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Properties and mechanisms of fast-setting calcium phosphate cements

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The setting time of a calcium phosphate cement consisting of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA) was reduced from 30 to 5 min by use of a cement liquid that contained a phosphate concentration of 0.25 mol/l or higher. The diametral tensile strength and conversion of the cement ingredients to hydroxyapatite (OHAp) during the first 3 h were also significantly increased by the phosphate. However, the phosphate produced no significant effects on the properties of the 24-h cement samples. Results from additional experiments in a slurry system verified that the high phosphate concentration in the solution accelerated the formation of OHAp in the TTCP + DCPA system, and this reaction could explain the fast-setting properties of the cements.

1. Introduction

Brown and Chow reported [1] a calcium phosphate cement (CPC) that consists of tetracalcium phosphate (TTCP), $Ca_4(PO_4)_2O$, and dicalcium phosphate anhydrous (DCPA), $CaHPO_4$, or dicalcium phosphate dihydrate (DCPD), $CaHPO_4 \cdot 2H_2O$. CPC has generated considerable interest in the field of calcium phosphate biomaterials because it possesses self-hardening cement properties and forms, as the only end product, hydroxyapatite (OHAp), $Ca_{10}(PO_4)_6(OH)_2$, the putative mineral in teeth and bones. Because the OHAp was formed in an aqueous environment, it is more similar to biological apatites than is the OHAp formed in high temperature processes. As a consequence, CPC was found to be highly osteoconductive [2–7] and is readily osseointegrated. CPC also was found to adapt to the contours of the defect surfaces at a microscopic level. Thus, it is more effective than granular OHAp in blocking the infiltration of fibrous tissues into the implant–tissue interface. While CPC does appear to have several advantages over presently used calcium phosphate biomaterials, an apparent shortcoming is the relatively long setting time of approximately 30 min.

A number of studies have reported methods for reducing the setting time of CPC, for example by addition of OHAp seed crystals in CPC [8] and by controlling the particle sizes of CPC ingredients [9]. Results from these studies showed that the setting times can be shortened somewhat but not significantly. Several recent studies have shown that phosphate in the cement liquid was effective in reducing the setting time or accelerating the formation of OHAp in different calcium phosphate cement systems. Chow *et al.* [10] reported that the formation of OHAp in TTCP + DCPA cements was greatly accelerated by using sodium phosphate solutions, 0.25 to 1.0 mol/l in concentration, as the cement liquids. The phosphate also reduced the setting time from 25 to 5 min. Driessens *et al.* reported [11] that addition of either $NaH_2PO_4 \cdot 2H_2O$ or NaH_2PO_4 to the cement liquid reduced the setting time of a cement consisting of α-tricalcium phosphate (α-TCP) and precipitated OHAp. Recently Fernandez *et al.* [12] showed that these phosphate compounds also reduced the setting times of two other calcium phosphate cements that consisted of α-TCP alone or α-TCP and DCPA. The objectives of the present investigation were to study the physical properties of cements that set rapidly in a phosphate solution and to elucidate possible mechanisms of the rapid setting reaction.

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2. Materials and methods

2.1. Materials

TTCP was prepared by heating an equimolar mixture of commercially obtained $CaHPO_4$ and $CaCO_3$ (Baker Analyzed Reagents, J. T. Baker Chemical Co., NJ) at 1500 °C for 24 h in a furnace (Model 51333, Lindberg, Watertown, WI) and quenched at room temperature in a desiccator. The solid was first crushed by mortar and pestle, followed by further grinding in water-free cyclohexane in a ball mill (Retsch PM4, Brinkman, NY) to obtain a median particle size of 4.1 μm. The commercial DCPA was also ground for 24 h in the ball mill in 95% ethanol to obtain a median particle size of 0.70 μm. The particle size distributions of the ground TTCP and DCPA were measured in isopropanol by a sedimentation method based on Stokes' law with the use of a centrifugal particle analyser (SA-CP3, Shimazu, Kyoto, Japan). The CPC powder was prepared by mixing equimolar amounts of the ground TTCP and DCPA in a micromill (Bel-Art Products, Pequannock, NJ). The phosphate solutions, with concentrations in the range from 0.25 to 2.5 mol/l, were used as the cement liquids. The solutions were prepared by dissolving commercially obtained reagent grade NaH_2PO_4, KH_2PO_4, or $(NH_4)\,H_2PO_4$ in CO_2-free double distilled H_2O, and the pH levels of the solutions were adjusted to 7.4 by adding NaOH, KOH, or NH_4OH, respectively.

