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ABSTRACT

Calcium hydroxide cements can lack long-term stability and achieve sustained release by matrixcontrolled diffusion of hydroxyl ions. Tetracalcium phosphate (TTCP) hydrolyzes slowly to form calcium hydroxide and a thin insoluble apatite layer that prevents further reaction. In this study, mechanical amorphization was used to create a setting calcium-hydroxide-releasing cement from TTCP. The effect of high-energy ball milling of TTCP on the mechanical properties of the cement was investigated. X-ray diffraction data were used to determine the phase composition of the set cements. An accelerated in vitro test compared pH of water after prolonged boiling of nanocrystalline TTCP cements and a calcium salicylate material. As milling time increased, cement compressive strength and degree of conversion increased. Hydroxyl ion release from the cement was comparable with that from a calcium salicylate material. This new cement system offers the antimicrobial potential of calcium salicylate materials combined with the long-term stability of insoluble apatite cements.

KEY WORDS: tetracalcium phosphate, calcium phosphate cement, hydrolysis, calcium hydroxide.

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Nanocrystalline Tetracalcium Phosphate Cement

INTRODUCTION

alcium-hydroxide-containing cavity liners and pulp-capping materials may be formed by the reaction of Ca(OH), and salicylate ions to form calcium salicylate [with an unreacted Ca(OH)₂ component], or Ca(OH)₂ may be an additive in light-cured methacrylate-based resins (Craig, 1993). While salicylate-based systems release sufficient calcium hydroxide for significant antibacterial properties (Staehle et al., 1989) and the stimulation of secondary dentin (About et al., 2001), they are thought to be soluble (Prosser et al., 1982). Light-cured systems are less soluble; however, there is little evidence that they possess any considerable antibacterial effect (Staehle et al., 1989). Although adhesive resins have replaced Ca(OH)₂ cements for largely bacteria-free pulp-capping indications, there remains a need for a stable system with sustained calcium hydroxide release for lining deep cavities and treating infected root canals. Recently, a CaO/NaH₂PO₄ cement has been developed that sets to form hydroxyapatite (el-Briak et al., 2002), but a considerable excess of CaO was required for any antibacterial activity (Serraj et al., 2002). Hence, like the salicylate systems, the Ca(OH)₂ originated from a limited unreacted component. In this study, a new cement system based on nanocrystalline tetracalcium phosphate that sets to form hydroxyapatite (HA) and Ca(OH)₂ was developed. Since the reaction was incomplete shortly after setting, it was hypothesized that this system would deliver Ca(OH), over extended periods while maintaining mechanical integrity.

Hydrolysis of TTCP to HA and calcium hydroxide (Eq. 1) is slow under physiological conditions.

$$3Ca_4(PO_4)_2O + 3H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 2Ca(OH)_2$$
 (1)

TTCP remains unreacted in water at room temperature for 4 wks, but at 80°C complete hydrolysis occurs within 80 hrs (Elliott, 1994). The low reactivity has been attributed to the formation of a thin insoluble HA layer around the TTCP particles, inhibiting further hydrolysis (Martin and Brown, 1993).

In this study, phase-pure and highly crystalline TTCPs were ground in ethanol for up to 24 hrs. The setting reaction of nanocrystalline (nc) TTCP with a sodium phosphate solution was examined, and mechanical performance was monitored as a function of grinding time. The Ca(OH)₂ released from this cement was compared with that released from a commercial salicylate-based product.

