:  $\text{Ca}_4(\text{PO}_4)_2\text{O}$ ) and dicalcium phosphate anhydrous (DCPA:  $\text{CaHPO}_4$ ) set to form hydroxyapatite (HAP:  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) in a much shorter period, 30–60 minutes, at physiological temperature<sup>9,10</sup>. Although the apatite cement consisting of TTCP and DCPA set much faster than  $\alpha$ -TCP-based cement, its setting time was still too long — and hence impractical — for clinical use.

In 1995, fast-setting apatite cement (fs-AC) was invented by employing neutral hydrogen phosphate as its liquid phase<sup>11,12</sup>. Setting time of fs-AC was five minutes, and this setting time was appropriate and acceptable for clinical use. Presently, fs-AC is commercially available as Bone Source<sup>®</sup>.

Setting reaction of apatite cement is based on

dissolution-precipitation reaction<sup>11,13,14</sup>. When powder phase of apatite cement is mixed with the liquid phase, TTCP and DCPA dissolve to supply  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  as shown in Equations (1) and (2). The solution will be supersaturated with respect to hydroxyapatite, and HAP crystals would thus be precipitated as shown in Equation (3). Precipitated HAP crystals then form a cluster upon setting.



It has also been reported that powder size influences the basic properties of apatite cement. For example, a mixture of large TTCP particles and small DCPA particles would render apatite cement with good mechanical strength, whereas combination of small TTCP and large DCPA particles showed no setting reaction<sup>10</sup>. This is also closely related to the function of the powder phase. In the setting reaction of apatite cement, the chief role of the powder phase is to supply  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  in the reaction medium. Against this background, addition of other calcium phosphates might inadvertently affect the basic properties of apatite cement.

In the present study, the aim was to investigate the effects of added  $\alpha$ -TCP and  $\beta$ -TCP on the basic properties of TTCP-DCPA apatite cement by examining the setting reaction of apatite cement. To this end, setting time and diametral tensile strength were measured, and compositional and SEM analyses

were performed.

## MATERIALS AND METHODS

### *Preparation of base apatite cement*

For base apatite cement, an equimolar mixture of TTCP and DCPA was chosen. TTCP was synthesized by heating a mixture of DCPA ( $\text{CaHPO}_4$ ; Aldrich, USA) and calcium carbonate ( $\text{CaCO}_3$ ; Wako Pure Chemical Industries Ltd., Osaka, Japan) at 1500 °C for six hours in an alumina crucible, followed by air-quenched to room temperature inside a desiccator to avoid transformation to HAP. Synthesized TTCP was crushed using a manual mortar and pestle and sieved using a 45- $\mu\text{m}$  screen. Commercial DCPA was ground in 90% ethanol using a planetary rotating mill for several hours to get an average particle size of about 1  $\mu\text{m}$ .

### *Preparation of experimental apatite cement*

Commercially available  $\beta$ -TCP powder ( $\beta$ -TCP B, Taihei Chemical Co., Japan) was used as an additive. As for  $\alpha$ -TCP, commercially obtained  $\alpha$ -TCP was heated at 950 °C for 24 hours. This heat treatment produced  $\beta$ -TCP based on phase transformation reaction keeping its original morphology. TCP thus obtained was mixed as an additive to the base cement powder in ratios of 5, 10, 20, 40, and 60 wt%. In this paper, type and content of added TCP were given in parentheses. For example, apatite cement containing 10 wt%  $\beta$ -TCP was given as AC( $\beta$ :10%).

For the liquid phase of apatite cement, 0.2 mol/L of neutral sodium hydrogen phosphate was prepared as described previously<sup>11,12</sup>. In short, 0.2 mol/L of sodium dihydrogen phosphate was mixed with 0.2 mol/L of disodium hydrogen phosphate to yield pH 7.4. The resultant solution had approximately  $\text{Na}_{1.8}\text{H}_{1.2}\text{PO}_4$  as its chemical formula.

### *Cement preparation and aging*

The powder thus prepared was mixed with the liquid phase at a powder to liquid ratio of 4.0 with a stainless steel spatula, and then packed into a split stainless mold (6 mm in diameter  $\times$  3 mm in height). Both ends of the mold were covered with glass plates and aged at 37 °C and 100% relative humidity for 1, 2, 4, 8, 24, and 168 hours.

