RESEARCH

Development of a nonrigid, durable calcium phosphate cement for use in periodontal bone repair

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ABSTRACT

Background. Calcium phosphate cement (CPC) hardens in situ to form hydroxyapatite and has been used in dental and craniofacial restorative applications. However, when CPC was used in periodontal osseous repair, tooth mobility resulted in the fracture and exfoliation of the brittle CPC implant. The objective of the authors' study was to develop a strong and nonrigid CPC to provide compliance for tooth mobility without fracturing the implant.

Methods. The authors used tetracalcium phosphate, dicalcium phosphate anhydrous and biopolymer chitosan to develop a strong and nonrigid CPC. They used a powder:liquid ratio of 2:1, compared with the 1:1 ratio of a previously developed nonrigid CPC control. Specimens were characterized using a flexural test, scanning electron microscopy and powder X-ray diffraction.

Results. After 28 days of immersion, the new cement had a flexural strength (mean ± standard deviation; n = 6) of 5.2 ± 1.0 megapascals, higher than 1.8 ± 1.5 MPa for the control ($P < .05$) and overlapping the reported strengths of sintered hydroxyapatite implants and cancellous bone. This cement showed a high ductility with a strain at peak load of 6.5 ± 1.3 percent, compared with 4.4 ± 1.9 percent for the control; both were 20-fold higher than the 0.2 percent of the conventional CPC. Nanosized hydroxyapatite crystals, similar to those in teeth and bones, were formed in the cements.

Conclusions. The new nonrigid cement, containing nanohydroxyapatite crystals, possessed a high ductility and superior fracture resistance. This strong, tough and nonrigid CPC may be useful in periodontal repair to provide compliance for tooth mobility without fracture.

Clinical Implications. The results of this study may yield the first self-hardening and nonrigid hydroxyapatite composite with high strength and durability and large deformation capability to be useful in the regeneration of periodontal osseous defects.

Key Words. Periodontal bone repair; calcium phosphate cement; nanohydroxyapatite crystals; nonrigidity.

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Fifteen percent of people 13 years of age or older in the United States have advanced periodontal bone destruction.[1] Such destruction causes tooth loss. Ninety-eight percent of all people aged 65 years and older in the United States have lost eight to 12 teeth.[2] Furthermore, the need for hard-tissue treatment is predicted to increase dramatically as the world's population ages.[3] Periodontal therapy includes bone graft and guided tissue regeneration.[4,5] The use of autogenous bone can be problematic in terms of donor site pain and limited supply. Allografts such as demineralized bone matrix have raised concerns about pathogen transmission and immunorejection. Therefore, synthetic biomaterials continue to be of interest in alveolar bone repair in the treatment of periodontal disease.

Hydroxyapatite has found wide use in hard-tissue repair owing to its similarity to the apatite found in human bones and teeth.[6-8] Initial recommendations stated that hydroxyapatite implants should

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be considered acceptable for periodontal bone repair.[9] However, owing to their inability to be readily resorbed or replaced by new bone, hydroxyapatite implants have shown limited long-term clinical benefit.[10] Histologic analysis showed that nonresorbable hydroxyapatite implants had induced little new bone fill and limited, if any, periodontal bone regeneration.[11]

In light of all of this, a resorbable hydroxyapatite capable of regenerating new bone would be highly desirable. Calcium phosphate cement (CPC) is composed of a mixture of tetracalcium phosphate (TTCP) (Ca4[PO4]2O) and dicalcium phosphate anhydrous (DCPA) (CaHPO4).[12] When mixed with water, CPC powder forms a paste that can be sculpted during surgery to conform to the defects in hard tissues. This paste self-hardens to form resorbable hydroxyapatite with excellent osteoconductivity and bone-replacing capability.[12-14] As a result, CPC (referred to in this article as "conventional CPC") was approved in 1996 by the U.S. Food and Drug Administration (FDA) for repairing craniofacial defects in humans, thus becoming the first CPC available for clinical use.[14] However, the use of CPC was "limited to the reconstruction of non–stress-bearing bone,"[13] and "clinical usage was limited by … brittleness … ."[14] In periodontal osseous repair, tooth mobility resulted in the exfoliation and failure of the brittle CPC.[15] Periodontal bone supports the root surface of the tooth and so is stress-bearing under vertical and lateral occlusal forces.[15] Consequently, a mechanism for the failure of CPC was "the lack of sufficient flexural stress resistance."[15]

Calcium phosphate cement, when mixed with water, forms a paste that self-hardens to form resorbable hydroxyapatite with excellent osteoconductivity and bone-replacing capability.

