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ABSTRACT

The main challenges facing composite restorations are secondary caries and bulk fracture. The objective of this study was to develop nano DCPA (dicalcium phosphate anhydrous)-whisker composites with high strength and Ca and PO₄ ion release to combat caries. Flexural strength for the nano DCPA-whisker composites at a nano DCPA:whisker mass ratio of 1:2 ranged from (148 ± 9) MPa to (167 ± 23) MPa, significantly higher than the (103 ± 32) MPa of an inlay/onlay commercial control composite without Ca-PO₄ release. The nano DCPA-whisker composite released PO₄ to a concentration of (1.95 ± 0.13) mmol/L and Ca of (0.68 ± 0.05) mmol/L. Compared with previous conventional Ca- and PO₄-releasing composites, the nano DCPA-whisker composites had strengths two-fold higher, and released comparable or higher levels of Ca and PO₄. In conclusion, combining nano-DCPA with whiskers yielded novel composites that released high levels of Ca and PO₄ requisite for remineralization. These high-strength composites may provide a unique combination of stress-bearing and caries-inhibiting capabilities.

KEY WORDS: dental resin composite, nano particles, whiskers, strength, Ca and PO₄ ion release.

Nano DCPA-Whisker Composites with High Strength and Ca and PO₄ Release

INTRODUCTION

Extensive efforts have been made to improve the fillers and filler-matrix interfaces of resin composites (Söderholm *et al.*, 1984; Goldberg *et al.*, 1994; Ferracane *et al.*, 1998; Drummond and Bapna, 2003; Anusavice *et al.*, 2005). Resin compositions and cure conditions have also been improved (Eick *et al.*, 1993; Bayne *et al.*, 1998; Loza-Herrero *et al.*, 1998). Nonetheless, while composites may be satisfactory for smaller restorations, they are not recommended for large stress-bearing restorations (Sakaguchi, 2005). A recent study reported that "clinical data indicate that the two main challenges are secondary caries and bulk fracture" (Sarrett, 2005).

To overcome the fracture problem, investigators have used whiskers to improve composite strength and fracture resistance (Xu *et al.*, 1999). Nano-sized silica particles were fused onto the whiskers to enhance silanization and retention in the resin by roughening the whisker surfaces. Compared with glass-particle-filled composites, the whisker composites exhibited superior performance in thermal-cycling (Xu *et al.*, 2002), long-term water-aging (Xu, 2003), and three-body wear (Xu *et al.*, 2004a). The whisker composites were non-cytotoxic and supported cell proliferation and viability *in vitro* (Xu *et al.*, 2004b).

To combat secondary caries, researchers have developed composites with calcium (Ca) and phosphate (PO₄) ion release (Dickens-Venz *et al.*, 1994; Skrtic *et al.*, 1996a,b; Dickens *et al.*, 2003). These composites demonstrated the ability to remineralize tooth lesions *in vitro*. However, the low strength of these Ca- and PO₄-releasing composites was "inadequate to make these composites acceptable as bulk restoratives" (Skrtic *et al.*, 2000).

In the present study, nano-sized dicalcium phosphate anhydrous (DCPA, CaHPO₄) particles were used as fillers with whiskers in resins. DCPA has been extensively used in calcium phosphate cements (Chow, 2000) and Ca- and PO₄-releasing materials (Dickens *et al.*, 2003). However, while nano forms of hydroxyapatite and tricalcium phosphate have been synthesized previously (Zhang and Gonsalves, 1997; Sutorik *et al.*, 2003; Bow *et al.*, 2004), nano-sized DCPA particles have never been reported. The nano DCPA particles in the present study were synthesized for the first time. It was hypothesized that adding nano-DCPA would result in Ca and PO₄ release for the composite, and adding whiskers would result in strength matching that of control composites without release.

