Effects of liquid phase on basic properties of α-tricalcium phosphate-based apatite cement

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Effects of liquid phase on the basic properties of α-tricalcuim phosphate (α-TCP)-based cement, BIOPEX®, were investigated by employing three liquid phases: distilled water, neutral sodium hydrogen phosphate solution, and succinic acid disodium salt solution containing sodium salt of chondroitin sulfate. When mixed with neutral sodium hydrogen phosphate or succinic acid disodium salt solution, the initial setting times of the cement were 19.4 ± 0.55 and 11.8 ± 0.45 minutes respectively. These setting times were much shorter than that of distilled water, 88.4 ± 0.55 minutes. Formation of needle-like crystals typical of apatite was much faster when neutral sodium hydrogen phosphate solution was used, as compared to distilled water or succinic acid disodium salt solution. Moreover, at 24 hours after mixing, the largest amount of apatite was formed when neutral sodium hydrogen phosphate solution was used, whereas use of succinic acid resulted in the least. On the final mechanical strength of the cement, that yielded with neutral sodium hydrogen phosphate solution was the highest. In contrast, lower mechanical strength was observed — especially at the initial stage — when succinic acid sodium salt was used. It was thus concluded that α-TCP-based cement allowed accelerated transformation to apatite, and that higher mechanical strength since the initial stage was achieved when neutral sodium hydrogen phosphate solution was used as the liquid phase.

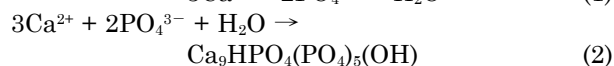*Key words:* Apatite cement, Tricalcium phosphate, Apatite, Phosphate

INTRODUCTION

Apatite cement (AC) has attracted much attention and generated much interest in orthopedic and dental fields for bone reconstruction. This is largely because of its twofold benefits: AC paste can set *in situ* and the final product after complete setting is apatite[1-11]. Due to the formation of apatitic structure, set AC shows excellent tissue response and good osteoconductivity.

At the same time, it must be duly highlighted that if AC fails to set and if calcium phosphate powder is released into the soft tissue, it will invoke an inflammatory response called crystalline inflammatory response. In plastic and reconstructive surgery, as well as oral and maxillofacial surgery, AC paste may be exposed to body fluid before its setting reaction. Consequently, due to the failed setting of AC, many clinical failure cases occurred because unset AC paste lacked the mechanical strength to withstand external stress. Therefore, a key factor for the success of bone defect reconstruction using AC is to guarantee the setting reaction of AC. However, understanding of the setting reaction of AC sorely pales in comparison to its importance for clinical use.

The setting reaction of AC is fundamentally a dissolution-precipitation reaction. In the case of α-TCP-based cement, α-TCP dissolves to supply Ca^{2+} and PO_4^{3-} into the liquid phase as shown in Eq. (1). Subsequently, the solution becomes supersaturated with apatite, leading to the formation of needle-like crystals of apatite as shown in Eq. (2). These apatite crystals interlock with each other to form a set mass, a phenomenon similar to the setting reaction of gypsum[12-14].

$$3Ca_3(PO_4)_2 + H_2O \rightarrow$$
$$3Ca^{2+} + 2PO_4^{3-} + H_2O \qquad (1)$$
$$3Ca^{2+} + 2PO_4^{3-} + H_2O \rightarrow$$
$$Ca_9HPO_4(PO_4)_5(OH) \qquad (2)$$

On setting time, Ishikawa *et al.*[13-16] have reported that tetracalcium phosphate (TTCP: $Ca_4(PO_4)_2O$)-dicalcium phosphate anhydrous (DCPA: $CaHPO_4$)-based fast-setting AC (fs-AC). Distinctive difference between a conventional AC (c-AC) and a fs-AC lies in their liquid phase: fs-AC used neutral sodium phosphate aqueous solution instead of distilled water, which is used for c-AC. With TTCP-DCPA-based cement, the dissolution of DCPA is the rate-determining step due to its limited solubility *versus* that of TTCP. Furthermore, PO_4^{3-} ions are required for apatite formation and that the dissolution of DCPA supplies PO_4^{3-} ions more

dominantly than TTCP. Since the supply of PO_4^{3-} ions is critical to apatite formation, it was reasonable to use neutral sodium hydrogen phosphate solution as the liquid phase to accelerate the setting reaction. On the contrary, the setting reaction of α-TCP based cement was historically modified by employing dicarboxylic acid. Dicarboxylic acid forms chelate bonds with calcium-containing compounds. Therefore, a faster setting reaction can be achieved by employing dicarboxylic acid as the liquid phase. However, dicarboxylic acid is known to inhibit the formation of apatite.