In response to these limitations, a nonrigid CPC with increased fracture resistance was developed.[16] The nonrigid CPC's strain capability—the extent to which the specimen can deform without fracture—was increased by an order of magnitude over that of the conventional CPC, which was rigid and brittle.[16] However, these properties were determined after specimens were immersed in a physiological solution for one day.[16] In unpublished studies using longer immersion times, we detected specimen cracking and property degradation, which raised concern that this nonrigid CPC might not be sufficiently strong and durable for periodontal osseous repair.

Therefore, we undertook a study with the objective of developing a strong and nonrigid CPC that would accomplish two important goals:

- achieving a higher strength and fracture resistance than the previous nonrigid CPC without compromise of its high elasticity;
- maintaining its mechanical properties during prolonged immersion in a physiological solution without cracking or loss of strength.

MATERIALS AND METHODS

Specimen fabrication. The CPC powder consisted of a mixture of TTCP and DCPA powders at a molar ratio of 1:1.[12,16] The cement liquid was made by mixing chitosan lactate (Vanson [now Halosource], Redmond, Wash.) with distilled water at a mass fraction of 15 percent, on the basis of the results of a previous study.[16] Chitosan and its derivatives are natural biopolymers that are elastomeric and biocompatible.[17]

In preliminary studies, the addition of extra TTCP to the CPC powder together with the use of 15 percent chitosan at a powder:liquid ratio of 2:1 resulted in a nonrigid cement with a strain at peak load exceeding 5 percent. Conventional CPC without chitosan had a strain at peak load of 0.2 percent.[16] This increase in strain likely occurred because the extra TTCP, which was alkaline, caused the chitosan solution to jell more rapidly, while the CPC setting reaction occurred more slowly in the chitosan matrix. This resulted in the elastomeric chitosan's becoming the continuous phase in the set cement, thereby forming a more elastomeric composite.

Accordingly, we fabricated and investigated four cements: three experimental cements and a control cement. Cement A was a CPC-TTCP mixture at a 1:2 mass ratio. Cement B had a CPC:TTCP mass ratio of 1:5. Cement C had a CPC:TTCP mass ratio of 0:1. The corresponding TTCP:DCPA molar ratios were 3.7:1, 7.9:1 and 1:0, respectively. We selected these ratios on the basis of the results of the preliminary study with the requirement that the set cement possess a strain-at-peak-load value of at least one order of magnitude larger than the 0.2 percent for the conventional CPC.[16] In mixing each cement

powder with the liquid to make the specimens, cements A, B and C had the same powder:liquid mass ratio of 2:1. We selected the fourth cement, which served as the control, from one of our previous studies using CPC as the powder with no extra TTCP and mixed at a powder:liquid ratio of 1:1.[16] The liquid we used with all cements was water with 15 percent chitosan.

Setting time measurement. We mixed the paste, placed it into a mold of 6-millimeter diameter and 3-mm depth and incubated it at 100 percent relative humidity and 37 C.[18] When the powder component of the specimen did not scrub off when gently rubbed with fingers,[18] we determined that the setting had occurred enough to hold the specimen together. We used the time measured from the powder and liquid mixing to this point as the setting time.[18] The estimated uncertainty was ± 0.5 minutes.

Mechanical testing. We used molds of $3 \times 4 \times 25$ cubic millimeters^3 to make specimens.[19] We sandwiched the paste in the mold between two glass slides and set it in the humidor for four hours. We then demolded and immersed the specimens in a simulated physiological solution (as used in Xu and colleagues[16]) (1.15 millimolars per liter calcium, 1.2 mmol/L phosphorus, 133 mmol/L sodium chloride, 50 mmol/L 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid, buffered to a pH of 7.4) and stored them in an oven at 37 C for different periods as described below.