MATERIALS & METHODS

Nano DCPA Particles

Nano-sized DCPA was prepared *via* a spray-drying process (Chow *et al.*, 2004). We prepared a solution by dissolving 1.088 g of DCPA (J.T. Baker, Phillipsburg, NJ, USA) in 1 L of an acetic acid at 16 mmol/L concentration to obtain a Ca and PO₄ ionic concentration of 8 mmol/L. The solution was sprayed through a nozzle (PNR America, Poughkeepsie, NY, USA) that was situated on the top of a heated

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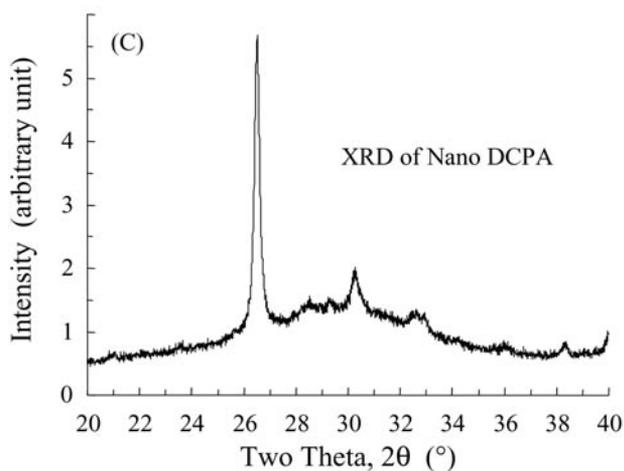
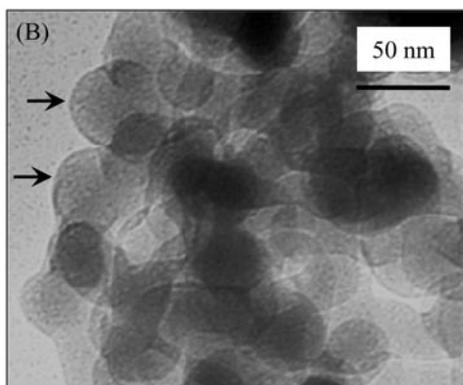
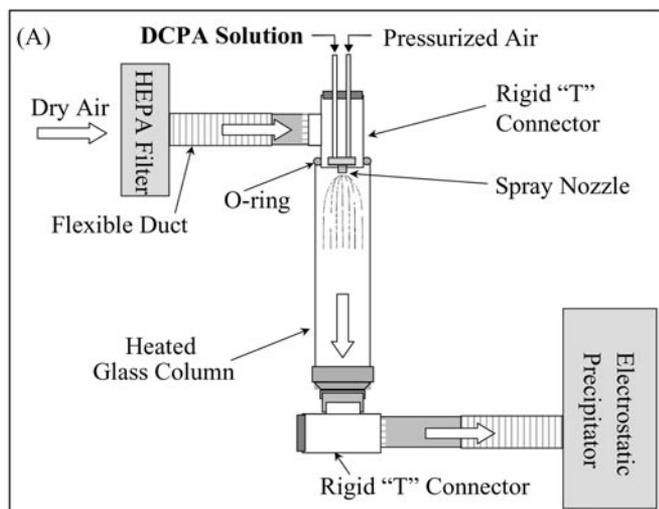


Figure 1. Synthesis of nano DCPA particles. (A) Schematic of the spray-drying apparatus. "HEPA" refers to High Efficiency Particulate Air Filter. The glass column was heated with electrical heating tapes and thermally insulated with a fiberglass tape, reaching an average temperature in the column of 80°C. (B) TEM of nano DCPA particles. Arrows indicate particles about 50 nm in diameter. (C) XRD pattern indicates that the powder was a poorly crystalline DCPA.

glass column (Fig. 1A). An electrostatic precipitator (MistBuster, AirQuality Engineering, Minneapolis, MN, USA) was connected to the lower end of the column and drew air from the column to create

Table. Matrix Compositions of the 3 Groups of Composites (mass %)*

	Bis-GMA	TEGDMA	HEMA	BHT	BPO	DHEPT	MEHQ
Group 1							
part 1	36.475	36.475	25	0.05	2		
part 2	37	37	25			1	
Group 2							
part 1	48.975	48.975		0.05	2		
part 2	49.5	49.5				1	
Group 3	48.965	48.965			2		0.07

* Bis-GMA = bisphenol glycidyl dimethacrylate. TEGDMA = triethylene glycol dimethacrylate. HEMA = 2-hydroxyethyl methacrylate. BHT = 2,6-di-*tert*-butyl-4-methylphenol. BPO = benzoyl peroxide. DHEPT = *N,N*-dihydroxyethyl-*p*-toluidine. MEHQ = 4-methoxyphenol. Group 1 used a two-part chemically activated resin system containing HEMA. Group 2 used the two-part chemically activated resin system without HEMA. The purpose was to examine the effects of HEMA on the composite properties. Specimens of Group 3 were heat-cured for indirect applications.

a steady flow of air/mist. The water/volatile acid were evaporated into the dry, heated column and expelled from the precipitator into an exhaust hood. The dried particles were collected by the electrostatic precipitator.

