Effect of powder grinding on hydroxyapatite formation in a polymeric calcium phosphate cement prepared from tetracalcium phosphate and poly(methyl vinyl ether-maleic acid)

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Abstract

The primary aim of this study was to determine if cements based on poly(methyl vinyl ether-maleic acid) (PMVE-Ma) and tetracalcium phosphate resulted in hydroxyapatite formation. In addition, the mechanical strength of this type of polymeric calcium phosphate cement was evaluated. Cements were prepared by mixing, in a powder/liquid mass ratio of 3.0, an aqueous solution of PMVE-Ma (mass fraction = 25%) and tetracalcium phosphate powders ground for various periods of time. The tetracalcium phosphate powders and set cements were characterized by means of X-ray powder diffraction and scanning electron microscopy. Mechanical strengths of the cements were tested 24 h after mixing. Prolonged grinding of tetracalcium phosphate powder decreased particle size and/or crystallite size and increased lattice distortion. This enhanced the reactivity of the tetracalcium phosphate powder and elevated the extent of crosslinking between PMVE-Ma molecules, resulting in improved mechanical strength. Hydroxyapatite formation was detected in the cement prepared with the most finely ground tetracalcium phosphate powder. The conversion of residual tetracalcium phosphate particles to more thermodynamically stable hydroxyapatite crystals will reduce the solubility of the polymeric cement and increase its biocompatibility. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Matsuya et al. [1] recently reported that a polymeric calcium phosphate cement can be prepared by mixing an equimolar mixture of tetracalcium phosphate and dicalcium phosphate anhydrous with an aqueous solution of poly(methyl vinyl ether-maleic acid), PMVE-Ma (mass fraction = 20–30%). The polymeric calcium phosphate cement set within 30 min, and showed mechanical strengths comparable to those of zinc polycarboxylate cements. The hardening mechanism of the polymeric calcium phosphate cement depends primarily on the acid–base reaction between the carboxylic acid groups of PMVE-Ma and the basic tetracalcium phosphate [1]. This hardening mechanism is similar to that of the polycarboxylate cement that depends on the reaction between the carboxyl groups of the poly(acrylic acid) and zinc oxide [2, 3]. In the case of the polymeric calcium phosphate cement derived from PMVE-Ma and tetracalcium phosphate alone, the carboxyl groups of the polyacid react with calcium ions from tetracalcium phosphate. Because the overall Ca/P molar ratio is reduced to approximately the stoichiometric ratio (1.67) of hydroxyapatite by the calcium consumption, this reaction may also induce the formation of hydroxyapatite, the prototype of the inorganic component of teeth and bone. Thus, the polymeric calcium phosphate cement should be expected to be more biocompatible than the zinc

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polycarboxylate cement and other types of dental cement. In addition, the solubility of the cement will decrease with hydroxyapatite formation from residual tetracalcium phosphate particles, because the former is thermodynamically more stable than the latter. However, hydroxyapatite formation has not been confirmed in the previously prepared polymeric calcium phosphate cements. This may be attributed to the delayed reaction caused by the slow dissolution of tetracalcium phosphate powder into the cement liquid. The hypothesis of this study was that the dissolution of tetracalcium phosphate and hydroxyapatite formation can be accelerated by additional grinding of the powder, a process that increases the surface area of tetracalcium phosphate. However, additional grinding may affect not only hydroxyapatite formation, but also the mechanical strength and setting time of the resulting cement.

The objectives of this study were to assess the mechanical strengths and setting times of several polymeric calcium phosphate cements that were prepared by mixing PMVE-Ma with tetracalcium phosphate powders that were ground for various periods of time, and to confirm that hydroxyapatite formation occurred in the set cements.

2. Materials and methods

2.1. Cement powder

Tetracalcium phosphate was synthesized by the solid-state reaction of an equimolar mixture of calcium carbonate and dicalcium phosphate anhydrous (Ca/P molar ratio = 2.0) at 1450°C for 6 h. The tetracalcium phosphate was manually ground in an agate mortar with a pestle; the powder having particle sizes less than 45 μm were collected. This original powder was further ground for 0.5, 1, 2 and 5 h in a planetary micro-mill (Pulverisette 7, Fritsch GmbH, Idar-Oberstein, Germany) under dry conditions. The original and the various milled tetracalcium phosphate powders were used in the preparation of the polymeric calcium phosphate cements.