MATERIALS & METHODS

We synthesized TTCP by heating a mixture of monetite and calcium carbonate at 1500°C for 18 hrs, a phase-pure and highly crystalline product was verified by x-ray diffraction (D-5005, Siemens, Karlsruhe, Germany). The TTCP was milled in a planetary ball mill (PM-400, Retsch, Germany; diameter, 400 mm

Grinding Time [hrs]	P/L Ratio	Setting Time [min], n = 3	Compressive Strength [MPa], n = 7	HA Content [wt%], n = 1	Cement Density [g min ⁻¹], n = 3	Relative Porosity [%], n = 3
0	2.50	-	-	15	-	-
1	2.67	52 ± 2.6	5.6 ± 0.8 ^b	39	2.884 ± 0.009	40 ± 1.0
2	2.50	46 ± 2.0	7.2 ± 1.6 ^b	48	2.842 ± 0.006	38 ± 0.2
4	2.35	42 ± 1.7	7.5 ± 1.0	55	2.830 ± 0.004	40 ± 0.5
8	2.22	36 ± 1.7	8.5 ± 1.9	67	2.748 ± 0.004	40 ± 0.9
12	2.10	30 ± 2.6	10.3 ± 1.4°	73	2.759 ± 0.009	42 ± 0.9
24	2.00	19 ± 4.4	10.0 ± 2.1°	88	2.784 ± 0.007	45 ± 0.2
1	2.00	-	5.3 ± 1.3 ^b	39	3.100 ± 0.005	44 ± 0.2
2	2.00	-	6.6±1.1 ^b	46	3.060 ± 0.005	46 ± 1.6
4	2.00	-	8.7 ± 1.0	56	2.938 ± 0.007	45 ± 0.7

Compressive strengths are significantly (p < 0.001) higher than those marked ^b.

unidirectionally at 250 rpm with 200 agate balls [10 mm], 125 g TTCP, and 125 mL ethanol). The particle size distribution was determined by laser particle size analysis (LA-300, Horiba, Kyoto, Japan).

XRD patterns of the starting materials, ncTTCPs and set

Intensity / cps



Figure 1. XRD patterns of reaction products of unground TTCP and ncTTCP ground for 1 hr, 4 hrs, and 24 hrs mixed with 2.5% Na₂HPO₄ solution at 37°C at a powder-to-liquid ratio of 2 g/mL, followed by storage in water until 24 hrs after being mixed. Prominent diffraction peaks are labeled: + TTCP, * HA.

cements, were recorded from $2\theta = 20-40^{\circ}$ (step size of 0.02° , count time 1 sec). Quantitative phase compositions of the cements were calculated by means of total Rietveld refinement analysis with the TOPAS software (AXS, Bruker, Karlsruhe, Germany). As references, the system's internal database structures of TTCP and HA were used, together with a Chebychev fourth-order background model and a Cu K_{α} emission profile.

The cement was produced by the mixture of ncTTCP and 2.5 wt% Na_2HPO_4 solution at powder-to-liquid (P:L) ratios

between 2.0 and 2.67 g/mL. The initial setting time of the cement was measured by the Gilmore needle test (ASTM, 2002). The densities of the cement reactants were found by helium pycnometry (Accupyc 1330, Micromeritics, Dunstable, UK). These data, combined with mass and dimension measurements, enabled us to calculate the relative porosity of the cement.

We prepared mechanical test samples by mixing 800 mg of powder with the required liquid volume on a glass slab for 30 sec. The cement paste was transferred in stainless steel molds (6 mm in diameter), and biaxially pressed at a pressure of 9 MPa for 5 sec, followed by a constant load of 0.7 MPa (Chow et al., 2000). After 2 hrs, the specimens were removed and stored in water for an additional 22 hrs at 37° C. Samples (n = 7 per condition) with an aspect ratio of 2:1 were tested in compression at a crosshead speed of 1 mm/min (Zwick-1440, Ulm, Germany). Statistical significance between mean strength values was determined by a one-way analysis of variance (ANOVA), and significance differences of means were evaluated by Tukey's post hoc test (SPSS v10.0, Chicago, IL, USA). Linear correlation between mean strength values and degree of conversion was performed with the use of Microsoft Excel 2000. Micrographs of carbon-coated fracture surfaces were recorded by scanning electron microscopy (Phillips FEI, XL30 ESEM).

To compare calcium hydroxide release and long-term stability of this cement system with that of a commercial calcium hydroxide product, Life (Kerr, Scafati, Italy), we boiled 600-700 mg of hardened cements (n = 3) in 100 mL of water for up to 600 min. The mass of the materials and the pH of the water were measured, and the water was refreshed after 1 min, 10 min, and every 100 min of boiling thereafter.