The powder was examined with x-ray diffractometry (XRD, DMAX2200, Rigaku Denki, Woodlands, TX, USA). The specific surface area of the powder was analyzed by multipoint-BET (AUTOSORB-1, Quantachrome Instruments, Boynton Beach, FL, USA) and transmission electron microscopy (TEM, 3010-HREM, JEOL, Peabody, MA, USA). To minimize agglomeration, we ultrasonicated an acetone suspension of the particles, and drops of the suspension were deposited onto the TEM grids.

Nano-silica-fused Whiskers

Silicon carbide whiskers (ART, Buffalo, NY, USA) having a mean diameter of about 0.9 μm and a mean length of 14 μm were mixed with nano-silica (Aerosil-OX50, Degussa, Ridgefield, NJ, USA), with a particle size of 40 nm, at a whisker:silica mass ratio of 5:1. The mixture was heated at 800°C for 30 min. The powder was silanized with 4% 3-methacryloxypropyltrimethoxysilane and 2% *n*-propylamine in cyclohexane (all mass fractions). The nano-silica-fused whiskers are hereinafter referred to as whiskers.

Fabrication of Nano DCPA-whisker Composites

Three groups of specimens were fabricated (Table). A 3x3 design was used, with 3 resins (direct-filling with HEMA; direct-filling without HEMA; indirect heat-cured without HEMA) and 3 DCPA:whisker mass ratios (1:2, 1:1, 2:1) (designated as "D:W").

For group 1, the nano-DCPA and whiskers were blended with resin part 1 to form the initiator paste (Table). The filler mass fraction was 60% for all 3 groups and yielded a flowable paste. The accelerator paste consisted of the same amount of powder mixed with resin part 2. Equal masses of the 2 pastes were blended and filled into a 2 x 2 x 25 mm³ mold. For group 2, the initiator and accelerator monomers are listed as parts 1 and 2, respectively (Table). For group 3, the specimens were heat-cured at 120°C for 30 min.