2.2. Methods

Setting times of the CPC samples were measured according to the method of international standard ISO1566 for dental zinc phosphate cements. In this method, the cement is considered set when a needle with a tip diameter of 1 mm and loaded with a 400-g weight fails to make a perceptible circular indentation on the surface of the cement. ISO requires that the cement be maintained at a temperature of 37 °C and a relative humidity of at least 30%. In the present study, the conditions of 37 °C and 100% humidity were used. A powder-to-liquid (P/L) weight/weight ratio of 4.0 was used in preparing the cement specimens.

CPC specimens for diametral tensile strength (DTS) measurements were prepared by mixing the CPC powder and liquid on a glass slab for approximately 30 s and the cement paste was packed into in a split mould (6 mm diameter × 3 mm height, typical sample weight 0.2 g) and placed in a incubator kept at 37 °C and 100% humidity. Specimens were removed from the molds at 0.5, 1, 3, 6, or 24 h after sample preparation for DTS measurement on a universal testing machine (United Calibration Corp, Garden Grove, CA). The diameter and length of each specimen were measured with a micrometer, and the specimen was placed between steel platens that were covered with one thickness of wet filter paper and crushed at a loading rate of 1 cm/min. The DTS value was the average values obtained from six specimens.

The conversion of CPC starting materials to OHAp was assessed by powder X-ray diffraction (XRD) analyses. After the specimens had been in the incubator for the prescribed time, they were removed from the moulds and freeze-dried in liquid N_2 (Automatic Freeze-Dryer 10 – 010, Virtis Co., Gardiner, NY). This procedure made it possible to stop the process of CPC conversion to OHAp at specific times. The freeze-dried samples were ground to powder and characterized by XRD. The XRD patterns of the specimens were recorded with the use of a vertical-mounted diffractometer system (Rigaku Denki Co. Ltd., Tokyo, Japan) that utilized graphite–monochromatized CuK_α radiation ($\lambda = 0.1540$ nm) generated at 40 kV and 25 mA. The sample was scanned from 3 to 60 degrees in 2Θ in a continuous mode (1°/min, time constant 2 s), and the intensities were recorded on a strip-chart recorder. The relative peak areas of the 0 1 3 (29.2°) and 0 4 0 (29.8°) reflections for TTCP, the 1 1 0 (26.6°) reflection for DCPA, and 0 0 2 (25.9°) reflection for OHAp were measured a minimum of three times, and the averaged values were used to estimate the extent of CPC conversion to OHAp. The percentage conversion of CPC to OHAp was calculated with a formula modified from that reported by Fukase *et al.* [13]:

$$\%\,\text{conversion} = \frac{\dfrac{OHAp_t}{OHAp_\infty} + \dfrac{1 - (DCPA_t/DCPA_0) + 1 - (TTCP_t/TTCP_0)}{2}}{2} \times 100 \qquad (1)$$

where $DCPA_0$ and $TTCP_0$ are the peak intensities of DCPA and TTCP in the unreacted CPC, $OHAp_\infty$ is the peak intensity of OHAp in the fully cured CPC (3 to 4 weeks in water), and $DCPA_t$, $TTCP_t$ and $OHAp_t$ are the corresponding values in the specimen at time t (0.5, 1, 3, 6, or 24 h) after mixing. In the study by Fukase *et al.* [13], the formula used for calculating the percentage conversion to OHAp in the cement was based on the decreases in the peak intensities of TTCP and DCPA, whereas in the present study Equation 1 also takes into account the increase in the peak intensity of the OHAp. This equation is used to reflect the fact that the crystallinity OHAp does not reach the maximum at 24 h when the starting materials, TTCP and DCPA, are nearly completely exhausted.