### *Setting time measurement*

Setting time was measured using a standard Vicat needle apparatus based on measurement method set forth for dental zinc phosphate cement (ISO 1566). The powder component was mixed with the liquid phase on a glass slab and packed into a Teflon mold. Mixed cements were kept inside these molds at 37 °C and 100% relative humidity. Specimens were checked from time to time using the testing apparatus. The needle with 1-mm diameter was gently placed on the

surface of the specimen at a load of 300g. Setting time was defined as the elapsed time until the needle failed to penetrate or produces flaws on the surface of the cement. An average setting time was obtained from three specimens.

### *Scanning electron microscopic observation*

For scanning electron microscopic (SEM) analysis, set specimen was critical point-dried (Hitachi) and mounted on a metal holder for gold coating. Microstructure of the specimen was then observed using a scanning electron microscope (FE-SEM; S-4300 SE, Hitachi Ltd., Tokyo, Japan) with an accelerating voltage of 15 kV.

### *Compositional analysis*

The composition of set apatite cement was evaluated by means of powder X-ray diffraction (XRD). The XRD patterns of vacuum-dried and powdered samples were recorded with a vertically mounted diffractometer system (Rint 2000, Rigaku, Tokyo, Japan) using  $\text{CuK}\alpha$  radiation generated at 40 kV and 100 mA.

Fourier transform infrared (FT-IR) spectra were also taken with a FT-IR spectrophotometer (Perkin Elmer FTIR Spectrometer, Spectrum 2000LX, USA) by using a KBr disk method.

### *Mechanical strength measurement*

Mechanical strength of the aged specimens was evaluated in terms of diametral tensile strength (DTS). After the diameter and height of each specimen were measured with a micrometer (156-101, Mitsutoyo Co. Ltd., Kanagawa, Japan), the specimen was crushed using a universal testing machine (SV-301, IMADA, Japan) at a crosshead speed of 1 mm/min. Each DTS value was an average of at least five specimens.

### *Statistical analysis*

For statistical analysis, one-way factorial analysis of variance and Fisher's PLSD method as a *post hoc* test were performed using a computer software, Stat View 4.02 (Abacus Concept, Berkeley, CA).

## RESULTS

Table 1 summarizes the effects of added  $\beta$ -TCP or  $\alpha$ -TCP on the setting time of apatite cements consisting of an equimolar mixture of TTCP and DCPA. As shown in the table, setting time was extended significantly ( $p < 0.01$ ) with TCP addition regardless of TCP type, except for 5% addition which showed no significant differences. For an equivalent amount of added TCP, it was clearly shown that  $\beta$ -TCP addition would result in a longer setting time as compared to  $\alpha$ -TCP.

Table 1 Setting times of apatite cements containing  $\alpha$ -TCP or  $\beta$ -TCP

Amount of added TCP (wt%)	Setting time (min)	
	$\alpha$ -TCP	$\beta$ -TCP
0	8.0 $\pm$ 0.6	
5	9.0 $\pm$ 1.2	9.0 $\pm$ 0.6
10	13.0 $\pm$ 1.7	23.0 $\pm$ 2.9
20	22.0 $\pm$ 2.9	28.0 $\pm$ 2.9

Figure 1 shows the SEM photographs of added  $\alpha$ -TCP and  $\beta$ -TCP, as well as the fracture surfaces of apatite cement aged for 168 hours. Since  $\beta$ -TCP was prepared based on the phase transformation of  $\alpha$ -TCP, the morphologies of added  $\alpha$ -TCP (Fig. 1(a)) and  $\beta$ -TCP (Fig. 1(b)) were the same. With respect to the morphology of apatite cement after aging for 168 hours, set apatite cement containing no TCP demonstrated needle-like crystals typical of apatite crystals (Fig. 1(c)). Likewise, similar morphology was found for apatite cements containing  $\alpha$ -TCP (Figs. 1(d), (e), (f)). Interestingly, plate-like crystals as shown in Fig. 1(f) were also observed in  $\beta$ -TCP-