A hybrid composite (TPH, Caulk/Dentsply, Milford, DE,

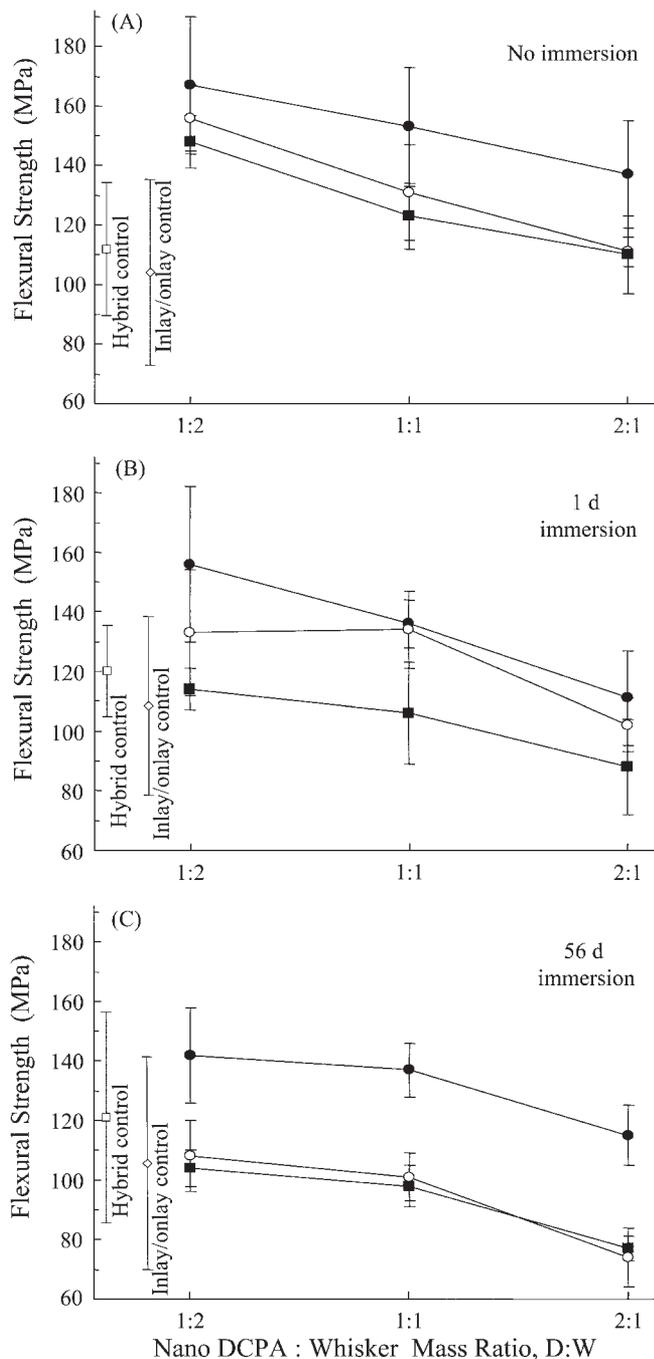


Figure 2. Composite flexural strength. Heat-cured nano DCPA composite: ●. Chemically cured nano DCPA composite with HEMA: ○. Chemically cured nano DCPA composite without HEMA: ■. Specimens (A) without immersion, (B) after 1 day's immersion, and (C) after 56 days' immersion. Each value is the mean of 5 measurements, with the error bar showing 1 standard deviation (SD) (mean \pm SD; $n = 5$). Two-way ANOVA identified significant effects of resin composition and DCPA:whisker ratio ($p < 0.05$), with no significant interaction between the two factors ($p = 0.86$). The 56-day immersion decreased the strength of the chemically cured DCPA-whisker composites, but not the heat-cured DCPA-whisker composites and the controls ($p > 0.1$).

USA), containing 78% of silicate particles, about 0.8 μm in diameter, was used as a control and is referred to as hybrid control. The specimens were light-cured (Triad-2000, Dentsply, York, PA,

USA) for 2 min. Specimens of an inlay/onlay composite (Concept, Ivoclar, Amherst, NY, USA; designated as inlay/onlay control) were cured in a Concept Heat-Integrated Processor at 120°C for 10 min under a pressure of 0.6 MPa.

Measurements of Strength and Ca and PO₄ Release

Flexural strength was measured by a three-point flexural test with a 10-mm span at a crosshead speed of 1 mm/min on a Universal Testing Machine (5500R, MTS, Cary, NC, USA). A NaCl solution (133 mmol/L), buffered with 50 mmol/L HEPES (pH = 7.4; 37°C), was used for specimen immersion. The specimens were: (1) stored in an oven at a relative humidity of approximately 50% for 1 day; (2) immersed for 1 day; or (3) immersed for 56 days, all at 37°C, prior to mechanical testing.

To measure the Ca-PO₄ release, we used the NaCl solution described above. In a previous study, a composite disk of approximately 15.8 mm in diameter and 1.55 mm in thickness was immersed in 100 mL solution (Skrtic *et al.*, 1996a), yielding a composite volume *per* solution of 3.0 mm³/mL and a surface area/solution of 4.7 mm²/mL. In the present study, 3 specimens of approximately 2 x 2 x 12 mm³ were immersed in 50 mL solution, yielding a specimen volume/solution of 2.9 mm³/mL and a surface area/solution of 6.2 mm²/mL. The immersion times were: 1 day, 2, 4, 7, 14, 21, 28, 35, 42, 49, and 56 days. At each time, aliquots of 0.5 mL were removed and analyzed for Ca and PO₄ concentrations with a spectrophotometer (DMS-80 UV-visible, Varian, Palo Alto, CA, USA), according to established standards and calibration methods (Vogel *et al.*, 1983; Skrtic *et al.*, 1996a; Dickens *et al.*, 2003).