The setting reaction of CPC was studied with suspensions (P/L = 1.0) of CPC powder in water or a phosphate solution. At various times, a small sample was taken from the suspension and filtered through a 0.22 μm filter (Millex-GS filter assemblies, Millipore Corp., Bedford, MA). The filtrate was analysed for pH and the calcium (atomic absorption spectrophotometry, Model 603, Perkin Elmer, Norwalk, CT) and phosphate (phosphomolybdate complex) [14] concentrations. Activities of the ions in the solution were calculated with the use of a commercial software EQUIL (MicroMath Scientific Software, Salt Lake City, UT).

The potential phase diagram for calcium phosphates was constructed as described previously [2].

3. Results

The setting time for the conventional CPC, i.e. with water used as the cement liquid, was approximately 30 min. When a sodium phosphate solution with a concentration of 0.25 mol/l or higher was used as the liquid, the cement setting time was found to be between $4.6 \pm 0.5 (n = 5)$ to $5.4 \pm 0.5 (n = 5)$ min. Increasing the phosphate concentration from 0.25 mol/l to 2.5 mol/l did not have a significant effect on the setting time. The same rapid setting time was observed when potassium phosphate or ammonium phosphate solutions were used as the liquid phase.

The DTS values (MPa) of the various fast setting CPC (FSCPC) formulations are shown in Table I. In the conventional CPC, no significant strengths were developed until approximately 3 h after a specimen was prepared. In contrast, the FSCPCs showed measurable DTS at 0.5 h, the earliest time point when the DTS measurements were conducted. FSCPC specimens had a DTS of 3.2 to 4.1 MPa at 0.5 h when the sodium phosphate concentration was between 0.25 and 1.00 mol/l. However, a significantly lower DTS value of 1.7 ± 0.4 MPa was observed for cements prepared with the 2.5 mol/l phosphate solution. At a given P/L ratio of 4.0, the cement paste was found to become dryer and less cohesive with increasing phosphate concentration in the cement liquid. No significant differences in DTS were observed in samples prepared with the sodium phosphate, potassium phosphate, or ammonium phosphate solution at 0.5 h or 24 h (Table I). Some slight but statistically significant differences were observed in DTS among cements prepared with the different solutions at 1,3, and 6 h. Although the FSCPC specimen showed higher DTS values than the conventional CPC at the early stages, no significant differences in DTS were observed after 24 h. The only exceptions were the cements prepared with the 2.5 mol/l sodium phosphate solution, which had significantly lower DTS values than samples prepared with the more dilute phosphate solutions or water (Table I).

Table II summarizes that data on the conversion of the cement ingredients to OHAp at 0.5, 1, 3, 6, and 24 h in the cements prepared with the various phosphate solutions. During the first 3 h, significantly greater amounts of OHAp were formed in the FSCPCs than in the conventional CPC. These differences diminished by 6 h. When the DTS values were plotted against the corresponding percentage OHAp conversion values at various time points (Fig. 1), data points for all the cement formulations studied fell on one broad curve. The least-squares regression line had

TABLE I Diametral tensile strength (MPa) of calcium phosphate cements with various phosphate solutions as the cement liquid (P/L = 4)

