Polymeric Calcium Phosphate Cements Incorporated with Poly-γ-Glutamic Acid: Comparative Study of Poly-γ-Glutamic Acid and Citric Acid

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ABSTRACT: Polymeric calcium phosphate cements (PCPC) derived from biodegradable poly-γ-glutamic acid (γ-PGA) were prepared in an attempt to improve the mechanical strength of calcium phosphate cement (CPC). The characteristics of the PCPCs were compared with those of cement incorporated with citric acid. The diametral tensile and compressive strengths of the CPC incorporated with γ-PGA were significantly higher than that of cement incorporated with citric acid at equivalent concentrations ($P < 0.05$). The maximal diametral tensile and compressive strengths of the CPC incubated for 1 week in physiological saline solution were approximately 18.0 and 50.0 MPa, respectively. However, the initial setting time of the PCPC was slower than that of CPC incorporated with citric acid. The formation of ionic complexes between calcium ions and γ-PGA was observed using FTIR spectroscopy. Hydroxyapatite (HA) formation was retarded by γ-PGA incorporation according to scanning electronic microscopy (SEM) and powder X-ray diffraction (XRD) observations. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 1223–1231, 2009

Key words: biological applications of polymers; biomaterials

INTRODUCTION

Calcium phosphate cements (CPCs) have received increasing interest in biomedical applications for repairing hard tissues in orthopedics, dentistry, and drug delivery due to the following advantages: (1) the ability to be used under ambient conditions, (2) the ability for self-setting through a low exothermic reaction in an aqueous environment, and (3) excellent biocompatibility because of their compositional similarity to bone and teeth.[1,2] Clinically, the main application of self-setting CPCs is currently cranio and maxillofacial surgery for reconstruction of bone defects.[3,4] The location of these sites is such that these materials bear little loading since they are weak and brittle compared with natural bone. To further widen their clinical applications, such as in vertebroplasty, CPCs must be stronger. It is of utmost importance that the CPC provide adequate mechanical support not only during the initial phase following surgery, but also until the bone healing process has restored the mechanical integrity of the bone.

In an attempt to increase the mechanical strength of CPC, polyacids, polymers with acid groups, have been incorporated into CPCs. Poly(acrylic acid) produced cements that set fast and had high diametral tensile and compressive strengths.[5,6] Matsuya et al.[7,8] reported that CPC incorporated with poly (methyl vinyl ether-maleic acid) (PMVE-Ma), the hydrolysis product of a 1 : 1 copolymer of methyl vinyl ether and maleic anhydride, had high mechanical strength and set slower than that incorporated with poly(acrylic acid). A polymeric acrylic system supporting a derivative of aminosalicylic acid was incorporated into CPC, with the aim of achieving pharmacological effects as well as improving its mechanical and rheological properties.[9]

The polyacids dissolve calcium phosphate compounds and provide ionic crosslinks with calcium ions.[7–9] Therefore, the mechanical properties of CPCs were improved in the previous studies. However, the previously studied polyacids are hardly biodegradable and thus may lead to adverse effects, such as decreased mechanical strength of the reconstructed bone tissue, when the CPCs are implanted in the body.

In the present study, we have studied CPC incorporated with the polyacid poly-γ-glutamic acid (γ-

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PGA), which is a biodegradable and non-toxic polyamino acid. The γ-PGA powder was incorporated with α-tricalcium phosphate (α-TCP), which is one of the main reactants of the powder phase of several CPCs. The properties, such as the mechanical strength, of CPC incorporated with γ-PGA were compared with those of CPC incorporated with citric acid, which is frequently used for setting.[10,11]

MATERIALS AND METHODS

Preparation and characterization of cement powders

Dicalcium phosphate ($CaHPO_4$, Sigma Chemical) and calcium carbonate ($CaCO_3$, Sigma Chemical) at a 2 : 1 molar ratio were used as starting materials for fabrication of α-TCP [$Ca_3(PO_4)_2$]. The batch mixture with a Ca/P molar ratio of 1.5 was homogenized by ball milling in ethanol for 24 h and dried. The dried powder was calcined at 1300°C for 12 h in an electronic furnace and then quenched at room temperature. The material obtained was ground in a ball mill for 24 h and then sieved (aperture size = 38 μm).

The α-TCP was admixed with the citric acid powder (Sigma Chemical) at weight ratios of 0, 5, 10, and 15% to make cement powders with citric acid, and the powders were then ground in a ball mill. To make cement powders with γ-PGA, the α-TCP was admixed and ground with the γ-PGA powder (MW: about 2000 kg/mol) (BLS 200, Bioleaders Co., Korea) at weight ratios of 0, 5, 10, and 15%.

The morphology of the α-TCP, citric acid, and γ-PGA particles were examined by scanning electronic microscopy (SEM) (JSM-840A, JEOL Ltd.) and transmission electron microscopy (TEM, JEM-2010, JEOL Ltd.). The particle size and distribution were measured using a particle size analyzer (BI-DCP, Brookhaven instruments Ltd.).

The formation of α-TCP was assessed by powder X-ray diffraction (XRD) analysis. The XRD pattern was recorded on a Rigaku Rotaflex diffractometer (D/Max-2200 Ultima/PC, rotating Cu target, 3 kW X-ray and set to 40 kV and 40 mA) at an X-ray incident angle of 0.02°. The sample was scanned 20–40° in 2θ using a step-scanning mode with an integration time of 10 s at intervals of 0.02°. Peak indexing was carried out by means of cards: JCPDS-29-359 for α-TCP, JCPDS-9-169 for β-TCP, and JCPDS-9-432 for apatite (Joint Committee on Powder Diffraction Standards, 1988).