We had two study goals, which led to our testing two groups of specimens. Our first goal was to determine the effect of cement composition; therefore, the first group consisted of cements A, B and C and the control, all immersed for three days. Our second goal was to determine the effect of immersion time. We identified Cement A as the strongest candidate in the first group. Hence, the second group consisted of Cement A and the control, and we immersed both for 21 days and 28 days before testing them.

We used a three-point flexural test with a span of 20 mm to fracture the specimens at a crosshead speed of 1 mm/minute on a universal testing machine (5500R, MTS Systems, Cary, N.C.).[20] We determined flexural strength, strain at peak load and work of fracture (toughness).[16,20,21]

Powder X-ray diffraction analysis of conversion to hydroxyapatite. We used powder X-ray diffraction (XRD) analysis to examine the percentage of CPC conversion to hydroxyapatite.[16,22] We used a 2×3 full factorial design for Cement A and the control with three immersion times (three days, 21 days and 28 days). We recorded the XRD patterns with a powder X-ray diffractometer (DMAX 2000, Rigaku, Danvers, Mass.) using graphite-monochromatized copper Kα radiation ($\lambda = 0.154$ nanometers) generated at 40 kilovolts and 40 milliamperes. We prepared a series of samples that contained known amounts of hydroxyapatite using 100 percent converted CPC and known amounts of unreacted CPC powder. We constructed a standard curve describing the relationship between the mass fractions of hydroxyapatite and the intensities of (002) peak of hydroxyapatite. We obtained the hydroxyapatite conversion by comparing the standard curve with the measured (002) peak intensity of the experimental CPC specimen. The estimated uncertainty for this measurement was 1 percent.

Our first goal was to determine the effect of cement composition; our second goal was to determine the effect of immersion time.

Scanning electron microscopy and statistics. We examined specimens with a scanning electron microscope (SEM) (JSM-5300, JEOL, Peabody, Mass.). We performed two-way and one-way analysis of variance (ANOVA) and the Tukey multiple comparison tests to detect significant ($P \leq .05$) effects of the variables.

RESULTS

Table 1 lists the setting time results. Cements A through C had significantly shorter setting times than did the control (Tukey at $P = .05$). As shown in Figure 1, Cement A had a flexural strength (mean ± standard deviation; n = 6) of (5.3 ± 1.1) megapascals, significantly higher than (3.5 ± 0.5) MPa for Cement B, (3.0 ± 0.5) MPa for Cement C, and (2.9 ± 0.9) MPa for the control ($P < .05$). We detected no significant difference in strain at peak load ($P = .09$) and work of fracture ($P = .37$) between the cements.

Figure 2 (page 1135) is a scanning electron micrograph of the fracture surface of Cement A. We observed nanosized crystals with a width of approximately 100 nm and a length ranging from 200 to 500 nm; the other cements had similar features.

At 21 days, as shown in Figure 3 (page 1135),

TABLE 1

Calcium phosphate cement setting time.*†

MATERIAL	SETTING TIME (MINUTES)
Cement A	4.8 ± 0.5
Cement B	4.4 ± 0.5
Cement C	4.0 ± 0.0
Control	17.0 ± 0.8

* Mean ± standard deviation.
† Number of repeats for each cement = 4.

Cement A had a flexural strength (mean ± SD; n = 6) of (6.0 ± 0.8) MPa, significantly higher than (1.3 ± 0.8) MPa of the control ($P < .05$). Cement A had a work-of-fracture value of (843 ± 167) joules per square meter, sevenfold greater than the (116 ± 34) J/m² for the control. The two materials had strain-at-peak-load values that were not significantly different ($P > .05$).

At 28 days, as shown in Figure 3, Cement A had a flexural strength value of (5.2 ± 1.0) MPa, higher than (1.8 ± 1.5) MPa of the control ($P < .05$). The work-of-fracture value of Cement A was (688 ± 122) J/m², also higher than (130 ± 66) J/m² for the control ($P < .05$). The strain-at-peak-load value of Cement A was 6.5 ± 1.3 percent, not significantly different from that of the control (4.4 ± 1.9 percent) ($P > .05$).