Cement liquid (pH = 7.4)		Incubation time (h) 0.5	1	3	6	24
Conventional CPC						
Water		0	0	5.7 ± 0.6[a]	9.0 ± 0.8	10.6 ± 1.1
FSCPC						
NaH_2PO_4	0.25 mol/l	3.2 ± 0.8	5.9 ± 0.4	6.5 ± 0.8	10.8 ± 1.3	10.8 ± 1.5
	0.50	4.1 ± 0.4	8.9 ± 1.0	8.6 ± 0.9	9.2 ± 0.5	9.6 ± 1.8
	0.75	3.4 ± 0.7	8.2 ± 1.2	8.1 ± 0.8	10.3 ± 1.2	11.3 ± 1.6
	1.00	3.7 ± 0.4	6.2 ± 1.1	8.2 ± 1.2	11.4 ± 2.0	9.6 ± 1.7
	2.50	1.7 ± 0.4	3.5 ± 0.5	5.2 ± 1.2	5.0 ± 0.9	4.8 ± 0.8
KH_2PO_4	0.50 mol/l	4.2 ± 0.8	5.9 ± 0.8	5.3 ± 1.3	10.8 ± 1.6	8.7 ± 1.5
	1.00	4.1 ± 0.4	8.9 ± 1.0	8.6 ± 0.6	9.2 ± 1.2	10.1 ± 0.8
$(NH_4)H_2PO_4$	0.50 mol/l	4.2 ± 0.8	5.9 ± 0.6	5.3 ± 0.6	10.8 ± 1.2	8.7 ± 1.5
	1.00	4.1 ± 0.4	8.9 ± 1.0	8.6 ± 0.9	9.2 ± 0.5	10.1 ± 0.8

[a] mean ± s.d. ($n = 6$)

Newman–Keuls multiple comparison summary

Ranking of DTS at various incubation times (h) 0.5	1	3	6	24
NaH_2PO_4 Concentration (mol/l)				
0.50[b]	0.50	0.50	1.00	0.75
1.00	0.75	1.00	0.25	0.25
0.75	1.00	0.75	0.75	0.00
0.25	0.25	0.25	0.50	1.00
2.50	2.50	0.00	0.00	0.50
		2.50	2.50	2.50

[b] Groups connected by a vertical line are not significantly ($p > 0.05$) different.

TABLE II Percentage conversion of CPC components to OHAp in cements with various phosphate solutions as the liquid phase (P/L = 4)

Cement liquid (pH = 7.4)		Incubation time (h) 0.5	1	3	6	24
Conventional CPC						
Water		4 ± 1[a]	8 ± 2	35 ± 3	68 ± 8	82 ± 7
FSCPC						
NaH_2PO_4	0.25 mol/l	36 ± 5	58 ± 4	70 ± 9	71 ± 9	81 ± 10
	0.50	49 ± 6	68 ± 2	70 ± 4	73 ± 9	79 ± 7
	0.75	35 ± 3	66 ± 2	66 ± 5	70 ± 8	75 ± 10
	1.00	30 ± 4	30 ± 4	63 ± 5	68 ± 7	81 ± 8
	2.50	23 ± 2	29 ± 2	45 ± 3	43 ± 7	65 ± 9
KH_2PO_4	0.50 mol/l	40 ± 2	45 ± 3	58 ± 6	75 ± 8	83 ± 9
	1.00	45 ± 4	42 ± 4	59 ± 7	67 ± 8	79 ± 10
$(NH_4)H_2PO_4$	0.50 mol/l	36 ± 3	58 ± 3	70 ± 7	71 ± 5	81 ± 5
	1.00	50 ± 4	59 ± 4	65 ± 9	71 ± 3	83 ± 7

[a] mean ± s.d. ($n = 3$)

Newman–Keuls multiple comparison summary

Ranking of OHAp conversion at various times (h) 0.5	1	3	6	24
NaH_2PO_4 Concentration (mol/l)				
0.50 [b]	0.50	0.50	0.50	0.00
0.25	0.75	0.25	0.25	1.00
0.75	0.25	0.75	0.75	0.25
1.00	1.00	1.00	1.00	0.50
2.50	2.50	2.50	0.00	0.75
0.00	0.00	0.00	2.50	1.00

[b] Groups connected by a vertical line are not significantly ($p > 0.05$) different.