The XRD patterns of mixtures of α- and β-TCP at weight ratios of 10/0, 7.5/2.5, 5/5, 2.5/7.5, and 0/10 were obtained to estimate the α-TCP content in prepared TCP powders. The α- and β-TCP, which were purchased from Fluka were pure phase for analysis of bone cement. The amounts of α-TCP incorporated in the mixtures were plotted against the relative peak area (R) of α-TCP. R was calculated from the following equation, where P is peak area:

$$R = P_\alpha/(P_\alpha + P_\beta)$$

where P_α and P_β are peak areas of α- and β-TCP. The 2θ of peaks chosen for α- and β-TCP, which did not overlap, was 27.80° and 22.86°, respectively.

Preparation and characterization of cements

The pH changes in the cements were indirectly evaluated by measuring the pH of 30 mL of physiological saline (0.9% NaCl) solution containing 10 g of cement paste. To make the cement paste, the cement powders and deionized water were added together at a 5 : 2 weight ratio in a PTFE beaker and mixed manually with a spatula for approximately 40 s. The pH of the physiological saline solution incubated at 37°C was measured at 5-min intervals for an hour. The concentration of Ca^{2+} ions in the physiological saline solution after 1-h incubation was measured by atomic absorption spectrophotometry (Perkin-Elmer 2380, Atomic Absorption Spectrometer).

The hardening times of cement pastes were measured using the Gilmore needle method. Three wells of a 24-well tissue culture plate were filled with each cement paste. The plate was kept in physiological saline solution at 37°C to simulate a physiological environment. The sample was considered set when a 100-g mass loaded on a needle with a tip diameter of 1 mm failed to make a perceptible circular indentation on the surface of the cement.

A rheometric dynamic spectrometer (Rheometrics RDA-III, Rheometric scientific™) in dynamic oscillation mode with a parallel plate configuration was used to measure the rheological characteristics of cement pastes as a function of time. The cement pastes were prepared as mentioned above and 5 g of the paste was then loaded into the rheometer. The plates were brought together and, after the excess cement had been removed, the rheometer was started. The rheometer was used in dynamic oscillation mode at a frequency of 5 Hz and a temperature of 27°C. The diameter of the upper plate was 25 mm and the gap between the plates was 2 mm. The instrument was used in 5% constant strain mode. The dynamic viscosity was measured using a single sample for each cement group.

For diametral tensile strength (DTS) measurements, the cement pastes were prepared as mentioned above and subsequently packing the cement paste into a glass tube (8 mm diameter × 50 mm length). The samples were cured for 2 h in an incubator at 37°C and 100% relative humidity prior to

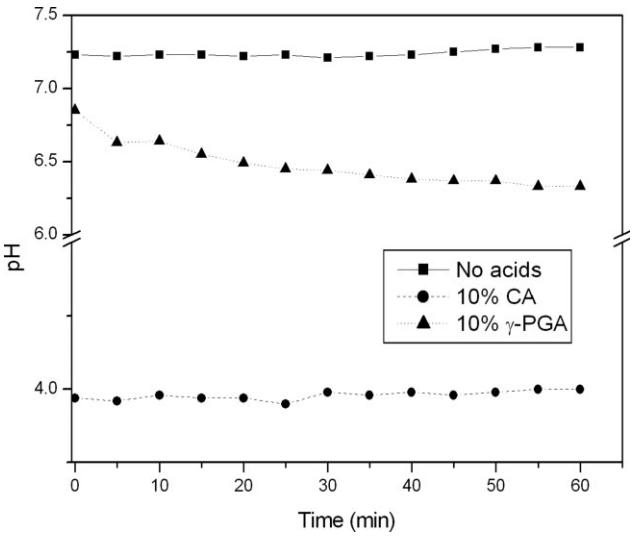

Figure 1 The pH changes in physiological saline solution containing calcium phosphate cements. TCP only: cement without organic acids; 10% CA: cement prepared with powders containing 10 wt % citric acid; 10% γ-PGA: cement prepared with powders containing 10 wt % γ-PGA.

their removal from the glass tubes and subsequent storage in physiological saline solution at 37°C for 3 and 7 days. The cement samples ($n = 5$, where n is sample number) were cut into approximately 4-mm lengths using a diamond saw and subsequently quenched in methanol for 2 h to arrest the setting reactions and thoroughly dried at 80°C. The diameter and length of the dried samples were measured with a micrometer. The DTS measurements were conducted on a universal testing machine (Instron 4482) at a crosshead speed of 1 mm/min. The DTS (T) was calculated from the formula $T = 2F/\pi DL$ in which F is the crushing force, L is the length, and D is the diameter. The compression tests were carried out on cylindrical specimens (8 mm diameter × 16 mm height, $n = 5$ for each group). The aforementioned method was used to prepare the cylindrical cement specimens. The compression test was carried out according to the ASTM F451-86 recommendations. The compression load was applied along the axis using a crosshead speed of 1 mm/min. In mechanical tests, the data were expressed as mean and standard deviation. Student's t-test was used to assess whether the observed differences between groups were statistically significant.

Following the mechanical tests, the fractured surfaces were observed by SEM after the samples had been coated with platinum. Fourier transform infrared (FTIR) spectroscopy (EQUINOX 55, Buker) and XRD were performed to determine chemical reactions and material formation.

RESULTS

α-TCP and γ-PGA were irregularly shaped according to SEM and TEM observation (data not shown). The particle size of α-TCP was 8.1 ± 4.8 μm by particle size analysis and that of γ-PGA was approximately 1–2 μm by SEM observation. Particle size analysis was not available for γ-PGA since it swelled in solvents such as water and ethanol.