The tetracalcium phosphate powders were characterized by X-ray powder diffraction (RINT 2500V, Rigaku Ltd, Tokyo, Japan) to examine the effect of grinding on crystallite size and lattice distortion. The crystallite size and lattice distortion were calculated using a computer program attached to the apparatus. In this calculation, it is assumed that the peak broadenings due to crystallite size and lattice distortion can be approximated by the Cauchy function and the Gauss function, respectively, and also assumed that the crystals are equidimensional (Operating instructions for the application software attached to RINT 2500V, Rigaku Ltd., Tokyo, Japan). The integral intensities of the 200, 032, 040, 212, 033, −204,

053 and 400 peaks of the tetracalcium phosphate diffraction pattern, automatically collected with monochromatized Cu K_α radiation ($\lambda = 0.1541$ nm) generated at 40 kV and 100 mA, were used in the calculation. Four samples were measured for each tetracalcium phosphate powder. A least-squares method was used to evaluate the dependence of crystallite size and lattice distortion on grinding time. In addition, the powder samples were sputter-coated with gold, and observed with the secondary emission mode using scanning electron microscope (SEM) (JSM5410, JEOL, Tokyo, Japan).

2.2. Cement liquid

The cement liquid used in this study was an aqueous solution of PMVE-Ma, mass fraction = 25%. This solution was prepared by heating the corresponding aqueous mixture of a commercial methyl vinyl ether-maleic anhydride copolymer (Gantrez AN-149, Batch No. 90430, GAF Corp., Wayne, NJ, USA) at 60°C for 48 h as described previously [1].

2.3. Cement preparation

The cement components were mixed at 23–25°C and 50–70% relative humidity using a powder/liquid mass ratio of 3.0. The cement pastes were mixed for 45 s, and setting times were determined under 100% relative humidity at 37°C using a standard Gillmore needle test [4]. The cement pastes were placed into Teflon molds of 6 mm in height and 4 mm in diameter for the preparation of compressive strength specimens and stainless steel molds of 25 mm long and 2 mm square for the preparation of flexural strength specimens. They were covered with two glass plates fixed in place with steel binder clips, and then cured for 1 h at 37°C and 100% relative humidity prior to their removal from the molds and subsequent storage in distilled water at 37°C.

2.4. Mechanical testing

Compressive strength and flexural strength were determined one day after mixing using a tensile and compressive testing machine (SV-301, Imada Ltd, Aichi, Japan) at loading rates of 1.0 and 0.5 mm/min, respectively. Three-point bonding specimens for flexural strength were supported by two rods set 20 mm apart. The dimensions of each specimen were measured with a micrometer prior to testing. Six specimens were made of each material for each strength test. The data are expressed as mean values ($n = 6$) with their standard deviations (SD). Differences among means were analyzed using ANOVA and Scheffe's multiple comparisons test.

2.5. X-ray powder diffraction analysis and SEM observation of set cement

The set cement specimens were immersed in distilled water kept at 37°C for 1, 7 and 196 d, and then dried at room temperature for more than 1 d. The 1, 7 and 196 d aged cements were manually ground using an agate mortar and a pestle, and then examined by means of X-ray powder diffraction in the same way as previously described. The fractured surfaces of 196 d aged cements were also sputter-coated with gold and observed by SEM.

3. Results

Figure 1 shows X-ray diffraction patterns of the tetracalcium phosphate powders used in this study. The patterns showed that there were no detectable amounts of other phases, such as hydroxyapatite and calcium hydroxide. The amount of those phases in the present system was estimated to be less than 5% mass fraction. The effect of grinding time on the crystallite size and lattice distortion of the tetracalcium phosphate powders is shown in Fig. 2. The crystallite size and lattice distortion were shown as mean and standard deviation ($n = 4$) in the figure. With increasing grinding time, the crystallite size decreased approximately linearly ($R = 0.9306$), while the lattice distortion increased ($R = 0.9312$). Figures 3 and 4 show the scanning electron micrographs of the original tetracalcium phosphate powder and the additionally ground powders, respectively. Aggregation of many smaller particles can be observed in the photomicrographs of the additionally ground powders.