We performed two-way ANOVA to detect the significant effects of variables. We performed Tukey's multiple comparison at $p = 0.05$ to compare the data.

RESULTS

TEM (Fig. 1B) showed agglomerated particles, with individual particles having a size of approximately 50 nm (arrows). The XRD pattern (Fig. 1C) had peaks corresponding to DCPA, while the hump suggests that it may be poorly crystalline. The BET measurement yielded a specific surface area of 18.6 m²/g.

The DCPA-whisker composites before immersion showed decreasing strength with increasing D:W ratio (Fig. 2A). For the composite with HEMA, the flexural strength in MPa at D:W = 1:2 was (156 \pm 11), significantly higher than (111 \pm 5) at D:W = 2:1 ($p < 0.05$).

For the composite without HEMA, the strength at D:W = 1:2 was (148 \pm 9), higher than (110 \pm 13) at D:W = 2:1 ($p < 0.05$).

For the heat-cured composite, the strength at D:W = 1:2 was (167 \pm 23), not significantly different from (137 \pm 18) at D:W = 2:1 ($p > 0.1$). The strength of the heat-cured composite at D:W = 1:2 was higher than (103 \pm 32) of the inlay/onlay control ($p < 0.05$). Those of the chemically cured composites at D:W = 1:2, both with and without HEMA, were higher than (112 \pm 22) of the hybrid control ($p < 0.05$).

Most composites did not show a significant decrease in strength after 1 day's immersion (Fig. 2B). The heat-cured and the chemically cured nano DCPA-whisker composites without HEMA showed no significant decrease in strength compared with those before immersion ($p > 0.1$). Only the chemically cured composite with HEMA at D:W = 1:2 had a significant strength loss ($p < 0.05$).