The XRD pattern of the prepared α-TCP indicated that it was not pure, but rather included β-TCP (Fig. 8). To estimate the α-TCP content in the prepared TCP powders, an equation was obtained by the least-squares method from the plot of the amount of α-TCP (A, wt%) against the relative peak area (R):

$$A = 99.61R - 0.28$$

where the regression coefficient was 0.999. The content of α-TCP in the prepared TCP powders was 76.8 ± 12.3% ($n = 5$).

Figure 1 shows the typical pH changes with time in the physiological saline solution containing cements. The initial pH of the solution containing cement without organic acids was 7.2 and hardly changed with time. The initial pH of the solution containing cement prepared with 10 wt % citric acid was approximately 4.0 and hardly changed with time because citric acid dissolved rapidly due to its high solubility. The initial pH of the solution containing cement prepared with 10 wt % γ-PGA was approximately 6.8, then decreased slightly to 6.3 after 1 h, presumably due to slow solvation of γ-PGA.

Table I shows the concentration of Ca^{2+} ions released into the physiological saline solution and the initial setting times against the amount of organic acids incorporated into powder components. The concentration of Ca^{2+} ions increased with increased organic acid content. The release of Ca^{2+} ions from citric-acid incorporated cements was

TABLE I
Concentration of Ca^{2+} Ions Released Into Physiological Saline Solution and Initial Setting Times of Calcium Phosphate Cements

Acid amount (wt%)[a]	Released Ca ions (ppm)		Initial setting time (min)[b]	
	Citric acid	γ-PGA	Citric acid	γ-PGA
0	16.9	16.9	180 ± 10	180 ± 10
5	389	183	15 ± 3	120 ± 10
10	680	277	10 ± 2	63 ± 6
15	880	487	4 ± 1	22 ± 3
20	1340	1160	3 ± 1	12 ± 3

[a] Concentration of organic acids (citric acid and γ-PGA) in cement powders.
[b] The results are reported as the mean ± standard deviation; $n = 3$.

higher than that from γ-PGA incorporated cements at equivalent concentrations. This was assumed to result from the lower pH of citric acid. According to our experiments, the pH of 5 wt % citric acid and γ-PGA aqueous solutions were 1.82 and 2.69, respectively.

The initial setting time of the cement paste with no organic acids was approximately 180 min. The initial setting time decreased with increasing amounts of incorporated organic acids. The initial setting time of citric acid-incorporated cements decreased rapidly with increases in the amount of citric acid, such that the setting time of cement with 20% citric acid was approximately 3 min. In contrast, the initial setting time of γ-PGA incorporated cements decreased more slowly and the initial setting time of cement with 20% γ-PGA was approximately 12 min.

Figure 2 shows the typical changes in the dynamic viscosity of dough stages of the cements with time. The dynamic viscosity of the cement without organic acids was initially 0.98 MP, where MP is 10^6 poise, and then increased gradually with time. The dynamic viscosity increased significantly with organic acid incorporation. The cement pastes prepared with powders containing more than 10 wt % citric acid showed a radical increase in dynamic viscosity between approximately 100 and 200 s. The cement pastes prepared with powders containing γ-PGA did not exhibit a radical increase in dynamic viscosity within the measured time.

Figure 3 shows the changes in initial dynamic viscosity against the amount of incorporated organic acids such as citric acid and γ-PGA. The initial dynamic viscosity is very relevant to the mixing characteristics of the cements. The initial dynamic viscosity was increased by citric acid incorporation. However, with incorporation above 5%, the initial dynamic viscosity was not further increased with increasing amounts of citric acid. The initial dynamic viscosity of cements containing γ-PGA increased with increasing amounts of γ-PGA. Indeed, cements prepared with cement powders containing more than 15 wt % γ-PGA were very hard to mix due to their high viscosity.

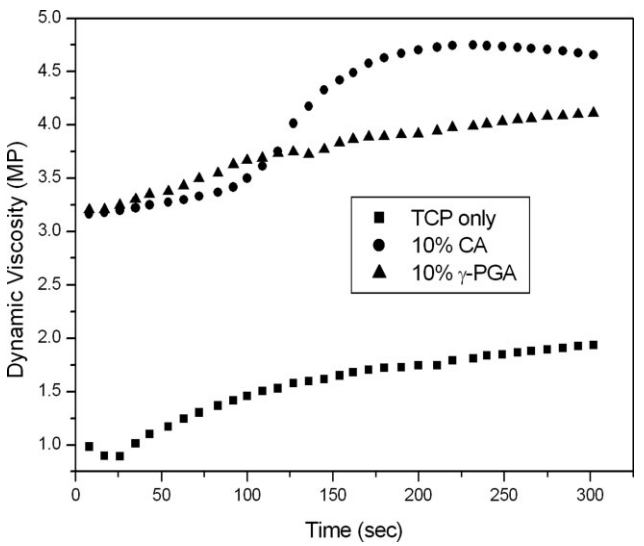

Figure 2 Dynamic viscosity as a function of time for the control cement (TCP only) and cements prepared with powders containing 10 wt % citric acid (10% CA) and γ-PGA (10% γ-PGA).