We performed two-way ANOVA on a 2 × 3 design with Cement A and the control at three days, 21 days and 28 days. Cement A had higher strengths than the control at all three points ($P < .05$). Cement A showed no significant decrease in strength from three days to 28 days ($P > .1$). The control showed a significant decrease in strain at peak load at 21 days and 28 days versus its three-day values ($P < .05$), while Cement A showed no significant decrease ($P > .05$). Cement A had significantly higher work-of-fracture values than did the control at 21 days and 28 days ($P < .05$), but not at three days ($P > .05$).

Cracks were visible in the wet control specimens when the specimens were immersed in the physiological solution for 21 days or 28 days. Figure 4 (page 1136) shows the cracks at 28 days (arrows indicate cracks). We did not find such cracks in Cement A. To determine whether these cracks were only surface-localized, we also examined the specimen cross-sections with SEM. We observed similar cracks throughout the cross-sections of the control (arrows in the cross-section of the control), but no such cracks in the cross-sections of Cement A.

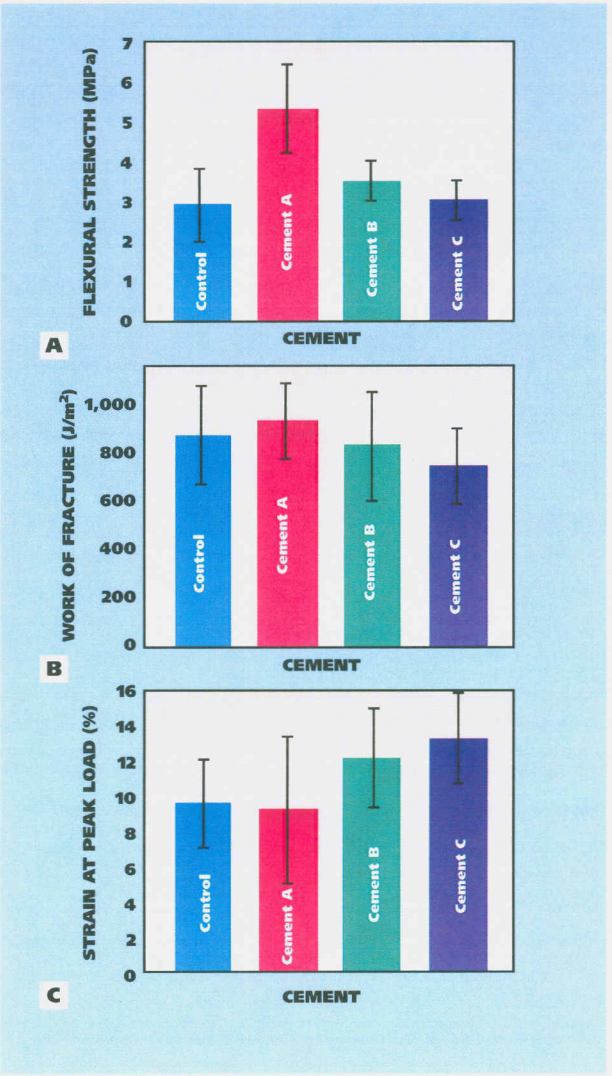

Figure 1. Mechanical properties of the nonrigid calcium phosphate cement (CPC) control that the authors developed previously and the three new nonrigid CPCs after three days of immersion in a physiological solution. Each value is the mean of six measurements, with the error bar showing one standard deviation (mean ± standard deviation; n = 6). J/m²: Joules per square meter. MPa: Megapascals.

Table 2 (page 1137) lists the percentages of conversion to hydroxyapatite. Two-way ANOVA detected significant effects of cement type and immersion time ($P < .05$), with a significant interaction between cement type and immersion time. The control had significantly higher percentages of hydroxyapatite than Cement A at all immersion times ($P < .05$). Both cements had increased conversion when the immersion time was increased from three days to 28 days.