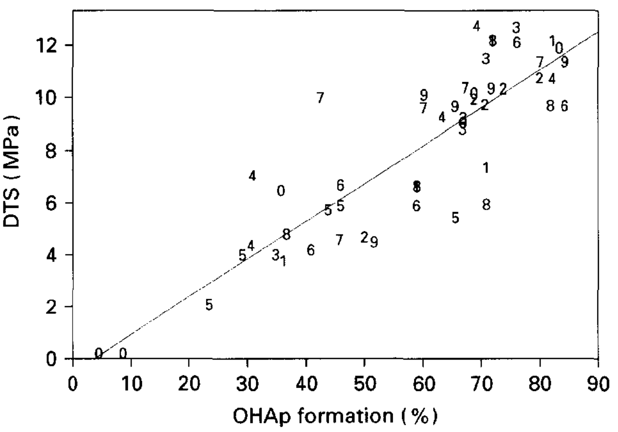

Figure 1 Relationship between DTS (Table I) and percentage OHAp conversion (Table II) in the various cements that were kept for various times (0.5, 1, 3, 6 and 24 h) at 37° C and 100% humidity. The numbers denote the cement liquids used: (0) water; (1) 0.25 mol/l NaH_2PO_4; (2) 0.5 mol/l NaH_2PO_4; (3) 0.75 mol/l NaH_2PO_4 (4) 1.0 mol/l NaH_2PO_4; (5) 2.5 mol/l NaH_2PO_4; (6) 0.5 mol/l KH_2PO_4; (7) 1.0 mol/l KH_2PO_4; (8) 0.5 mol/l (NH_4) H_2PO_4; and (9) 1.0 mol/l (NH_4) H_2PO_4.

a correlation coefficient of 0.87, a value suggesting that there was a strong linear correlation between DTS and OHAp formation when all the data points were considered as one group.

4. Discussion

Results from a previous study [15] showed that the 3-h DTS of CPC samples increased with increasing K_2HPO_4 concentration in the cement liquid up to 0.15 mol/l; the setting time was not significantly affected by phosphate in this concentration range. The present results indicated that with a phosphate concentration of 0.25 mol/l or higher, the setting time was reduced to 5 min, and the initial (0.5 to 6 h) strength (Table I) and the initial (0.5 to 3 h) rate of OHAp formation (Table II) were also significantly increased. With the exception of the 2.5 mol/l phosphate solutions, the phosphate-containing cement liquid did not reduce the DTS of 24-h specimens (Table I). This finding is in contrast to the reported effects of phosphate on calcium phosphate cements that consisted of α-TCP or α-TCP and DCPA [12]. The present results suggest that the use of phosphate-containing solutions as the cement liquid cause a more rapid setting without compromising the mechanical strength of the final product. Thus, these faster setting cements may be desirable in those clinically applications where rapid hardening of the cement is important.

Fig. 1 shows that the DTS of cements increased linearly with the amount of OHAp formation. The fact that the data points of the various FSCPC did not fall on separate lines suggests that the mechanisms for the hardening of the cement are similar in cements prepared with various types and concentrations of phosphate solutions as cement liquids. The relationship between percentage OHAp conversion and DTS, indicated by the regression line (Fig.1), suggests that the cement should begin to develop some mechanical strength when the OHAp content is above 5%. However, experimentally, the 1-h CPC specimens prepared with water as the cement liquid showed no measurable DTS even though 8% of the cement was converted to OHAp.