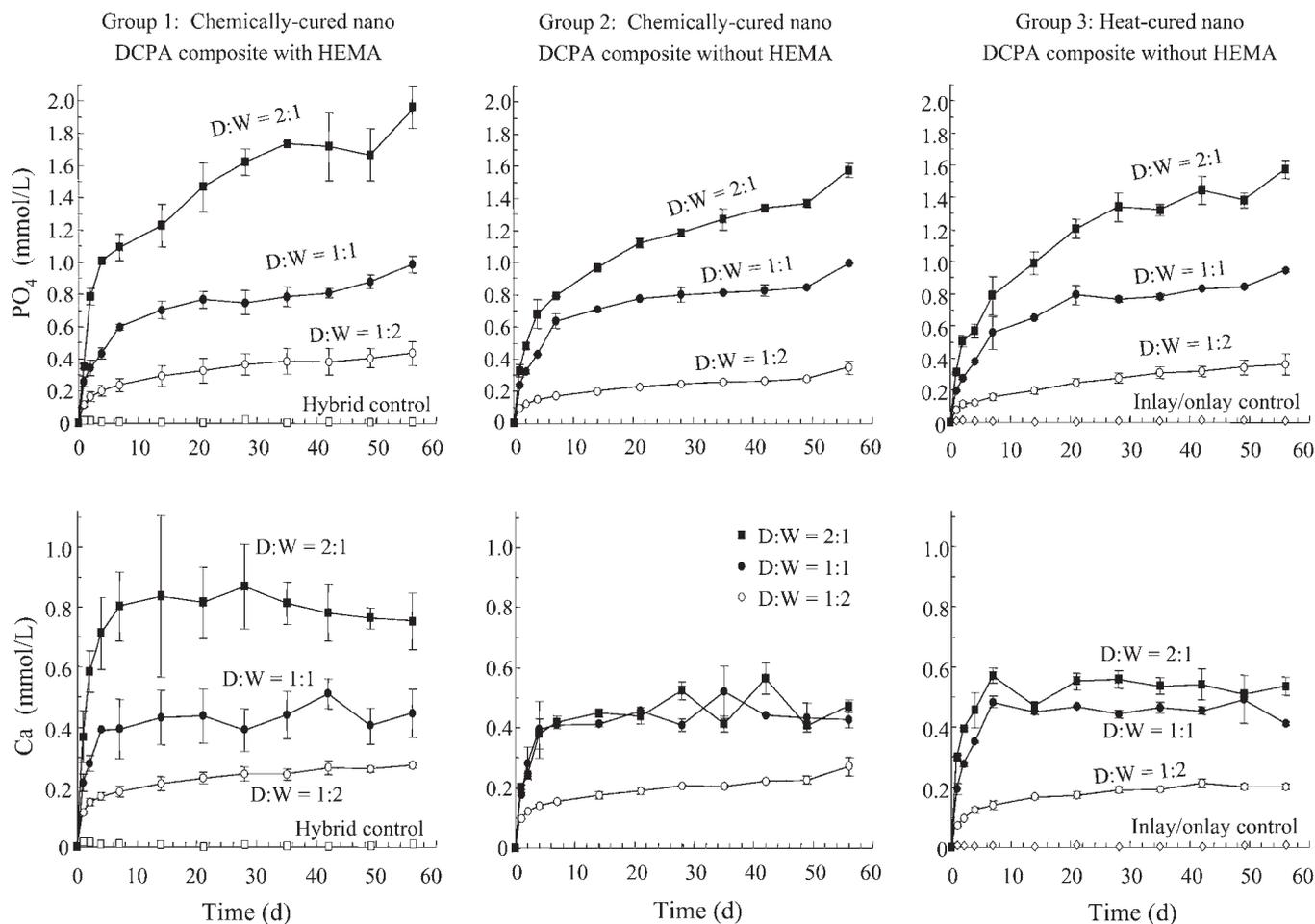


Figure 3. PO₄ and Ca release for the 3 groups of composites. Each value is mean \pm SD; n = 4. The nano DCPA:whisker mass ratio was designated as D:W. For each group, two-way ANOVA showed significant effects of D:W and immersion time, with a significant interaction between the 2 parameters ($P < 0.05$). Increasing the D:W ratio significantly increased the PO₄ and Ca concentrations ($P < 0.05$). At each D:W, increasing the immersion time significantly increased the PO₄ and Ca ion concentrations. The hybrid control and inlay/onlay control had no detectable release.

After the 56-day immersion (Fig. 2C), the heat-cured composite showed no significant decrease in strength ($p > 0.1$) compared with that of specimens before immersion. The chemically cured DCPA-whisker composites (with and without HEMA) showed significant strength losses ($p < 0.05$).

The ion release increased rapidly with time, then reached a plateau with further increase in time (Fig. 3). The composite with HEMA (D:W = 2:1) had higher release than other composites ($p < 0.05$). For group 1 at 56 days, the total ionic PO₄ concentration in mmol/L reached (1.95 ± 0.13) , (0.98 ± 0.05) , and (0.43 ± 0.07) , at D:W = 2:1, 1:1, and 1:2, respectively. The corresponding Ca concentrations reached (0.68 ± 0.07) , (0.38 ± 0.05) , and (0.27 ± 0.01) , respectively. Groups 2-3 had similar concentrations; both were slightly lower than those of group 1 ($p < 0.05$).

DISCUSSION

This study represented the first effort to combine nano-sized Ca- and PO₄-releasing fillers with reinforcing fillers in resins. In previous studies, nano-sized inert tantalum-oxide particles (Chan *et al.*, 1999), micron-sized fluoride-releasing fillers

(Anusavice *et al.*, 2005), and micron-sized DCPA were used in resins (Dickens *et al.*, 2003). The nano DCPA-whisker composites of the present study possessed flexural strengths similar to those of commercial composites without Ca and PO₄ release. The reinforcement mechanisms were whiskers pinning and bridging the cracks (Xu *et al.*, 1999, 2002). The whiskers have a tensile strength of about 50 GPa, compared with 2.6 GPa for glass fibers. Fracture toughness is $> 2 \text{ MPa}\cdot\text{m}^{1/2}$ for silicon carbide, compared with $0.7 \text{ MPa}\cdot\text{m}^{1/2}$ for glass. Hence, the whiskers were more effective in resisting cracks and less likely to be cut through by the cracks, compared with glass fillers in composites.