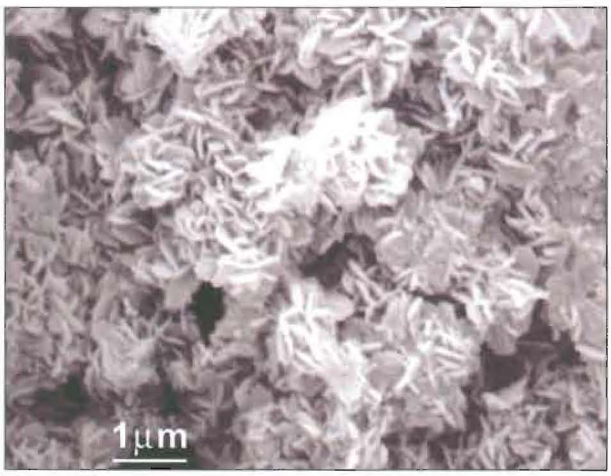

Figure 2. Scanning electron micrograph of the fracture surface of Cement A showing the formation of nanosized hydroxyapatite crystals. The four cements had similar fracture surface features at three days. μm: Micrometer.

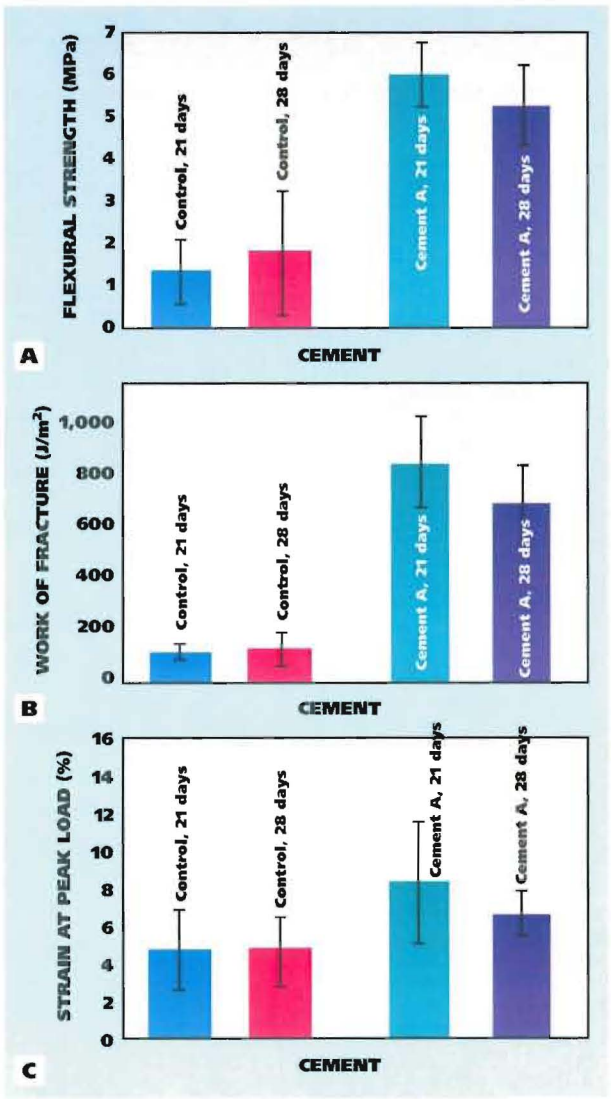

Figure 3. Effect of immersion time on the nonrigid calcium phosphate cement control and the new nonrigid Cement A at 21 days and 28 days of immersion in a physiological solution. Each value is mean ± standard deviation; n = 6. J/m²: Joules per square meter. MPa: Megapascals.