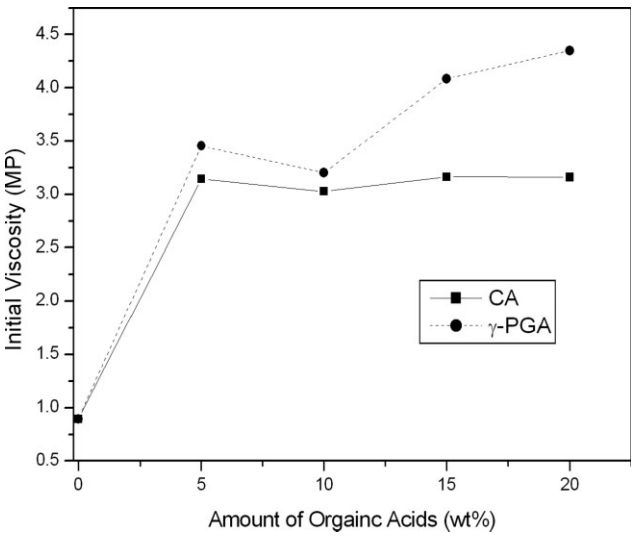

Figure 3 The changes of initial viscosity against amount of organic acids for cements prepared with powders containing citric acid (CA) and γ-PGA (γ-PGA).

Table II shows DTS of cements incubated in physiological saline solution for 3 and 7 days. The DTS of cement without organic acids was not measurable as it was very weak. After 3-day incubation, the DTS of cements prepared with citric acid increased from 0.55 ± 0.2 to 2.82 ± 0.34 MPa when the amount of citric acid was increased from 5.0 to 10.0 wt %. However, the DTS decreased as the amount of citric acid increased above 15.0 wt %. After 3-day incubation, the DTS of cement prepared with powder

TABLE II
Diametral Tensile Strength (DTS) of Calcium Phosphate Cements after Incubation for 3 and 7 days

Acid amount (wt%)[a]	Incubation time			
	3 days		1 week	
	Citric acid	γ-PGA	Citric acid	γ -PGA
5	0.55 ± 0.2	6.20 ± 1.72	3.03 ± 0.47	9.65 ± 1.33
10	2.82 ± 0.34	7.07 ± 1.04	2.38 ± 0.76	17.73 ± 4.50
15	2.7 ± 0.21	15.09 ± 2.50	2.10 ± 1.18	15.09 ± 0.32
20	1.12 ± 0.15	14.32 ± 0.95	1.46 ± 0.36	18.45 ± 5.00

(Results are reported as mean ± standard deviation; $n = 5$).
[a] Concentration of organic acids (citric acid and γ-PGA) in cement powders.

component containing 5.0 wt % γ-PGA was 6.20 ± 1.72 MPa. The DTS increased as the amount of γ-PGA increased to 15.0 wt % and then decreased thereafter. After 1-week incubation, the DTS of cement prepared with powder component containing 5 wt % of citric acid was 3.03 ± 0.47 MPa. The DTS decreased with increasing amounts of citric acid. After 1-week incubation, the DTS of cements prepared with γ-PGA increased from 9.65 ± 1.33 to 17.73 ± 4.50 MPa when the amount of γ-PGA was increased from 5.0 to 10.0 wt %. However, the DTS did not increase significantly with increasing amounts of γ-PGA above 15.0 wt %. In all the samples, the DTS of cements prepared with γ-PGA was significantly higher than that of cements prepared with citric acid ($P < 0.05$ according to the Student's t-test).

Table III shows the compressive strength (CS) of cements incubated in physiological saline solution for 3 and 7 days. After 3-day incubation, the CS of cement prepared with powder component containing 5 and 10 wt % citric acid was 2.65 ± 0.04 and 8.54 ± 1.72 MPa, respectively and then decreased thereafter. After 3-day incubation, the CS of cement prepared with powder component containing 5 wt % γ-PGA was 23.72 ± 4.23 MPa. The CS was increased with increasing amounts of γ-PGA and was maximal at 15.0 wt % γ-PGA (38.76 ± 3.8 MPa). After 3-day incubation, the CS of cements prepared with γ-PGA was significantly different from that of cements prepared with the corresponding concentration of citric acid ($P < 0.05$). After 1-week incubation, the CS of cement prepared with powder component containing 5 wt % citric acid was 10.43 ± 0.69 MPa. The CS increased slightly with increasing amounts of citric acid up to 15.0 wt % and then decreased thereafter. After 1-week incubation, the CS of cements prepared with γ-PGA increased from 21.18 ± 5.60 to 50.23 ± 2.23 MPa when the amount of γ-PGA was increased from 5.0 to 10.0 wt %. However, the CS decreased with further increases in the amount of γ-PGA. After 1-week incubation, the CS of cements prepared with γ-PGA was significantly different than that of the corresponding cements incorporated with citric acid ($P < 0.05$).

TABLE III
Compressive Strength (CS) of Calcium Phosphate Cements after Incubation for 3 and 7 days

Acid amount (wt %)[a]	Incubation time			
	3 days		1 week	
	Citric acid	γ -PGA	Citric acid	γ -PGA
5	2.65 ± 0.04	23.72 ± 4.23	10.43 ± 0.69	21.18 ± 5.60
10	9.4 ± 0.49	31.08 ± 3.4	12.69 ± 1.21	50.23 ± 0.23
15	8.54 ± 1.72	38.76 ± 3.8	11.77 ± 0.74	34.68 ± 1.38
20	4.8 ± 0.19	24.44 ± 12.06	6.00 ± 0.74	25.03 ± 3.00

(Results are reported as mean ± standard deviation; $n = 5$).

[a] Concentration of organic acids (citric acid and γ-PGA) in cement powders.