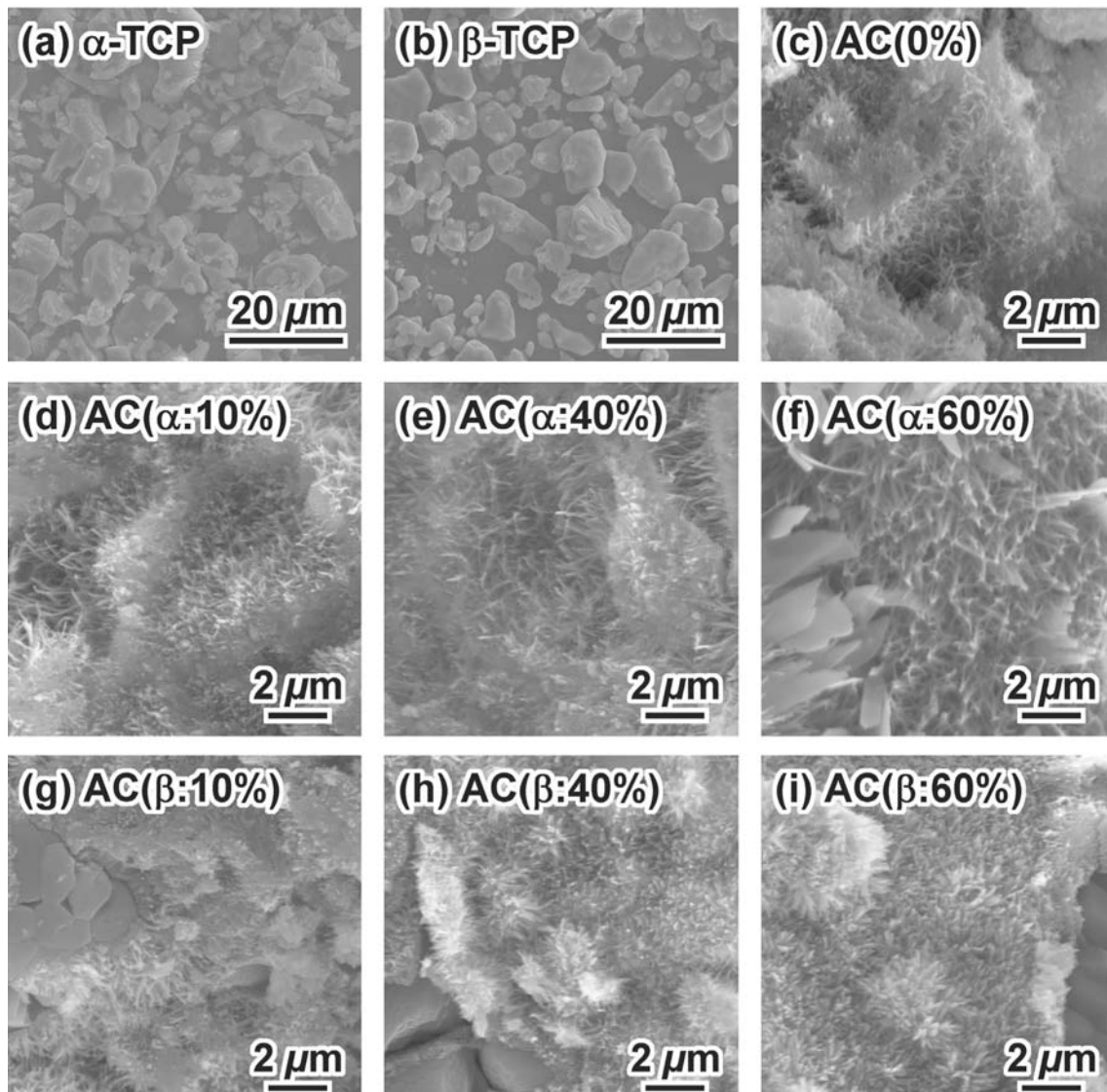


Fig. 1 SEM photographs of added  $\alpha$ -TCP (a) and  $\beta$ -TCP (b), and fracture surfaces of apatite cements aged for 168 hours ((c)-(i)).