Table 1 shows the mechanical strength of the polymeric cements prepared from those tetracalcium phosphate powders one day after mixing. The additional 0.5 h grinding of the powder increased the mechanical strength of the polymeric calcium phosphate cement. However,

Fig. 2. Effect of grinding time on the crystallite size and lattice distortion of tetracalcium phosphate powder. It was evaluated by a least-squares method. Vertical lines indicate the standard uncertainty ($n = 4$).

Fig. 3. Scanning electron micrograph of original tetracalcium phosphate powder.

further grinding did not result in increases in mechanical strength. The compressive strength and flexural strength of the cement prepared using 5 h ground tetracalcium phosphate powder were about 48.3 and 16.1 MPa, respectively. On the other hand, the setting time of the polymeric cements seemed to slightly decrease from 20 to 17 min with increased grinding time, but no statistical difference could be detected.

Figure 5 shows the X-ray diffraction patterns for the 1 d (a), 7 d (b) and 196 d (c) aged cements, respectively. No formation of hydroxyapatite could be observed in any of the cements aged in distilled water at 37°C for one day. In the case of 7 and 196 d aged cements, hydroxyapatite was detected in the cement prepared using the 5 h ground tetracalcium phosphate powder. Even at 196 d, however, hydroxyapatite formation was hardly detected in the other cements. The results of SEM observation (not shown) did not show clear differences between the fractured surfaces of the set cements prepared using the original tetracalcium phosphate powder and 5 h ground powder.
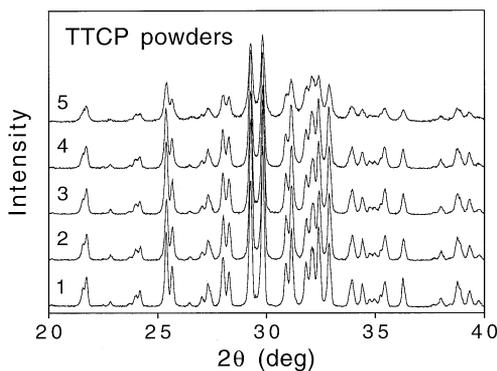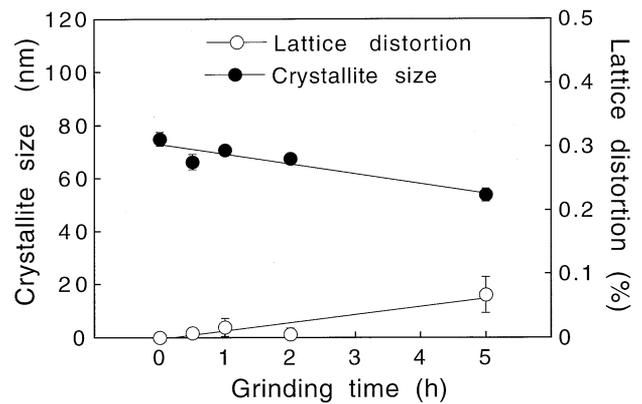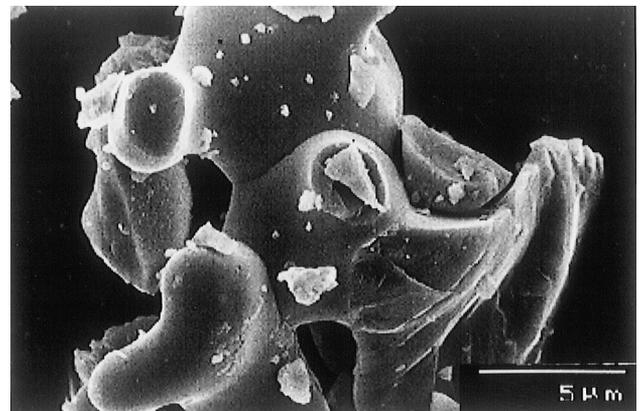