A previous study used amorphous calcium phosphate (ACP) fillers and developed an ACP remineralizing composite (Skrtec *et al.*, 1996a). When dry specimens without immersion were used, the ACP composite had a three-point flexural strength of $(47 \pm 5) \text{ MPa}$ with unmilled ACP and $(56 \pm 16) \text{ MPa}$ with milled ACP (O'Donnell *et al.*, 2006). In the present study, the nano DCPA-whisker composites for direct-filling had three-point flexural strengths of about 150 MPa before immersion at D:W = 1:2.

After the 56-day immersion, the strength of the chemically cured composite (D:W = 1:2) decreased to (104 ± 6) MPa without HEMA and (108 ± 12) MPa with HEMA. In comparison, a previous composite, with micron-sized DCPA, had a bi-axial flexural strength of 40-60 MPa before immersion; the strength decreased to 10-20 MPa after 90 days of immersion (Dickens *et al.*, 2004). The strength of the ACP composite decreased to 40 MPa after 11 days' immersion (Skrtic *et al.*, 1996a). Hence, the strengths of nano-DCPA-whisker composites were substantially higher than those of previous Ca- and PO_4 -releasing composites. The nano-silica-whiskers are relatively inert and stable, and previous studies showed that they were strongly bonded with the resin matrix (Xu *et al.*, 2002). Hence, the nano DCPA-whisker composites are expected to have improved long-term durability compared with the previous Ca- PO_4 composites that do not have a stable reinforcement phase. Further studies are needed to investigate the long-term water-aging behavior of the nano DCPA-whisker composites.

It should be noted that a 10-mm span was used in the three-point flexural test, while ISO Specification 4049 (2000) calls for the use of a 20-mm span. To examine any differences, we tested specimens of the hybrid control composite (TPH) using both the 10-mm span and the 20-mm span. The flexural strength values (mean \pm SD; $n = 5$) were measured to be (111.7 ± 22.0) MPa and (111.9 ± 14.1) MPa, at 10-mm and 20-mm spans, respectively. Specimens of the heat-cured DCPA-whisker composite (at an intermediate D:W = 1:1) were also tested with both 10-mm and 20-mm spans, yielding (135.7 ± 7.8) MPa and (134.4 ± 17.5) MPa, respectively. Although the strengths with 10-mm and 20-mm spans are nearly the same, future studies should use the 20-mm span, to be consistent with the specification. Another issue is the number of samples: The present study used $n = 5$, consistent with the ISO Specification 4049, on the three-point flexural test. To examine the effect of n , we tested the heat-cured DCPA-whisker composite (at an intermediate D:W = 1:1) using the 20-mm span. The strength was (134.4 ± 17.5) MPa at $n = 5$, (136.1 ± 15.1) MPa at $n = 10$, and (139.4 ± 14.2) MPa at $n = 14$. While these values were nearly the same, there was a slight decrease in the standard deviation with increasing n .

The ACP composites with HEMA yielded PO_4 concentrations of 0.2-0.7 mmol/L and Ca of 0.3-1.0 mmol/L (Figs. 2-3 in Skrtic *et al.*, 1996a). The nano-DCPA composite with HEMA produced a Ca concentration (0.68 mmol/L) similar to the ACP composites, and a PO_4 concentration (1.95 mmol/L) approximately twice that of ACP composites. Another study on remineralizing Ca- PO_4 composites reported a PO_4 concentration of 0.1 mmol/L and a Ca concentration of 0.5 mmol/L in buffered saline (Dickens *et al.*, 2003).

A reason for the high release from the nano-DCPA composites, even with the non-releasing whiskers serving as part of the fillers, was likely the high surface area of the nano-DCPA, measured to be $A = 18.6 \text{ m}^2/\text{g}$. In comparison, in a previous study (Dickens *et al.*, 2003), the DCPA particle size, d , was 1.1 μm , and the TTCP (tetracalcium phosphate) particle size was 16 μm . The density, ρ , is 2.89 g/cm^3 for DCPA and 3.07 g/cm^3 for TTCP. Hence, $A = 6/(\rho d) = 1.9 \text{ m}^2/\text{g}$ for DCPA, and $A = 0.12 \text{ m}^2/\text{g}$ for TTCP, much less than the 18.6 m^2/g for the nano DCPA. Smaller particles with a larger surface area may have faster release, resulting in higher ionic concentrations. Previous studies (Skrtic *et al.*, 1996b; Dickens

et al., 2003) have shown that when Ca and PO_4 were released, they re-precipitated to form hydroxyapatite outside the composite and inside the tooth lesions, significantly increasing the mineral content of the lesion. The fact that the Ca- PO_4 concentrations from the nano-DCPA-whisker composites matched or exceeded those of previous composites that were shown to remineralize tooth lesions suggests that these nano-DCPA-composites may also be effective remineralizers.