RESULTS

No hardening setting reaction occurred within 24 hrs when unground high-crystallinity TTCP was mixed with sodium phosphate solution. In contrast, a hardening system could be achieved by mixing ground ncTTCP with 2.5% sodium phosphate solution (Table). Depending on grinding time, it was found that different powder-to-liquid (P:L) ratios (2.67-2.0 g/mL) gave workable pastes of similar consistency, as determined qualitatively. An increase in milling time resulted



Figure 2. Scanning electron micrographs of ncTTCP cement microstructures after 24 hrs of setting with a 2.5% Na₂HPO₄ solution at a powder-to-liquid ratio of 2 g/mL; (a) two-hour-ground ncTTCP, (b) 24-hour-ground ncTTCP, and (c) particle size distributions of the unground, two-hour-, and 24-hour-ground materials.

in a decrease of the initial setting time from nearly 1 hr (onehour milling) to 19 min (24-hour milling). Cement (P:L = 2.67) made with ncTTCP ground for 1 hr in ethanol had a mean compressive strength of ~ 5 MPa (24 hrs after setting), but after 24 hours' grinding (P:L = 2), strength was ~ 10 MPa, despite a higher porosity being the result of the lower P:L mixing ratio. Mean compressive strengths of cements made from TTCPs ground for 12 hrs and 24 hrs were significantly (p < 0.001) higher than those of cements made from TTCP ground for only 1 hr and 2 hrs.

After the cements set, the only detectable phases were TTCP and HA; the presence of $Ca(OH)_2$ could not be confirmed by XRD analyses, suggesting that it had a very small (< 10 nm) crystal size (Fig. 1). The low crystallinity of the HA meant that characteristic diffraction peaks at and near 31.5° overlapped with TTCP peaks, but TTCP peak intensity

12.5

12

11.5

11

10.5

10

9.5

펍



Figure 3. Mean pH values (\pm SD; n = 3) of water in which ncTTCPs ground for 1 hr and 24 hrs and a commercial Ca(OH)₂/salicylate cement (Life, Kerr) were boiled. ncTTCP cements were prepared at a powder-to-liquid ratio of 2 g/mL with a 2.5% Na₂HPO₄ solution. All samples (600-700 mg) were boiled in 100 mL water for up to 600 min, and the immersion liquid was replaced after 1 min, 10 min, and thereafter every 100 min. *Values are significantly different (p < 0.001).

200

300

Boiling Time (h)

400

500

600

10

1

100

decreased as the extent of reaction increased. The raw material converted to form 14% HA when mixed with 2.5% Na_2HPO_4 , which increased as grinding time of the TTCP component increased and was at maximum for the cement made from the 24-hour-ground material (88%, Table). Standard uncertainty of x-ray diffraction refinement analysis (rwp factor) was 7-11%.

Scanning electron microscopy revealed considerable difference in microstructure between cements prepared from ncTTCP ground for 2 hrs and 24 hrs (Figs. 2a, 2b). Cements formed from TTCP ground for 2 hrs consisted of ~ 2-10 μ m particles covered with ~ 100 nm blade-shaped crystals, whereas only much smaller particles (~ 1 μ m) were evident within the 24-hour-ground cement matrix, which was also nanocrystalline but spheroidal. The scale of microstructural features of the hardened cements corresponded to the particle size distributions of the ncTTCPs reactant powders (Fig. 2c). Before being ground, the TTCP material was found to have a median particle size of 21 μ m, which decreased with milling time to 1.1 μ m after 24-hour grinding.

It could be determined that, initially, the pH of the water in which the commercial material had been boiled increased from ~ 10.6 to 11.3; thereafter, it remained at 10.5-11.0. The aging media of the ncTTCP cements had higher initial pH (~ 11.7), but after 600 min the 24-hour-ground material had a significantly (p < 0.01) lower pH (9.8) compared with the calcium salicylate material (pH 10.9) (Fig. 3). No weight loss was detected in the ncTTCP cement samples, and only 3% weight loss was detected in the commercial product; however, this material fragmented after 10 min of boiling.