Fig. 1. X-ray diffraction patterns of original tetracalcium phosphate powder (1) and 0.5 h (2), 1 h (3), 2 h (4) and 5 h (5) ground tetracalcium phosphate powders.
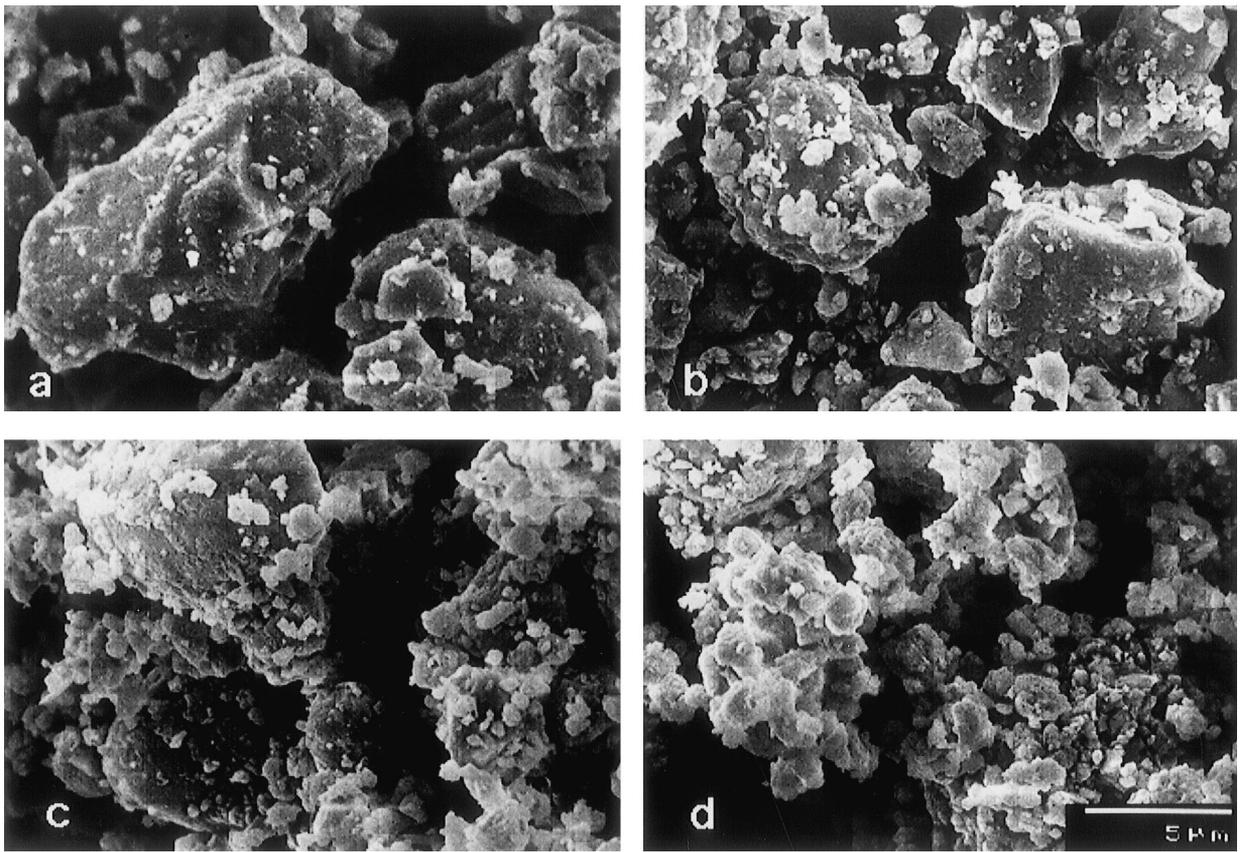
Fig. 4. Scanning electron micrographs of 0.5 h (a), 1 h (b), 2 h (c) and 5 h (d) ground tetracalcium phosphate powders.