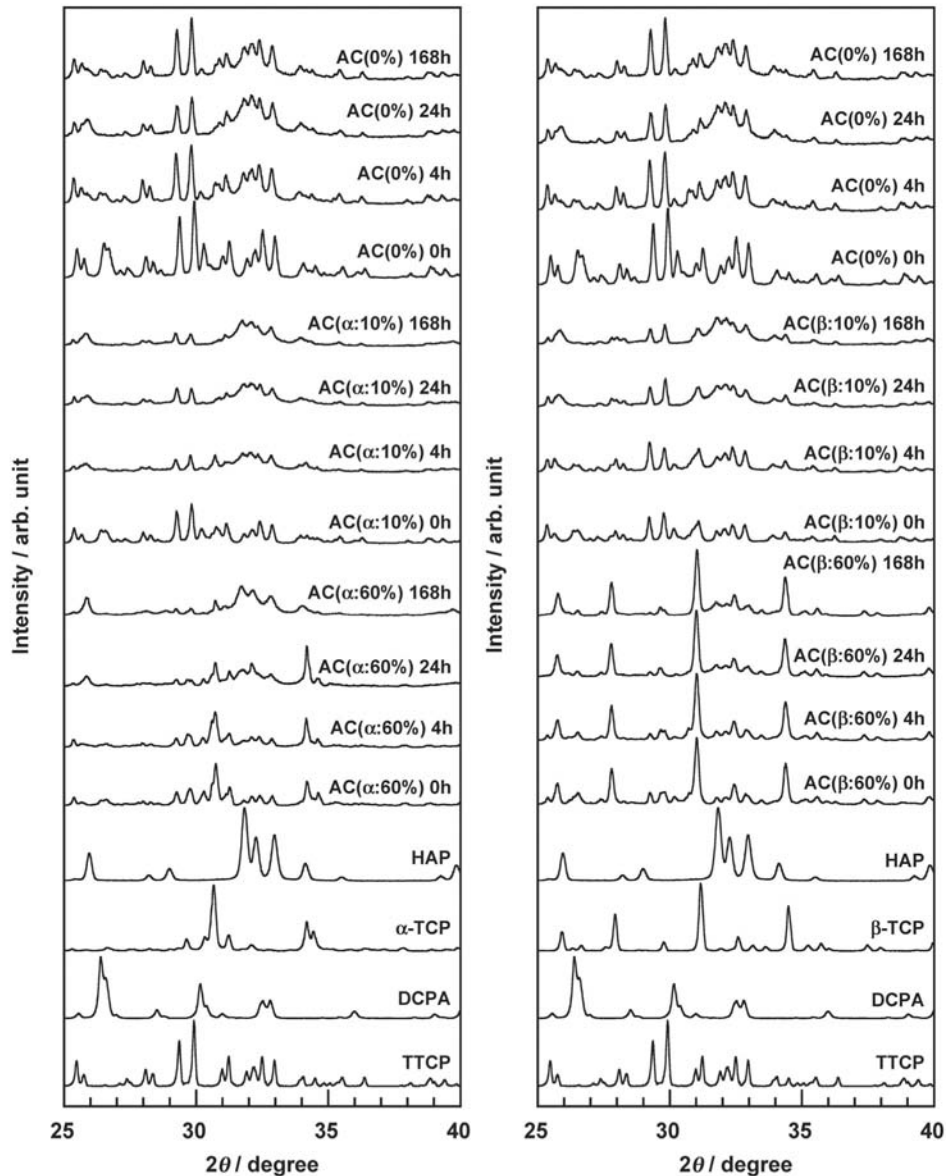


Fig. 2 XRD patterns of cements containing 0, 10, and 60% of  $\alpha$ -TCP or  $\beta$ -TCP aged for various times up to 168 hours.

added apatite cements, whereby the amount of plate-like crystals increased with increase in  $\alpha$ -TCP content. Moreover, the size of needle-like crystals also increased with the amount of added  $\alpha$ -TCP. No plate-like crystals were seen for apatite cements containing  $\beta$ -TCP, but particles similar to  $\alpha$ -TCP (Fig. 1(b)) were observed.

Figure 2 summarizes the XRD patterns of apatite cements containing 0, 10, and 60% of  $\alpha$ -TCP before and after aging for 4, 24, and 168 hours. XRD patterns of TTCP, DCPA, HAP,  $\alpha$ -TCP, and  $\beta$ -TCP were also shown for comparison.