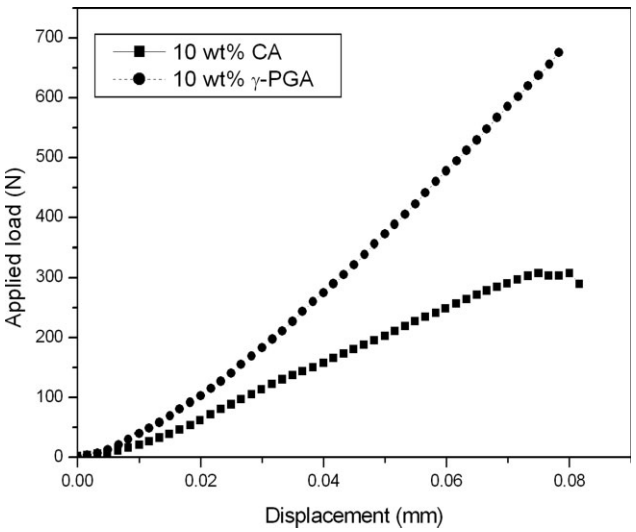

Figure 4 Typical load–displacement relationship of calcium phosphate cements prepared with powder component containing 10 wt % citric acid (10 wt % CA) or γ-PGA (10 wt % γ-PGA).

Figure 4 shows typical load–displacement relationship of cements prepared with powder component containing citric acid or γ-PGA. The slope of the set cement prepared with powder component containing 10 wt % γ-PGA was much higher than that of the set cement prepared with powder component containing 10 wt % citric acid. The area under the load–displacement curve of the set cement prepared with powder component containing 10 wt % γ-PGA was much larger than that of the set cement prepared with the powder component containing 10 wt % of citric acid. For other specimens and formulations, the experimental trends were similar to those shown in Figure 4. Therefore, the compressive modulus and toughness, as well as the mechanical strength, of set cements were increased with γ-PGA incorporation.

Figure 5 shows low magnification (×200) SEM micrographs of the fractured surface of the cements after 1-week incubation. The surface of the cement prepared with powder component containing 5.0 wt % citric acid was porous and coarse. In contrast, cements prepared with the higher concentration of citric acid were dense. In the case of cements with γ-PGA, air bubbles were involved and increased with increasing amounts of incorporated γ-PGA. The high viscosity of the cement pastes was thought to trap air during mixing.

Figure 6 shows high magnification (2000×) SEM micrographs of the fractured surface of the cements after 1-week incubation. The large needle dimensions were shown at the cement prepared with

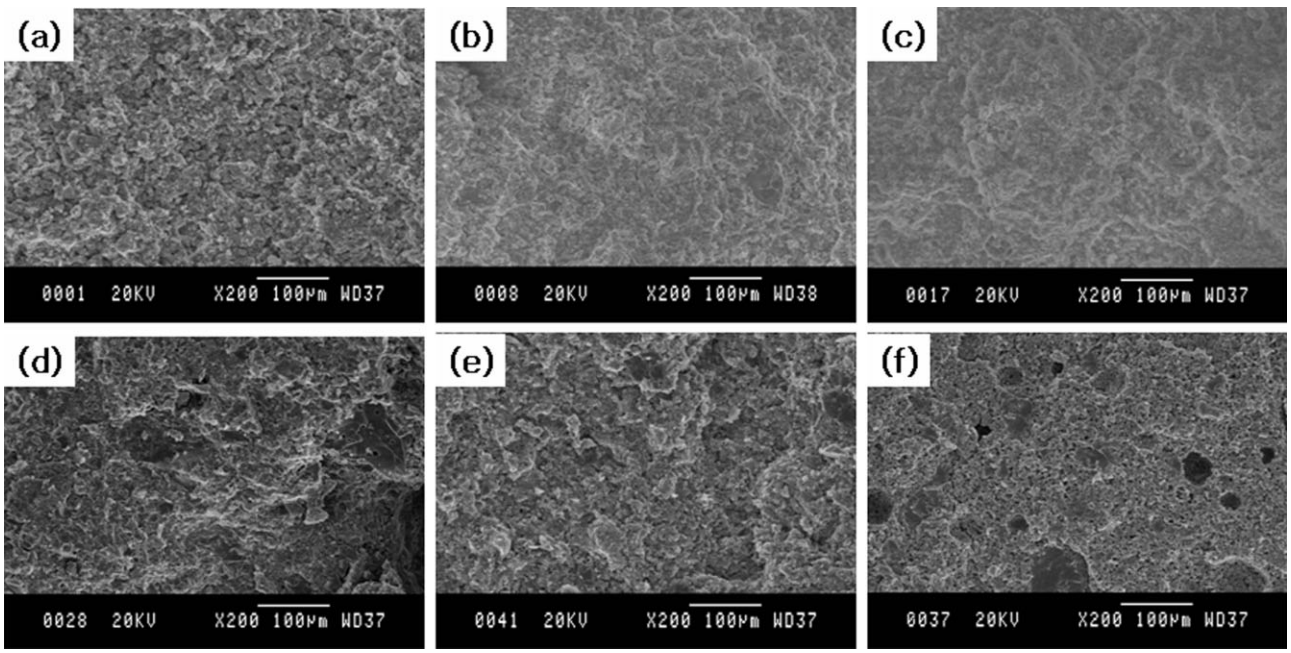

Figure 5 SEM images (×200) of calcium phosphate cements prepared with powder components containing 5 (a), 10 (b), and 20 (c) wt % citric acid and 5 (d), 10 (e), and 20 (f) wt % γ-PGA, after 1-week incubation.

powder containing 5 wt % citric acid. The needle size decreased with increasing amounts of citric acid. On the fractured surface of cements with γ-PGA, the needle dimensions were hardly observed. The surface became to be more porous when the amount of γ-PGA was increased.