Table 1
Effect of powder grinding on 24 h mechanical strength of polymeric calcium phosphate cement prepared from tetracalcium phosphate powder and PMVE-Ma (mass fraction = 25%)

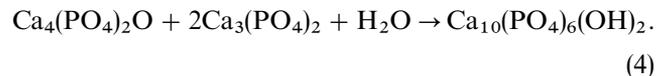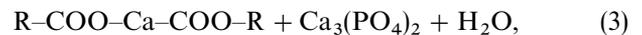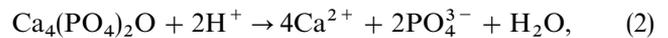| Additional grinding time (h)	Compressive strength (MPa)		Flexural strength (MPa)	
	n	Mean $\pm$ SD	n	Mean $\pm$ SD
0.0	6	22.9 ± 2.0	6	9.8 ± 1.2
0.5	6	46.4 ± 8.1^{a}	6	15.6 ± 1.8^{b}
1.0	6	49.0 ± 7.2^{a}	6	14.3 ± 1.4^{b}
2.0	6	48.4 ± 2.4^{a}	6	13.1 ± 1.0^{b}
5.0	6	48.3 ± 3.2^{a}	6	16.1 ± 2.5^{b}

n = number of samples.
SD = standard deviation.
Values with same superscript letter are not significantly different at $P = 0.05$.

4. Discussions

The following formulation is proposed for the cement forming reaction in the polymeric calcium phosphate cement where R–COOH represents PMVE-Ma.

$$R\text{–}COOH \rightarrow R\text{–}COO^- + H^+, \tag{1}$$

$$Ca_4(PO_4)_2O + 2H^+ \rightarrow 4Ca^{2+} + 2PO_4^{3-} + H_2O, \tag{2}$$

$$2R\text{–}COOH + Ca_4(PO_4)_2O \rightarrow$$
$$R\text{–}COO\text{–}Ca\text{–}COO\text{–}R + Ca_3(PO_4)_2 + H_2O, \tag{3}$$

$$Ca_4(PO_4)_2O + 2Ca_3(PO_4)_2 + H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2. \tag{4}$$

Calcium ions are released from tetracalcium phosphate powder by the dissolution/neutralization action of hydrogen ions from PMVE-Ma, as shown in the formulas (1) and (2). The Ca^{2+} ions form ionic crosslinks between the PMVE-Ma molecules of the liquid component in the cement mixture, as shown in reaction (3). The phosphate ions released in reaction (2) remain in the cement matrix until the cement is immersed in water, where they can then react with calcium and phosphate ions released by hydrolysis of residual tetracalcium phosphate to produce hydroxyapatite, as shown in formula (4).

The results of the X-ray powder diffraction analyses and SEM observations for the tetracalcium phosphate powders suggest that the additional grinding increased both their surface area and their reactivity (Figs. 1–4). Hence, it is likely that calcium ions are released more quickly from the finely ground tetracalcium phosphate powders than from the original powder, resulting in
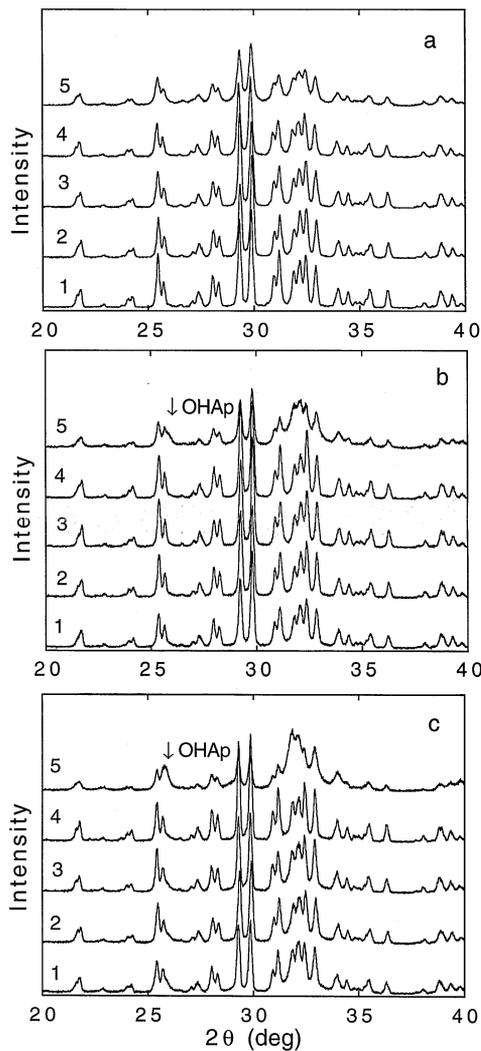
Fig. 5. Hydroxyapatite formation with aging time (a: 1 d aging, b: 7 d aging and c: 196 d aging) in the cements prepared using original tetracalcium phosphate powder (1) and 0.5 h (2), 1 h (3), 2 h (4) and 5 h (5) ground tetracalcium phosphate powders.