Formation of apatite was confirmed by the appearance of  $25.9^\circ (2\theta)$  and  $31.8^\circ (2\theta)$  peaks (corresponding to apatite) alongside a decrease in

DCPA doublet peak around  $26.5^\circ (2\theta)$  after 4 hours. The increase in peak corresponding to apatite and decrease in peak corresponding to DCPA continued with time, and no peaks corresponding to DCPA were observed at 24 hours and thereafter.

In the case of AC( $\alpha$ :10%), the reaction between TTCP and DCPA was slightly faster when compared to AC(0%). At 4 hours, a diffraction peak of HAP at  $25.9^\circ (2\theta)$  was seen and that of DCPA around  $26.5^\circ (2\theta)$  had almost disappeared. On the other hand, relative intensity of  $\alpha$ -TCP peaks was higher compared to that of TTCP at 4 hours. This finding suggested that the reaction between TTCP and DCPA preferentially occurred at an initial period, and that  $\alpha$ -TCP was not involved in the initial reaction.

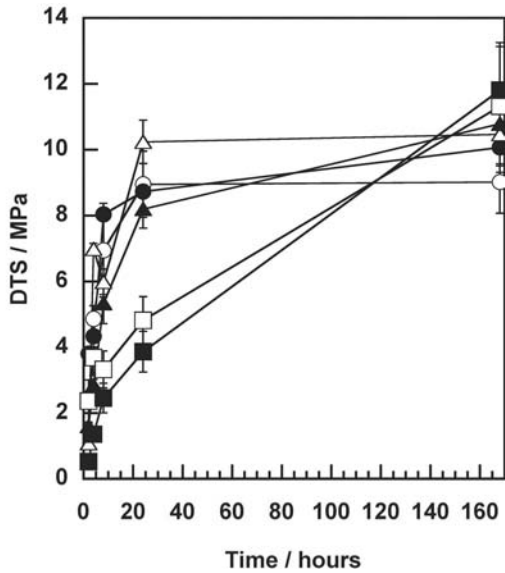


Fig. 3 Changes in DTS with time for cements doped with different amounts of  $\alpha$ -TCP. :AC(0%); :AC( :5%); :AC( :10%); :AC( :20%); :AC( :40%); :AC( :60%).

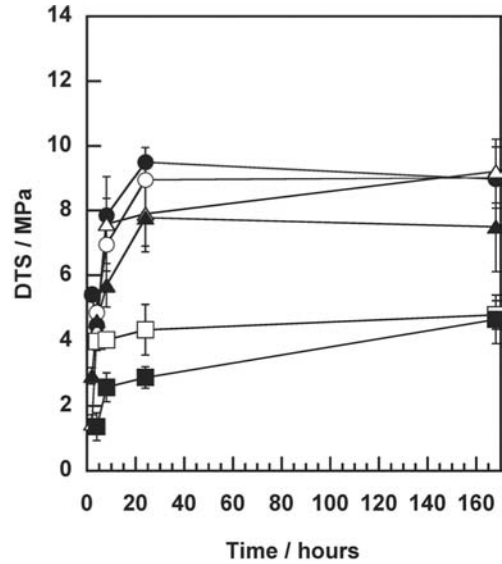


Fig. 5 Final changes in DTS at 168 hours according to the amount of  $\beta$ -TCP (a) or  $\alpha$ -TCP (b) added to the cement.

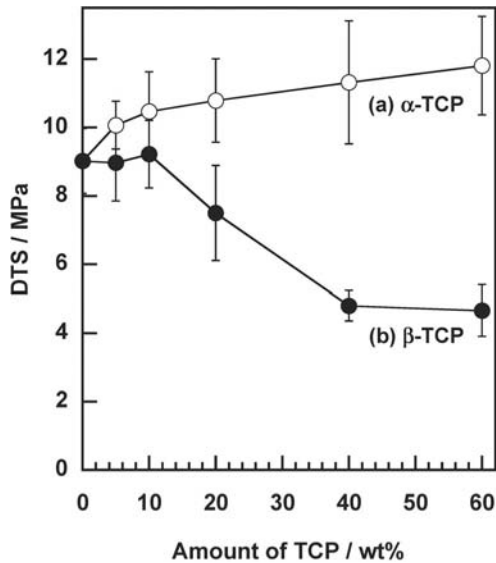


Fig. 4 Changes in DTS with time for cements doped with different amounts of  $\alpha$ -TCP. :AC(0%); :AC( :5%); :AC( :10%); :AC( :20%); :AC( :40%); :AC( :60 %).