Figure 7 shows FTIR spectra of carboxyl stretching region of cement powders and their corresponding set cements incubated for 3 days. In the spectrum of cement powder with citric acid, the carboxyl stretching peaks of carboxylic acid in citric acid appeared at 1755 and 1708 cm^{-1}. However, in the spectrum of set cement with citric acid, the peaks of carboxylic acid disappeared, whereas the carboxyl stretching peaks of calcium citrate were observed at 1543–1643 cm^{-1}. In the spectrum of cement powder with γ-PGA, the carboxyl stretching peak of carboxylic acid in γ-PGA appeared at 1736 cm^{-1}. However, in the

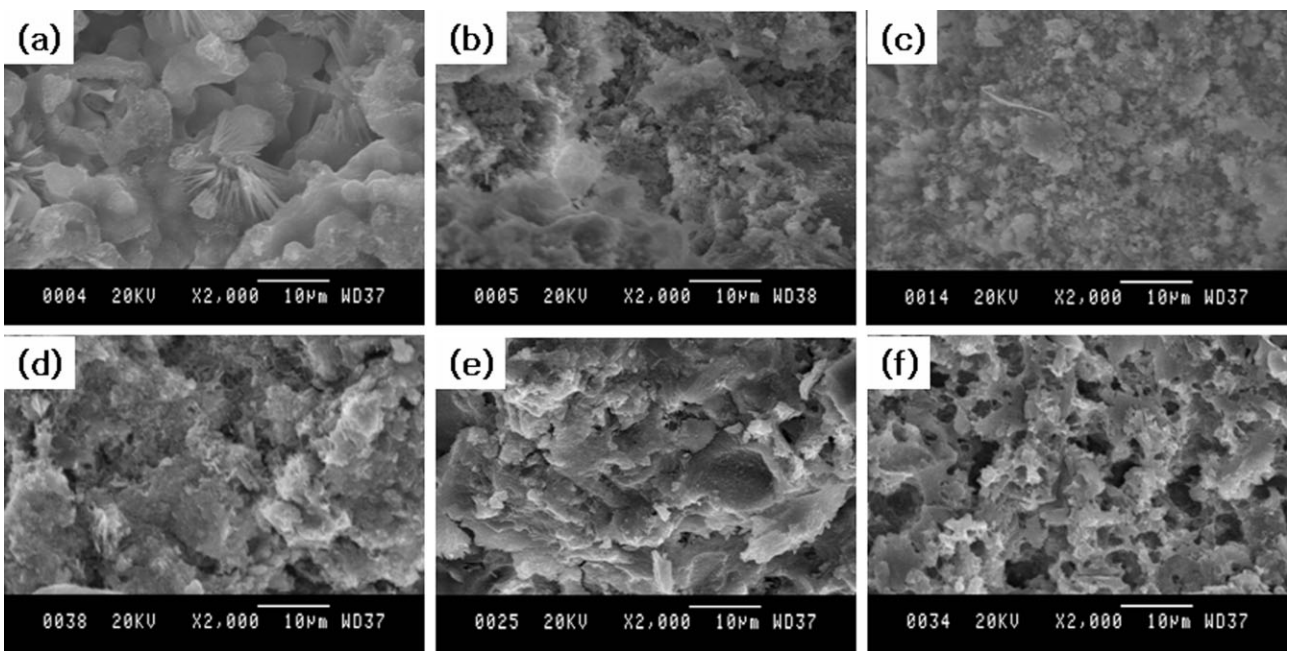

Figure 6 SEM images (×2000) of calcium phosphate cements prepared with powder components containing 5 (a), 10 (b), and 20 (c) wt % of citric acid, and 5 (d), 10 (e), and 20 (f) wt % of γ-PGA, after 1-week incubation.

spectrum of set cement with γ-PGA, the carboxylic acid peak disappeared, but the carboxyl stretching peak of calcium acetate was observed at 1643 cm^{-1}.

Figure 8 shows XRD patterns of the TCP powder and the set cements prepared with powder components without organic acids and with 10 wt % citric acid or γ-PGA. The incubation time of the set cements was 1 week. Peaks of α- and β-TCP were detected in the TCP powder used in this study. Thus, it was considered to be a mixture of α- and β-TCP. In the XRD pattern of cement prepared using the powder component without organic acids and with deionized water as the liquid phase, the intensity of α-TCP peaks decreased and hydroxyapatite (HA) peaks appeared. In the XRD patterns of set cements with citric acid or γ-PGA, HA peaks appeared but their intensity was weaker than those in set cement without organic acids. We could not discriminate between the peaks of dicalcium phosphate dehydrate (DCPD) and HA since their peaks almost overlapped. Therefore, formation of DCPD could not be determined by means of XRD analysis.

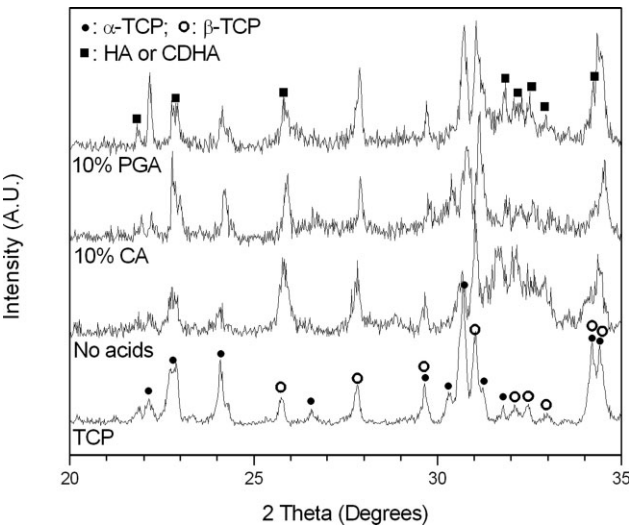

Figure 8 XRD patterns of TCP powder (cement powder) and set cements prepared with powder components without organic acids (TCP only) and with 10 wt % citric acid (10% CA) and γ-PGA (10% PGA), incubated for 1 week.