In the present study, therefore, the effect of liquid phase on the basic properties of α-TCP-based AC was investigated with a view to obtaining a deeper insight and better understanding of α-TCP-based AC.

MATERIALS AND METHODS

Cement preparation and aging
Commercially available AC, α-TCP-based BIOPEX®, was kindly donated by PENTAX Co. (Tokyo, Japan) and used as a base cement in this study without any modification. Powder phase of BIOPEX® was mixed with three kinds of liquid phases — namely distilled water, neutral sodium hydrogen phosphate solution, or succinic acid disodium salt solution containing chondroitin sulfate. Neutral sodium hydrogen phosphate solution was prepared by mixing 0.25 mol/L NaH_2PO_4 (Wako Pure Chemical Industries Ltd., Osaka, Japan) and 0.25 mol/L Na_2HPO_4 (Wako Pure Chemical Industries Ltd., Osaka, Japan) so that the solution pH became 7.4 at room temperature. Liquid phase of BIOPEX® was used as the succinic acid disodium salt solution containing sodium salt of chondroitin sulfate.

Powder phase was mixed with liquid phase at a powder-to-liquid ratio of 3.0 using a stainless steel cement spatula on a glass plate. The obtained cement paste was packed into split stainless molds (6 mm diameter×3 mm height). Both ends of the mold were covered with glass plates and aged at 37°C and 100% relative humidity for various time intervals of 4, 8, 24, and 168 hours in 100% relative humidity at 37°C. After aging according to the different designated periods, the set cements were taken out from the molds, immersed in acetone (Wako Pure Chemical Industries Ltd., Osaka, Japan) to terminate the setting reaction of the cement, and dried at 60°C.

Setting time measurement
Setting time was measured using a standard Vicat needle apparatus based on the measurement method set forth for dental zinc phosphate cements (ISO 1566). The powder component was mixed with the liquid phase on a glass slab and packed in 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 300 g. Setting time was defined as the elapsed time until the needle failed to penetrate or produce flaws on the surface of the cement. The average setting time was obtained from three specimens.

Characterization
Morphologies of the set cements were observed under a scanning electron microscope (SEM; JSM-5400LV, JEOL Co. Ltd., Tokyo, Japan) at 15 kV of accelerating voltage after gold sputter coating. Composition of the set cement was characterized using an X-ray diffractometer (XRD; RINT 2500V, Rigaku, Tokyo, Japan) using counter-monochromatized CuKα radiation generated at 40 kV and 100 mA at a scanning rate of 2°/min.

Mechanical strength measurement
Mechanical strength was evaluated in terms of diametral strength (DTS). Set cements of 6 mm diameter and 3 mm height were crushed using a universal testing machine (SV-301, Imada Manufacturing Co., Aichi, Japan) at a crosshead speed of 1 mm/min. Each DTS value was calculated and averaged from at least 10 specimens.

RESULTS

Table 1 summarizes the initial setting times of BIOPEX® mixed with various liquid phases: distilled water, neutral sodium hydrogen phosphate solution, or succinic acid disodium salt solution. The initial setting times of the cement mixed with neutral sodium hydrogen phosphate and succinic acid disodium salt solution were 19.4±0.55 and 11.8±0.45 minutes respectively, which were much faster than that mixed with distilled water, 88.4±0.55 minutes.

Figure 1 shows the SEM images of the fractured surfaces of BIOPEX® pre-hardened in an incubator kept at 37°C and 100% relative humidity for 24 hours (Figs. 1(A)−(C)) and 168 hours (Figs. 1(D)−(F)) after mixing with one of these liquid phases: distilled water (Figs. 1(A) and (D)), neutral sodium hydrogen phosphate solution (Figs. 1(B) and (E)), or succinic acid disodium salt solution (Figs. 1(C) and (F)). After 24 hours of aging, particles of approximately 6 μm in size embedded in needle-like crystals were observed in the set cement mixed with succinic acid disodium salt solution (Fig. 1(C)). In the cement mixed with distilled water or neutral sodium hydrogen phosphate solution, assemblies of needle-like crystals were found (Figs. 1(A) and (B)).