greater calcium binding and crosslinking of the PMVE-Ma polymer. The crosslinked polymer chains, i.e., the cement matrix, surrounds the unreacted tetracalcium phosphate particles. Thus, cement matrices that have a greater degree of crosslinking sites should make for more rigid cements. In fact, the cements prepared from the additionally ground tetracalcium phosphate powders showed higher mechanical strength than the cement formulated from original tetracalcium phosphate, as shown in Table 1. Further, after the 1 d aging, no hydroxyapatite formation was observed even in the cement prepared using 5 h ground tetracalcium phosphate powder, as shown in Fig. 5a. All the peaks in the diffraction patterns for the 1 d aged cements were due to tetracalcium phosphate. This result shows that the set cements consist of only unreacted tetracalcium phosphate and an amorphous crosslinked copolymer matrix and that hydroxyapatite formation does not contribute to the hardening 1 d

after mixing. Therefore, it appears that the higher mechanical strengths of the 1 d aged cements prepared from the additionally ground powders resulted from the enhanced stabilization and rigidity of the cement matrix brought about Ca^{2+} bridges forming more highly crosslinked PMVE-Ma polymer chains. That is to say, the reactions expressed by the reactions (2) and (3) are accelerated in the cement prepared using additionally ground tetracalcium phosphate powders.

Hydroxyapatite formation was detected in the cement prepared using 5 h ground tetracalcium phosphate powder at 7 and 196 d, as shown in Fig. 5. Normally, tetracalcium phosphate is not stable in neutral and acidic aqueous solutions and converts to hydroxyapatite or dicalcium phosphate dihydrate depending on the solution pH. In the case of the water-setting calcium phosphate cements prepared using a mixed powder of tetracalcium phosphate and dicalcium phosphate anhydrous [5], conversion to hydroxyapatite occurs several hours after mixing [6]. Radin and Ducheyne [7] have reported that tetracalcium phosphate powder was extensively transformed into poorly crystallized carbonated apatite after 2 d immersion in a simulated physiological solution kept at 37°C. Matsuya et al. [8] also found that tetracalcium phosphate powder converted to hydroxyapatite in 0.01–0.1 mol/l H_3PO_4 or KH_2PO_4 solution at a powder-to-solution ratio of 2 g/100 ml within 24 h. However, it has been reported that hydroxyapatite formation was minimal in the cement-forming reaction between a mixed powder of dicalcium phosphate/tetracalcium phosphate, or only tetracalcium phosphate and polymeric carboxylic acids such as poly(acrylic acid) and PMVE-Ma [1,9,10]. Particularly TenHuisen and Brown [10] reported that the amount of hydroxyapatite formed decreased with the increase of the poly(acrylic acid) concentration in the cement liquid. In the presence of these multifunctional acids, calcium ions released from tetracalcium phosphate are initially used up mainly in crosslinking reactions between polymeric acid molecules, as previously described [1]. In addition, these polymeric Ca^{2+} salts quickly form an insoluble matrix around unreacted tetracalcium phosphate particles. Thus further dissolution of tetracalcium phosphate and subsequent conversion to hydroxyapatite are severely retarded. In addition, the dissolution rate of tetracalcium phosphate decreases with increasing pH [8]. In the set cements, the pH of the liquid phase becomes elevated by neutralization of the acid with tetracalcium phosphate powder which, therefore, even further delays secondary dissolution of tetracalcium phosphate and hydroxyapatite formation.