DISCUSSION

γ-PGA hardly dissolves in neutral or acidic aqueous solution. Therefore, in this study γ-PGA was admixed in the powder component. Citric acid was also incorporated in the powder component for comparison in equivalent conditions.

Matsuya et al.[8] proposed that ionic crosslinks formed between calcium ions and PMVE-Ma, used as a polyacid, in a cement-forming reaction of tetracalcium phosphate (TTCP) and PMVE-Ma. With slight modification of their work, we propose the following formulation for the cement-forming reaction in α-TCP/citric acid and α-TCP/γ-PGA systems where R—COOH represents organic acids such as citric acid and γ-PGA.

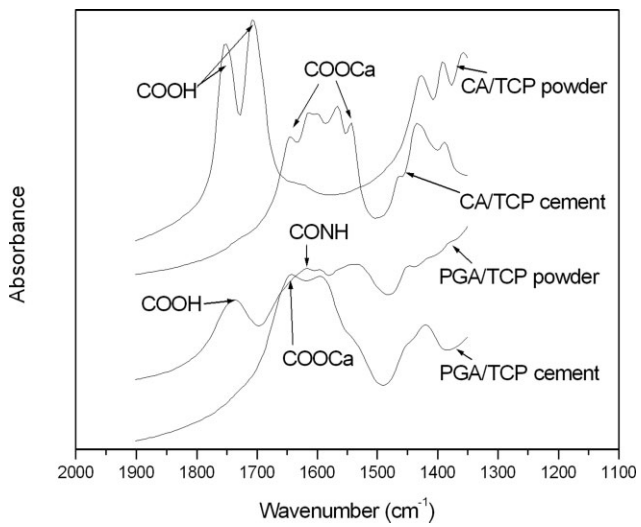

Figure 7 FTIR spectra of carboxyl stretching region of TCP cements incubated for 3 days. Powder with citric acid (CA/TCP powder) and its corresponding set cement (CA/TCP cement); powder with γ-PGA (PGA/TCP powder) and its corresponding set cement (PGA/TCP cement).

$$\text{R—COOH} \longrightarrow \text{R—COO}^- + \text{H}^+ \quad (1)$$

$$\text{Ca}_3(\text{PO4})_2 + 2\text{H}^+ \longrightarrow 3\text{Ca}^{2+} + 2\text{HPO}_4^{2-} \quad (2)$$

$$2\text{R—COOH} + \text{Ca}_3(\text{PO4})_2 + 2\text{H}_2\text{O} \longrightarrow \text{R—COO—Ca—OOC —R} + 2\text{CaHPO}_4 2\text{H}_2\text{O} \quad (3)$$

$$3\text{Ca}_3(\text{PO}_4)_2 + \text{H}_2\text{O} \longrightarrow \text{Ca}_{10}(\text{HPO}_4)(\text{PO}_4)_5(\text{OH}) \quad (4)$$

$$10\text{CaHPO}_4 2\text{H}_2\text{O} \longrightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 4\text{H}_3\text{PO}_4 + 18\text{H}_2\text{O} \quad (5)$$

Hydrogen ions dissociate from the organic acids in an aqueous environment, as shown in reaction (1). The hydrogen ions ionize/dissolve α-TCP, and then calcium (Ca^{2+}) and hydrogen phosphate (HPO_4^{2-}) ions are formed, as shown in reaction (2), which is a dissolution/neutralization action of hydrogen ions. The Ca^{2+} ions form ionic crosslinks with citric acid or γ-PGA molecules in the cement mixture. Furthermore, the Ca^{2+} ions may react with hydrogen phosphate ions to form DCPD, which then precipitates [reaction (3)]. In the cement-forming reaction, calcium-deficient hydroxyapatitie (CDHA) may be formed by the hydrolysis of α-TCP [reaction (4)].[12,13] The DCPD can be also changed to HA

in the physiological environment, as shown in reaction.[5,14]

α-TCP is basic in water, as shown in Figure 1. Therefore, an acid–base reaction occurs between α-TCP and hydrogen ions dissociated from organic acids, as shown in reaction (2). As shown in reaction (3), citric acid is converted to calcium citrate during the acid–base reaction, while γ-PGA forms a polymeric γ-PGA-calcium complex. The polymer complex may form ionic crosslinks by ionic bonding between calcium ions and carboxyl groups of γ-PGA because the γ-PGA molecule has many carboxylic acid groups. The acid–base reaction of citric acid-incorporated cements may be faster than that of γ-PGA incorporated cements because citric acid is more acidic when comparing equivalent concentrations of the organic acids, as shown in Figure 1. Therefore, the concentration of released calcium ions in the citric acid-incorporated cements was higher than that in the γ-PGA incorporated cements at equivalent concentrations of organic acids (Table I). The fast acid–base reaction of the citric acid-incorporated cements was very relevant to their initial setting time. That is, the initial setting times of the citric-acid incorporated cements were faster than those of the γ-PGA incorporated cements at equivalent concentrations (Table I).