It should be noted that features of needle-like crystals in the cement mixed with neutral sodium hydrogen phosphate solutions were more distinct and prominent than that mixed with distilled water. This indicated that the growth of needle-like crystals was accelerated in neutral sodium hydrogen phosphate when compared with distilled water. After 168 hours, assemblies of needle-like crystals were found in all specimens and no significant differences were found among them.

Figure 2 shows the powder XRD patterns of BIOPEX® pre-hardened in an incubator kept at 37°C and 100% relative humidity for 4, 8, 24, and 168 hours after mixing with one of these liquid phases: distilled water (Fig. 2(A)), neutral sodium phosphate solution (Fig. 2(B)), or succinic acid disodium salt solution (Fig. 2(C)). The pattern of commercial hydroxyapatite (HAp) is also shown for comparison. In the XRD patterns of powder phase before mixing with liquid phase, which were indicated as "0 h" in Fig. 2, diffraction peaks assigned to α-TCP were detected although small peaks assigned to other calcium phosphates contained in BIOPEX®, such as HAp, TTCP, and dicalcium phosphate dihydrate, were also detected. After mixing with liquid phase and aging, peaks corresponding to α-TCP gradually decreased. Broadened peaks assigned to HAp were simultaneously detected in all specimens and their intensities increased with time. When comparing the patterns at 24 hours, intensity of HAp peak was the highest for the cement mixed with neutral sodium hydrogen phosphate solution, and which decreased in the order of neutral sodium hydrogen phosphate solution > distilled water > succinic acid disodium salt solution. It seemed that the increase rate of apatitic phase in the cement was the highest when mixed with sodium hydrogen phosphate solution. No significant differences were found among them at 168 hours.

Figure 3 shows the changes in DTS of BIOPEX® pre-hardened in an incubator kept at 37°C and 100% relative humidity for 1, 4, 8, 24, and 168 hours after mixing with one of these liquid phases: distilled water (Fig. 3(A), neutral sodium hydrogen phosphate

Table 1 Initial setting times of Biopex® mixed with various liquid phases: distilled water, neutral sodium hydrogen phosphate solution, or succinic acid sodium salt solution

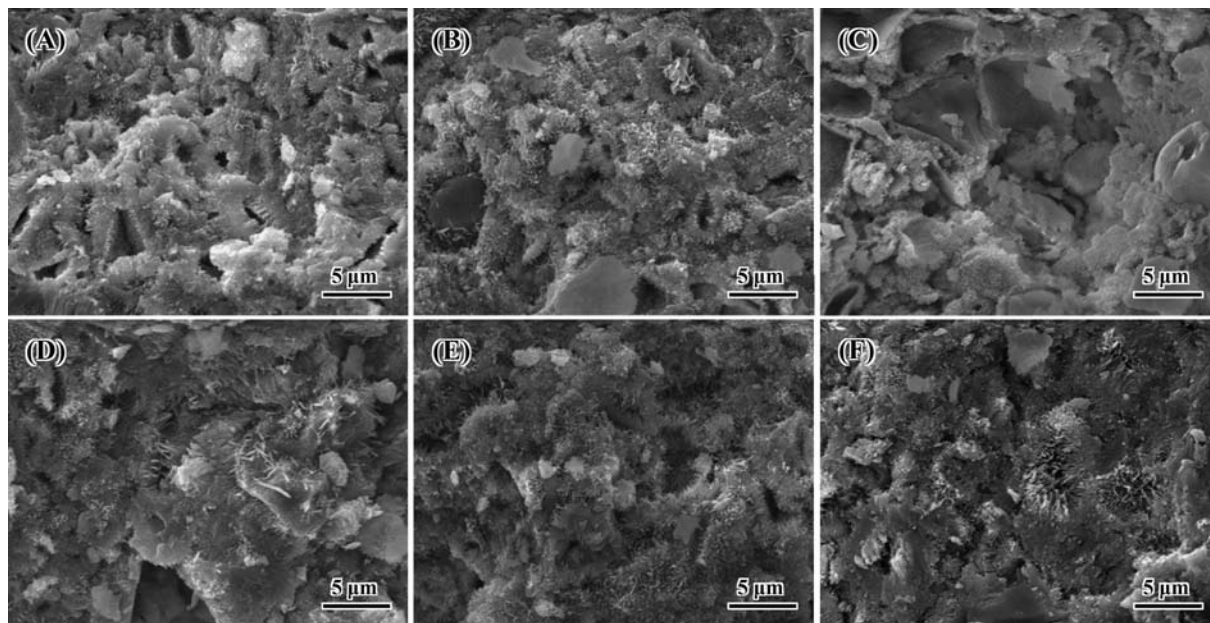| Liquid phase	Initial setting time/ min
Distilled water	88.4±0.55
Neutral sodium hydrogen phosphate solution	19.4±0.55
Succinic acid disodium salt solution	11.8±0.45