However, a large percentage of the crystals in the set cement prepared using 5 h ground tetracalcium phosphate powder was hydroxyapatite at 7 and 196 d (Fig. 5). The reactivity of the powder used in this cement seems to be significantly higher than in the other tetracalcium

phosphate powders (Figs. 1–3). It was expected, too, that the PMVE-Ma polymer chains in this cement would have the highest degree of crosslinking. Thus, the stability and rigidity of the binding matrix in this cement should have been greater than those in the other cements. However, we could not find a significant difference among the compressive strength and flexural strength values of the cements prepared using the additionally ground powders (Table 1). The mechanical strength values of the cement prepared using 5 h ground tetracalcium phosphate powder were almost the same as those of the cements prepared using 0.5, 1 and 2 h ground powders. This observation suggests that more calcium ions were initially released, especially from the 5 h ground tetracalcium phosphate powder, than required for optimal crosslinking. Thus, it is possible that not only free phosphate ions but also excess calcium ions remained in the binding matrix of the cement prepared using the 5 h ground tetracalcium phosphate powder. The resulting supersaturation of the liquid phase of this set cement may nucleate rapid hydroxyapatite formation. Although the additional 0.5 h grinding of the powder increased the mechanical strength of the polymeric calcium phosphate cement, further grinding did not result in increases in mechanical strength. This phenomenon might be attributed to an excessively high degree of crosslinking that can yield a rigid, brittle cement matrix, as mentioned in the case of glass ionomer cement [11]. However, since the cements based on tetracalcium phosphate and PMVE-Ma are not so brittle as glass ionomer cement, it may be that the nucleation of hydroxyapatite crystals also might have produced internal strain in the crosslinked PMVE-Ma, cement matrix, contributing to a reduction in plastic flow and enhanced stress concentration.

The setting time of this type of polymeric calcium phosphate cement was about 20 min even in the cements prepared using 5 h ground tetracalcium phosphate powders. This value is a little too long for clinical use. The slight decrease observed in setting time when the grinding time of the tetracalcium phosphate powder was increased was not statistically significant. Thus, extended grinding of the tetracalcium phosphate powder alone was not effective in shortening setting time. Nevertheless, the formation of the less soluble hydroxyapatite would make the polymeric calcium phosphate cement more useful in clinical applications, especially as a cavity liner and/or base cement in restorative dentistry. Recently, various calcium phosphate cements using organic acids or polyacids have been designed and reported in dentistry [1, 9, 10, 12–15]. These cements are based on acid base reaction and some of them are already used in clinical applications. However, their solubility is rather high [12–15]. The high solubility of the cement matrix formed from the calcium compound can be lowered by using polymeric acid as an acid component [12]. However, high solubility of the unreacted basic powder still re-

mains a problem, since the total solubility of a set cement prepared from powder and liquid depends on both the solubility of the unreacted powder and that of the cement matrix that forms from the reaction between the powder and liquid. It is expected that the hydroxyapatite-forming polymeric cement described in this paper would show greater durability in the mouth because of the lower solubility of hydroxyapatite compared to tetracalcium phosphate as well as that of the more highly crosslinked poly salt matrix. However, hydroxyapatite formation in a polymeric cement does not necessarily elevate its mechanical strength. Other factors such as the degree of crosslinking and residual stress in the poly salt matrix, internal strains and flaws in the hydroxyapatite crytals and the quality of the interface and/or inter phase all can affect mechanical strength. Further studies of these poly acid/tetracalcium phosphate cements are needed to better elucidate the effect of hydroxyapatite formation on the mechanical strength and durability of polymeric calcium phosphate cements.

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