The amount of  $\alpha$ -TCP decreased with time. This clearly demonstrated that  $\alpha$ -TCP was involved in the setting reaction of apatite cement and served as the source of apatite formation. The same tendency was observed in the case of AC( :60%).

In contrast, AC( :10%) showed a similar XRD pattern as that of AC(0%) at each aging period,

except for the existence of peaks corresponding to  $\alpha$ -TCP.

Figures 3 and 4 summarize the DTS values of apatite cements containing  $\alpha$ -TCP and  $\beta$ -TCP as a function of aging time, respectively. Basically, DTS increased with time to reach the highest value regardless of the presence or absence of TCP, nor the type or amount of added TCP. Time required to reach the maximum value was 4 - 24 hours for apatite cements containing no TCP or  $\alpha$ -TCP, or apatite cements containing a small amount of  $\alpha$ -TCP (5 or 10%). However, apatite cements containing a large amount of  $\alpha$ -TCP (40% and 60%) took a much longer time to reach the maximum value.

Figure 5 summarizes the effects of added TCP on DTS value at 168 hours as a function of added TCP. As shown, higher DTS values were observed ( $p < 0.05$ ) for apatite cements containing  $\alpha$ -TCP versus  $\beta$ -TCP-free apatite cement.

## DISCUSSION

The present study clearly demonstrated that the addition of different types of TCP, *i.e.*,  $\alpha$ -TCP or  $\beta$ -TCP, wielded different effects on the basic properties of apatite cement. In short,  $\alpha$ -TCP was involved in the setting reaction of apatite cement, whereas  $\beta$ -TCP was not. In the same vein, Ishikawa *et al.* investigated the effect of Zn-doped  $\beta$ -tricalcium phosphate (ZnTCP) on the basic properties of and cell response to apatite cement<sup>15</sup>. They found that ZnTCP showed no reactivity during the setting

reaction of apatite cement. As for the cell study, it was found that proliferation of human osteoblastic cells significantly increased when the cells were incubated on the surface of apatite cement containing ZnTCP, as compared to ZnTCP-free apatite cement. Although the detailed mechanism was not clarified in the paper<sup>15)</sup>, Zn which is known to have osteogenesis stimulation effect was released from ZnTCP-containing apatite cement. Released Zn was thus thought to affect the cell response.

No reactivity of -TCP with TTCP-DCPA type apatite cement was found in the present study. This finding served to highlight the usefulness of -TCP as an effective carrier of trace elements in apatite cement. However, this very trait of no reactivity of -TCP with apatite cement also became a drawback. As a result of no reactivity, -TCP acted as a pore in AC. Indeed, decrease in mechanical strength is a typical outcome of -TCP's non-reactivity. Extended setting time was the other outcome of no reactivity in the present study. This was because the setting time used in this study measured the time required for the presence of some mechanical strength.

In contrast to -TCP, -TCP showed reactivity with apatite cement. However, it was demonstrated that -TCP was involved in the latter reaction of apatite cement. In other words, TTCP and DCPA reacted much faster than the reaction with -TCP. As a result, -TCP addition resulted in a delay of setting time since setting time in the present study focused on initial reaction. At the initial period, it seemed that -TCP reacted with neither TTCP nor DCPA. The outcomes were therefore a decrease in initial mechanical strength and extended setting time.

-TCP gradually dissolved to supply calcium and phosphate ions. SEM observation revealed that -TCP-added apatite cement showed larger crystals when compared to -TCP-free apatite cement. Calcium and phosphate ions released from -TCP were thought to precipitate on the apatite crystals formed by the setting reaction of TTCP and DCPA.

As stated above, the setting reaction of apatite cement hinged on the interlocking of precipitated apatite crystals. Further, the formation of larger apatite crystal seemed to be beneficial in increasing mechanical strength.

## CONCLUSIONS

The present study demonstrated the usefulness of TCP addition to apatite cement. Added -TCP showed no reactivity with apatite cement. Thus, it might be useful as a carrier of trace elements in apatite cement. On the contrary, -TCP was useful in increasing the mechanical strength of apatite cement. Based on these interesting, initial findings,

further studies ought to be carried out to thoroughly explore the usefulness of TCP addition.

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