DISCUSSION

CPC possesses excellent osteoconductivity and bone replacement capability and is highly promising for use in a number of restorative dental and craniofacial procedures.[12-14] The FDA approved it for craniofacial indications a decade ago.[14] However, when CPC was used in periodontal bone repair, tooth mobility resulted in early fracture and eventual exfoliation of the rigid and brittle implants.[15] CPC is a typical brittle ceramic and can fracture catastrophically at a small deformation strain of about 0.2 percent. Therefore, it is desirable to have CPC in a nonrigid form that can sustain large deformation strains without fracture, thereby providing the needed compliance for tooth motion in alveolar bone repair for periodontal disease. The addition of TTCP to the CPC powder together with 15 percent chitosan resulted in a nonrigid CPC. For the conventional CPC, the setting mechanism was the reaction between TTCP and DCPA: $2Ca_4(PO_4)_2O + 2CaHPO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2$ (hydroxyapatite). For the CPC-chitosan composite, another faster setting occurred. Chitosan is soluble in acidic solutions but insoluble at alkaline pH. The mixing of the chitosan liquid with the CPC powder increased the pH and caused the soft CPC-chitosan paste to transform to an elastomeric solid. Hence, the initial setting of the CPC-chitosan composite was caused not by the relatively slower TTCP-DCPA conversion to hydroxyapatite, but by the faster chitosan setting due to increasing pH. The TTCP-DCPA conversion to hydroxyapatite proceeded within the elastomeric chitosan matrix. The reason that cements A through C set faster than the control (Table 1) likely was due to the extra TTCP (which was alkaline), which further increased the pH. This caused faster setting than the control, which did not have extra TTCP.

The setting time was four to five minutes for the new cements, compared with 17 minutes for the control. Fast setting should enable the cement to attain mechanical strength and geometrical integrity within a short period postoperatively. In a study in which conventional CPC was mixed with water and implanted subcutaneously, it failed to set and elicited a severe inflammatory