The viscosity of cement pastes is very important for the handling characteristics of bone cements in clinical applications. That is, paste with too high viscosity is hard to mix and inject, while paste with too low viscosity can be washed-out by the body fluid. As shown in Figure 2, cement pastes with organic acids were highly viscous, while cement paste without organic acids had low viscosity. The dynamic viscosity of cement pastes increased with time. This change was considered to result from the precipitation of dissolved ionic materials such as calcium, phosphate, γ-PGA, and citrate ions due to their supersaturation in the liquid phase of cement pastes. The cement paste with 10 wt % citric acid exhibited a rapid increase in dynamic viscosity between 100 and 200 s (Fig. 2). This rapid increase appeared to be closely associated with the fast precipitation of dissolved ionic materials through the fast acid–base reaction.

The initial viscosity of cement pastes shown in Figure 3 closely influenced the handling property of cements. The increase in the concentration of citric acid did not increase the initial viscosity of the cement pastes. That is, small molecules such as citric acid may not affect the viscosity of the cement paste much. However, the initial viscosity of the cement pastes increased with increasing concentrations of γ-PGA. The cement paste prepared with the powder components containing more than 15 wt % γ-PGA was very hard to mix. Hence, large molecules such as γ-PGA may severely affect the viscosity of the cement paste, presumably due to their large molecular size.

Despite more air bubbles involved, the mechanical strength of set cements with γ-PGA was much higher than that of set cements with citric acid at equivalent concentrations of organic acids, as shown in Tables II and III and Figure 4. The SEM, FTIR, and XRD analyses were carried out to determine the chemical and physical behaviors in the set cements (Figs. 5–8).

According to SEM observations (Fig. 5), the matrix of the set cements became dense with increasing incorporation of organic acids. It was assumed that the precipitates, such as calcium citrate and DCPD, adsorbed between undissolved TCP particles led to the increased density. The precipitates might increase with an increase in the concentration of organic acids due to the increased rate of the acid–base reaction. Similarly, Tenhuisen and Brown reported that citrate ions adsorbed onto the surface of undissolved TTCP particles by substitution of phosphate ions in the cement-forming reaction of TTCP with citric acid.[15]

Tenhuisen and Brown[15] also proposed that the needle dimension might be formed by HA generation, decreasing with increasing citric acid concentration because the adsorption of citrate ions on the TTCP surface interfered with HA formation by blocking the release of calcium and phosphate ions from the TTCP particles. In this study, large needle dimensions were similarly observed in cement with a low concentration of citric acid, while the dimensions were reduced or disappeared with increasing concentrations of citric acid (Fig. 6). When γ-PGA was incorporated into the cements, polymeric complexes between Ca^{2+} ions and carboxyl groups of γ-PGA might form and then cover the surface of undissolved TCP particles like threads. Hence, the ion release and HA formation would be largely blocked and thus the needle dimensions would not be observed (Fig. 6).

According to FTIR spectra, in cements incorporated with either citric acid or γ-PGA, the carboxyl stretching region of carboxylic-acid groups disappeared during the cement-forming reaction, while the carboxyl stretching region of calcium carboxylate groups was observed (Fig. 7). Therefore, most carboxylic-acid groups might react with Ca^{2+} ions to form ionic complexes such as calcium citrate and γ-PGA-calcium complex. Similarly, the carboxyl stretching region of carboxylic-acid groups disappeared during the cement-forming reaction between TTCP and organic acids such as citric acid and acetic acid.[15]

Calcium citrate may form ionic crosslinks with a high degree of cross-linking because the citrate ion

is trivalent. Furthermore, γ-PGA-calcium complexes also form ionic crosslinks with a high degree of crosslinking because the γ-PGA molecule has numerous carboxylic-acid groups. The crosslinked polymer chains and calcium citrate molecules surround the unreacted TCP particles and thus increase the mechanical strength of CPC. However, despite more air bubbles involved, γ-PGA-incorporated cements were mechanically stronger than citric acid-incorporated cements. It was assumed that the molecular entanglement of polymer chains increased the mechanical strength of CPC, whereas small molecules such as calcium citrate were not entangled. The greater toughness of PCPCs was also presumably due to molecular entanglement of the polymer chains (Fig. 4).

According to XRD patterns of the set cements, the HA formation in cements incorporated with citric acid or γ-PGA was less than that in cement without organic acids (Fig. 8). Therefore, the mechanical strength of CPC does not appear to be dependent on HA formation. Similarly, it was reported that HA formation was minimal in the cement-forming reaction between TTCP and polymeric carboxylic acids such as poly(acrylic acid) and poly(methyl vinyl ether-maleic acid).[6,7,16] Furthermore Tenhuisen and Brown reported that the amount of HA formed decreased with increasing concentration of poly(acrylic acid)[6] and citric acid[15] in the cement liquid. In the presence of these multifunctional acids, calcium ions released from calcium phosphate are initially used up in crosslinking reactions between acid molecules. In addition, these ionic complexes quickly form an insoluble matrix around unreacted calcium phosphate particles. Thus, further dissolution of calcium phosphate and subsequent conversion to HA are severely retarded. In addition, the dissolution rate of calcium phosphate decreases with increasing pH.[17] In set cements, the pH of the liquid phase becomes elevated by neutralization of the acid with calcium phosphate powder which, therefore, delays secondary dissolution of calcium phosphate and HA formation even further.

CONCLUSION

In this work, we prepared polymeric CPCs derived from γ-PGA. Their properties were compared with cements incorporated with citric acid. Because γ-PGA formed polymer crosslinks, the mechanical strength of polymeric CPCs derived from γ-PGA was significantly higher than that of the cements incorporated with citric acid at the equivalent concentration. HA formation was retarded by γ-PGA incorporation, which was similar to that with citric acid incorporation.

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