DISCUSSION

This study demonstrated that the extent of reaction of TTCP can be increased by the input of mechanical energy into the particles prior to hydrolysis, which led to both a reduction of the particle size and a phase transformation to the nanocrystalline state. This nanocrystalline component was responsible for cement formation and hydrolysis to HA at physiological temperatures. This phenomenon has been reported for several metallic and ceramic compounds (Weeber and Bakker, 1988; Gaffet and Harmelin, 1990), but until recent work by our team (e.g., Gbureck *et al.*, 2003), it had not been applied to bioceramics, which is surprising, given the considerable need for deriving novel and useful properties from the limited number of

The presence of Ca(OH)₂ could be inferred from density measurements, since both HA and TTCP have a density > 3 g cm⁻³, and Ca(OH)₂ has a density of 2.3. A cement with a density of 2.8 g cm⁻³ therefore represented a cement containing ~ 30 wt% Ca(OH)₂; however, Eq. 1 appeared to be incorrect, since only 13 wt% Ca(OH)₂ could theoretically be formed. It seems more likely that calcium-deficient apatite was formed, yielding 20 wt% Ca(OH)₂, since calcium-deficient apatite has a density of ~ 2.9 g cm⁻³:

materials available for dental and medical use.

$$3Ca_{4}(PO_{4})_{2}O + 3H_{2}O \rightarrow$$
(2)
$$Ca_{0}(HPO_{4})(PO_{4})_{5}(OH) + 3Ca(OH)_{2}$$

The inverse exponential relationship between strength and porosity of ceramics and cements is well-documented (e.g., Takahashi et al., 1997); therefore it was notable that, in this system, no such correlation was found. Comparison between cements made from the same starting materials but different P:L ratios showed, first, that strengths were very similar for cements made from the same material and, second, that cements made with lower P:L ratios had higher density products than the cements made with less liquid component, yet the degrees of conversion were remarkably similar. This suggests that the calcium hydroxide leached out of the more porous cements made at P:L = 2 during setting while immersed in water, and that this phase contributed little if anything toward the strength of ncTTCPs cement. Furthermore, this verified that porosity could be used to control Ca(OH)₂ release kinetics. The mechanical properties of another mechanically activated cement system have been previously found to be linearly related to degree of conversion (Gbureck et al., 2003). In this system, a strong linear correlation was found between 39 and 73% conversion ($R^2 = 0.94$). However, when the degree of conversion was higher, little improvement in mean strength was noted, reducing the linear correlation coefficient to 0.86. Even this moderately positive correlation between strength and degree of setting suggested that, over the relative porosity ranges investigated (38-46%), the degree of reaction appeared to be the dominant factor affecting strength.

Fig. 3 illustrated that $Ca(OH)_2$ release from the cements made with ncTTCP milled for 1 hr and 24 hrs was not significantly different from that released from the commercial calcium salicylate material at a given measurement interval (p < 0.001), for up to 500 min. After 600 min of boiling, the pH of the water in which the cement made with ncTTCP ground for 24 hrs had been aged was lower than that of the calcium salicylate material. This may have been because of the increased porosity as a result of the lower P:L ratio of this cement, such that the more permeable cement matrix provided less of a barrier to diffusion. While this aging study did verify that similar concentrations of calcium hydroxide could be released from the ncTTCP and calcium salicylate cements, it was not sufficient for an accurate prediction of antimicrobial efficacy, since it is likely to be the pH at the cement surface and within its immediate microenvironment that determines antimicrobial activity. However, the observations that equivalent hydroxyl ion concentrations could be attained for the duration of the experiment, and that no disintegration of the ncTTCP was detected during accelerated aging, confirmed the potential of this cement as an antimicrobial dental material. Furthermore, this study demonstrated that mechanically induced nanocrystallinity represents a valuable route by which to alter the properties of the limited number of biologically acceptable materials available to the dental ceramicist.

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