Fig. 1　SEM images of the fractured surfaces of Biopex® pre-hardened in an incubator kept at 37°C and 100% relative humidity for 24 hours (A, B, C) and 168 hours (D, E, F) after mixing with various liquid phases: distilled water (A, D), neutral sodium hydrogen phosphate solution (B, E), or succinic acid sodium salt solution (C, F).
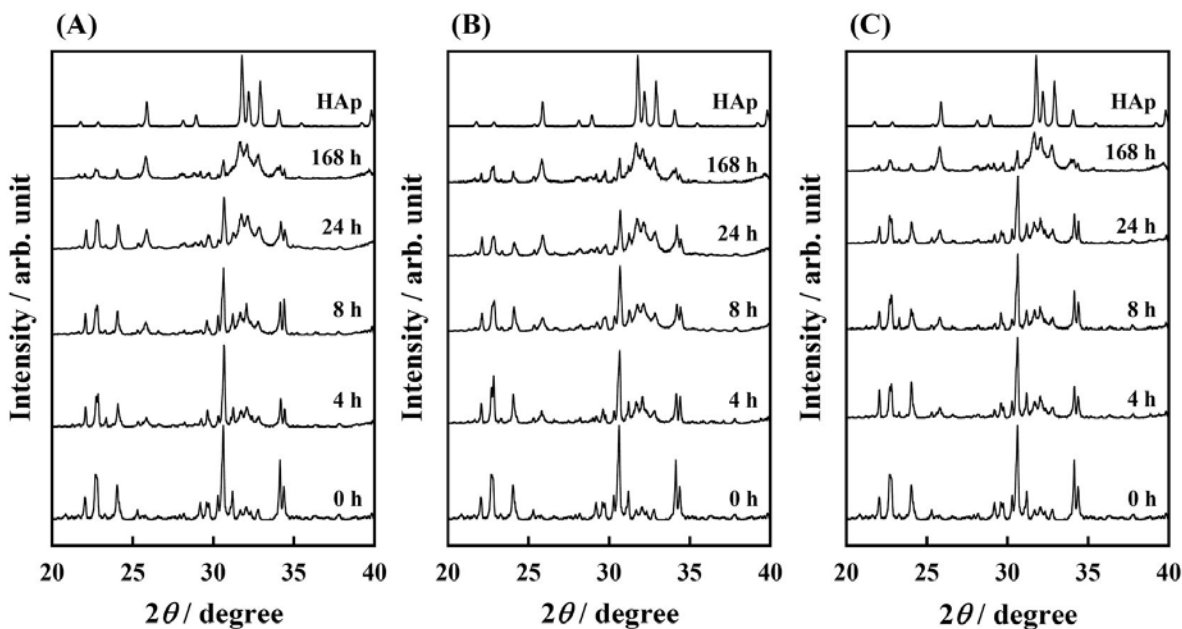
Fig. 2   XRD patterns of Biopex® pre-hardened in an incubator kept at 37°C and 100% relative humidity for 4, 8, 24, and 168 hours after mixing with various liquid phases: distilled water (A), neutral sodium hydrogen phosphate solution (B), or succinic acid sodium salt solution (C). "0 h" indicates the powder phase of Biopex®. The pattern of commercial hydroxyapatite (HAp) is also shown for comparison.
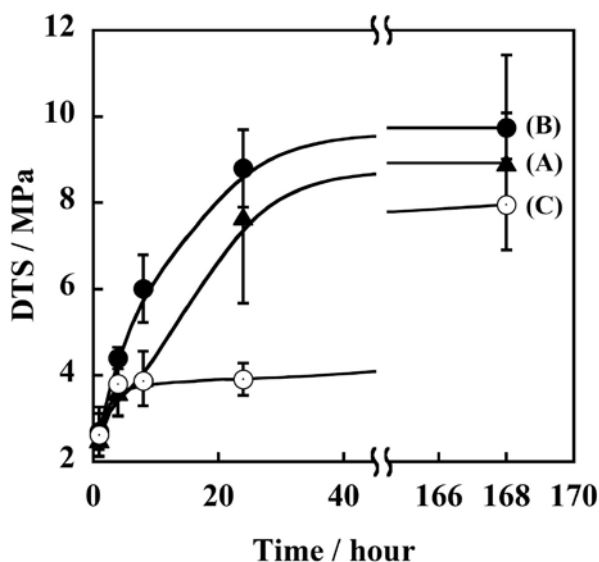