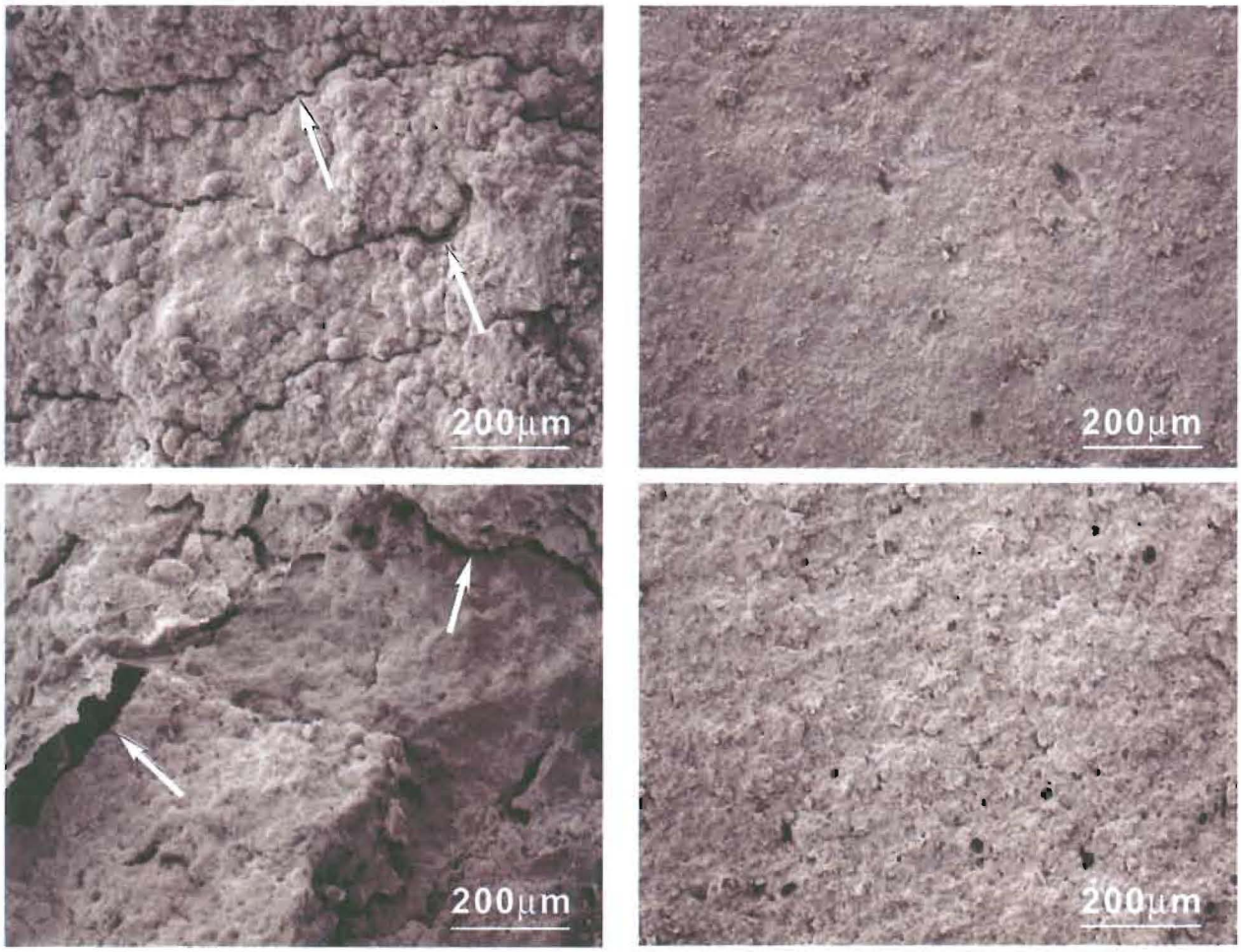

Figure 4. Scanning electron micrograph showing cracks in the surface and cross-section of the nonrigid calcium phosphate cement (CPC) control developed previously and the new nonrigid Cement A at 28 days of immersion in a physiological solution. Arrows indicate cracks in the control. No such cracks were observed in Cement A. μm: Micrometers.

response, probably owing to a low initial mechanical strength.[23] Hence, the rapid setting of the new cements should improve the initial mechanical strength, thereby avoiding implant disintegration. While a setting time of five minutes (Table 1) appeared to be appropriate for surgical placement, the cement remained nonrigid and could be molded after the initial setting.

The control had a strain-at-peak-load value of 4.4 percent at 28 days, compared with 0.2 percent for the conventional CPC.[16] The control, though nonrigid, had three deficiencies: low strength, low durability and a propensity to crack over time. The flexural strength of the control ranged from 1.3 MPa at 21 days to 1.8 MPa at 28 days. These values were lower than the reported flexural strength, which ranged from 2 to 11 MPa for sintered porous hydroxyapatite implants,[7] and a tensile strength of about 3.5 MPa for cancellous bone.[24] The control degraded during immersion with the occurrence of cracks. In contrast, the flexural strength of Cement A reached 5.5 MPa, overlapping the strengths of sintered porous hydroxyapatite and cancellous bone.

Cement A maintained its strength after immersion for 28 days. This improved durability in comparison with the control likely was a result of the higher powder:liquid ratio of 2:1, compared with the control's ratio of 1:1. A higher powder:liquid ratio increased the mineral content in the CPC-chitosan composite, thereby enhancing its structural integrity. This mechanism is not unlike that of dental resin-based composites, in which high levels of inorganic particles enhance the composite properties.[25,26] However, in our preliminary studies, when the powder:liquid ratio was increased to 3:1, the cement became brittle. While the optimal mineral:chitosan ratio possibly

occurred at a powder:liquid ratio of around 2:1, further studies are needed to investigate how the brittle-to-ductile transition is related to the mineral:chitosan ratio in the CPC-chitosan composite.

Comparisons should be made between Cement A, the control and sintered hydroxyapatite. Sintered hydroxyapatite requires machining to fit a bony defect and is not resorbable or replaceable by new bone.[10,11] Such implants have induced little new bone regeneration.[11] Compared with sintered hydroxyapatite, Cement A has three advantages. First, it can be molded to achieve intimate contact with neighboring bone. Second, the hydroxyapatite from CPC is bioresorbable.[13,14] This is because the hydroxyapatite from CPC is formed in an aqueous environment at body temperature and hence is more similar to biological apatites than is sintered hydroxyapatite formed at high temperatures.[13,14] Third, the nanohydroxyapatite crystals of Cement A (Figure 2) had sizes similar to those found in natural bones and teeth. For example, in the biomimetic fabrication of biomaterials, bone is considered a nanocomposite of nanosized apatite minerals and proteins.[27] Tooth enamel rods consist of apatite crystallites about 100 nm in diameter.[28] Dentin and bone have smaller apatite crystals, with dimensions of $5 \times 30 \times 100$ nm.[29] Compared with the control, Cement A had a lower percentage of conversion to hydroxyapatite (Table 2). This was likely because, with extra TTCP in Cement A, the DCPA was consumed in the conversion to hydroxyapatite while the TTCP was not fully consumed. TTCP can hydrolyze to form hydroxyapatite: $3Ca_4O(PO_4)_2 + 3H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 2Ca(OH)_2$.