The more rapid OHAp formation in cements prepared with the phosphate solutions could be explained by the rate-determining step in the kinetics of OHAp formation. It has been shown [16] that in the pH range of approximately 4.3 and higher, OHAp is the most stable (least soluble) calcium phosphate. Thus, any other calcium phosphate suspended in water has the tendency to dissolve and reprecipitate as OHAp. However, such a reaction is generally too slow to be utilized as a cement setting reaction. TTCP and DCPA (DCPD) are exceptions because of their high solubilities and rapid dissolution in acidic and neutral pHs and in neutral and alkaline pHs, respectively. The relatively high phosphate concentration resulting from the dissolution of TTCP and DCPA in the CPC system is probably a major factor responsible for the OHAp formation needed to cause cementation. The results of the present study show that since the rate of the OHAp formation is limited by the phosphate concentration, the rate of OHAp formation in the TTCP + DCPA system can be significantly increased by adding a phosphate salt in the cement liquid. OHAp formation accelerated by phosphate has also been reported in several previous studies [10,15,17]. The effect of phosphate on the rate of OHAp formation may be seen more clearly from the results of an experiment described below.

Fig. 2 is a chemical potential plot depicting the composition of solutions in terms of the chemical potentials of $Ca(OH)_2$ and H_3PO_4 in the solution. Shown in the figure are the compositions of the liquid phase of the suspension (P/L = 1.0) of CPC powder in water or in a 0.5 mol/l sodium phosphate solution (pH 7.4) at various times. The solubility isotherms of OHAp, OCP, and DCPA are also shown. In this plot, those compositions located below an isotherm are supersaturated with respect to the compound and those above the isotherm are undersaturated. It can be seen that in this region of the potential phase diagram the solubility of the salts increases in the order of OHAp, octacalcium phosphate (OCP), and DCPA. Thus, those solutions that are saturated with respect to DCPA are supersaturated with respect to OCP and OHAp, and those solutions that are saturated with respect to OCP are undersaturated with respect to DCPA but are supersaturated with respect to OHAp. In the suspension of CPC in water, the solution composition was initially nearly saturated with respect to DCPA and supersaturated with respect to both OCP and OHAp. The composition changed with time to become significantly undersaturated with respect to DCPA and was approximately saturated with respect to OCP throughout the remaining period of the experiment. In contrast, in the suspension of CPC in 0.5 mol/l sodium phosphate solution, the solution composition was only approximately saturated with respect to OHAp and was significantly undersaturated with respect to both OCP and DCPA throughout the experimental period. Since excess DCPA and TTCP were present in the suspensions until they were exhausted at the end of the experiment, the fact that the solution was grossly undersaturated with respect to DCPA and TTCP suggests that the formation of OHAp in this suspension was significantly faster than the dissolution of DCPA and TTCP. In comparison, the solution in the suspension of CPC in water was only slightly undersaturated with respect to DCPA and was significantly supersaturated with respect to OHAp. This condition suggests that the rate of OHAp formation in this system was slower than the rate of DCPA dissolution. OCP was not detected by XRD in either the conventional CPC or FSCPC at any of the time intervals.

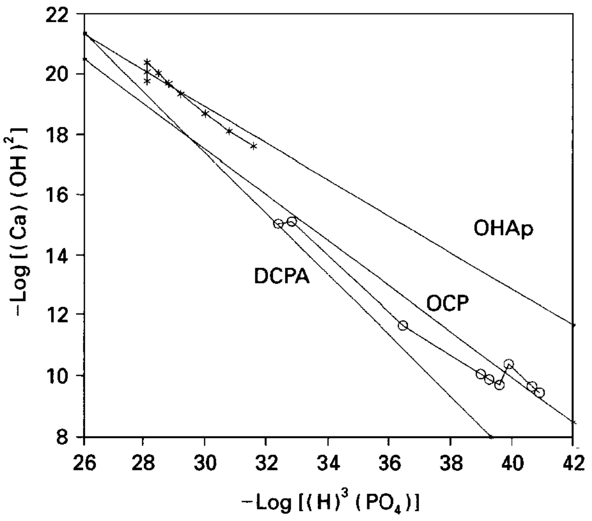

Figure 2 Negative logarithms of phosphoric acid and calcium hydroxide activities of the solutions in the slurries of TTCP and DCPA in water (○) and in a 0.5 mol/l phosphate solution (pH 7.4) (*).

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