Fig. 3   Changes in DTS of Biopex® pre-hardened in an incubator kept at 37°C and 100% relative humidity for 1, 4, 8, 24, and 168 hours after mixing with various liquid phases: distilled water (A), neutral sodium hydrogen phosphate solution (B), or succinic acid sodium salt solution (C).

solution (Fig. 3(B), or succinic acid disodium salt solution (Fig. 3(C)). Generally, DTS increased with time to reach the maximum value in the range from 8 to 10 MPa, and that there were no significant differences in the final DTS at 168 hours among the cements. Although no significant differences in DTS were found at 4 hours, the DTS of the cement mixed with neutral sodium hydrogen phosphate solution was higher than the others. The differences increased at 8 hours, and the DTS of the cement mixed with neutral sodium hydrogen phosphate was significantly higher than the other cements. When mixed with distilled water, DTS reached almost the same value with that mixed with neutral sodium hydrogen phosphate solution at 24 hours, although it was attained at a later time. When mixed with succinic acid disodium salt solution, the increase in DTS was limited even after 24 hours of aging.

DISCUSSION

The results obtained in the present study clearly demonstrated that neutral sodium hydrogen phosphate solution also accelerated the formation of apatite, such that the setting reaction rate of α-TCP-based AC was similar to that of TTCP-DCPA-based AC. However, the effects of neutral sodium hydrogen phosphate on the initial setting time, the ensuing apatite formation, and the development of mechanical strength were found to be somewhat limited when

compared to those of TTCP-DCPA-based cement. For example, use of neutral sodium hydrogen phosphate salt solution shortened the setting time of TTCP-DCPA-based AC from 30 to 5 minutes[13,14], whereas the same merely shortened the setting time of α-TCP-based cement from approximately 90 minutes to 20 minutes.

This could be explained, at least in part, by the different dissolution behaviors of the powder phase of ACs and the resulting supply of Ca^{2+} and PO_4^{3-}. In the case of TTCP-DCPA-based AC, the dissolution of DCPA — and the resulting supply of PO_4^{3-} ions — is the rate-determining step for apatite formation. In other words, there is speedy and sufficient supply of Ca^{2+} since TTCP, which supplies more Ca^{2+}, dissolves quickly. Therefore, presence of PO_4^{3-} in the liquid phase brings about an instant attainment to super-saturation with respect to apatite. Consequently, the setting time of TTCP-DCPA-based AC was shortened from 30 minutes to 5 minutes when phosphate salt solution was used as the liquid phase. In contrast, the dissolution rate of α-TCP is much slower when compared to TTCP, and thus a longer time is required for the slurry to be supersaturated with respect to apatite. Nonetheless, the presence of PO_4^{3-} shortened the time required to reach supersaturation. However, the effect was somewhat limited when compared to that of TTCP-DCPA cement.

Although the degree of contribution to shorten the setting time of ACs was different, use of phosphate salt aqueous solution increased the mechanical strength of both TTCP-DCPA-based and α-TCP-based ACs. To date, the detailed mechanism underlying the increase in mechanical strength has not been clarified. However, as PO_4^{3-} ions contained in the liquid phase of ACs contribute to the formation of apatite, a higher density of the set mass — by virtue of PO_4^{3-} from the liquid phase — may contribute to a higher mechanical strength of the ACs.

When succinic acid disodium salt solution was used as the liquid phase, mechanical strength of the set AC was even lower than that using distilled water as the liquid phase. Although initial apatite formation was inhibited by the presence of succinic acid disodium salt, there were no significant differences in the degree of apatite formation (Fig. 2). Besides, there were also no differences in the crystalline morphologies of the ACs after 168 h (Figs. 1(D), (E), (F)). It was thus speculated that succinic acid which remained on the surface of formed apatite crystals might have affected the interlocking of apatite crystals.

Although there remains many unresolved issues, it was straightforward that use of sodium hydrogen phosphate may be useful as a liquid phase for α-TCP-based AC. Based on the initial findings obtained in the present study, they should be leveraged to further elucidate the unresolved issues to attain a deeper and more thorough understanding of α-TCP-based AC.

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