Table 2 suggests that this process may take more than 28 days.

Meanwhile, TTCP is more basic and hence more soluble than hydroxyapatite at acidic pH,[13] such as that produced by osteoclast cells in vivo.[30] Therefore, Cement A with extra TTCP, once implanted in vivo, may be resorbed more rapidly than the conventional CPC. Further studies are needed to investigate whether Cement A, while being mechanically strong and nonrigid, also would possess a resorption rate similar to or faster than that of the conventional CPC in animal models.

While immersion times longer than 28 days also are of interest, new bone formation should have initiated in vivo after about one month.[8,31]

TABLE 2

X-ray diffraction measurement of hydroxyapatite conversion after different periods of immersion in a physiological solution.

MATERIAL	MASS PERCENTAGE OF HYDROXYAPATITE CONVERSION*, BY MEASUREMENT INTERVAL		
	3 Days	21 Days	28 Days
Cement A	(43.6 ± 2.1)	(65.7 ± 0.5)	(70.3 ± 2.3)
Control	(84.5 ± 2.9)	(98.2 ± 0.6)	(99.9 ± 1.1)

* Mean ± standard deviation; n = 4.

New bone is known to increase the strength of the implant[31]; hence, it is the strength of the implant in the early stage (that is, the first few weeks) after placement that is critically important. Further study is needed to examine the performance of the nonrigid CPC in the treatment of vertical intrabony periodontal defects, where sufficient elasticity and fracture resistance are needed to accommodate tooth movement associated with the occlusal function.[15] Another potential improvement would be to create interconnected macropores for bony ingrowth into Cement A by using porogens[19] and reinforcement fibers.[32] A previous study determined that "the lack of sufficient porosity in the set HAC [hydroxyapatite cement, referring to CPC] to allow bone ingrowth to occur" was a major reason for the failure of conventional CPC in periodontal bone repair.[15(p155)] Therefore, the favorable properties of Cement A, when coupled with macroporosity and fiber reinforcement,[19,32] may result in a nonrigid and macroporous scaffold with potential for regeneration of periodontal osseous defects.

CONCLUSION

We developed a nonrigid and high-strength CPC by incorporating TTCP and chitosan into the conventional CPC. Compared with a flexural strength of 1.8 MPa for an earlier nonrigid CPC, Cement A in this study had a flexural strength of 5.5 MPa, which it maintained during 28 days' immersion in a physiological solution. This strength overlapped the reported flexural strengths of 2 to 11 MPa for sintered porous hydroxyapatite implants and cancellous bone. Cement A had a strain-at-peak-load value of 7 percent, compared with 4 percent for the nonrigid CPC control; both were 20-fold greater than the 0.2 percent value for the conventional CPC.

Cement A had a work-of-fracture (toughness) value of 688 J/m^2, compared with 130 J/m^2 for the nonrigid CPC control, showing a substantially increased capacity to absorb energy and avoid catastrophic fracture. While several CPCs have been developed in previous studies, our Cement A may be the first nonrigid hydroxyapatite composite that possesses a high strength and durability and the needed large deformation capability for periodontal osseous repair. ■

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Certain commercial materials and equipment are identified in this article to specify experimental procedures. In no instance does such identification imply recommendation by the National Institute of Standards and Technology or the ADA Foundation or that the material identified necessarily is the best available for the purpose. The data reported in this article should not be compared with data obtained in other laboratories under different conditions.

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