It should be noted that this study focused on developing novel nano-composites with high strength and Ca and PO_4 release, without attempting to simulate the *in vivo* saliva flow and pH changes. Thus, this study demonstrated that the high-strength composites released Ca and PO_4 matching/exceeding the previous remineralizing composites measured by a similar method, without examining the kinetics of the release under *in vivo* conditions. Although this study measured the release up to 56 days, the release is expected to continue after 56 days. The ion concentrations in Fig. 3 plateaued, likely due to the measurement method in which the solution was not changed. Further studies should measure the concentration of the solution at certain time intervals, such as hourly or daily, and replenish the solution with the same volume of fresh solvent at each time interval. Studies are also needed to investigate the remineralization of enamel and dentin lesions with these high-strength nano DCPA-whisker composites.

In summary, nano DCPA particles were synthesized and incorporated into dental resins for the first time. Significant releases of Ca and PO_4 were obtained from these composites. The addition of nano-silica-fused whiskers resulted in high composite strengths, matching those of commercial stress-bearing, non-releasing composites. Furthermore, the Ca- PO_4 concentrations from the nano-DCPA-whisker composites matched/exceeded those of previous Ca- PO_4 composites that were shown to remineralize tooth lesions, while the flexural strengths of the nano-DCPA-whisker composites were two- to three-fold those of the previous Ca- PO_4 composites. In addition, the processing method of synergistically using calcium phosphate nano-fillers/reinforcement fillers in dental resins may yield new composites with high stress-bearing and caries-inhibiting capabilities, a combination not available in any current dental materials.

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DISCLAIMER

Certain commercial materials and equipment are identified to specify the experimental procedure. This does not imply recommendation or endorsement by NIST or ADAF or that the material or equipment identified is necessarily the best available for the purpose. One standard deviation was used as the estimated standard uncertainty of the measurements. These values should not be compared with data obtained in other laboratories under different conditions.

REFERENCES

- Anusavice KJ, Zhang NZ, Shen C (2005). Effect of CaF₂ content on rate of fluoride release from filled resins. *J Dent Res* 84:440-444.
- Bayne SC, Thompson JY, Swift EJ Jr, Stamatides P, Wilkerson M (1998). A characterization of first-generation flowable composites. *J Am Dent Assoc* 129:567-577.
- Bow JS, Liou SC, Chen SY (2004). Structural characterization of room-temperature synthesized nano-sized beta-tricalcium phosphate. *Biomaterials* 25:3155-3161.
- Chan DC, Titus HW, Chung KH, Dixon H, Wellinghoff ST, Rawls HR (1999). Radiopacity of tantalum oxide nanoparticle filled resins. *Dent Mater* 15:219-222.
- Chow LC (2000). Calcium phosphate cements: chemistry, properties, and applications. *Mat Res Soc Symp Proc* 599:27-37.
- Chow LC, Sun L, Hockey B (2004). Properties of nanostructured hydroxyapatite prepared by a spray drying technique. *J Res Natl Inst Stand Technol* 109:543-551.
- Dickens SH, Flaim GM, Takagi S (2003). Mechanical properties and biochemical activity of remineralizing resin-based Ca-PO₄ cements. *Dent Mater* 19:558-566.
- Dickens SH, Flaim GM, Floyd CJE (2004). Effect of resin composition on the mechanical and physical properties of calcium phosphate filled bonding systems. *Polymer Preprints* 45:329-330.
- Dickens-Venz SH, Takagi S, Chow LC, Bowen RL, Johnston AD, Dickens B (1994). Physical and chemical properties of resin-reinforced calcium phosphate cements. *Dent Mater* 10:100-106.
- Drummond JL, Bapna MS (2003). Static and cyclic loading of fiber-reinforced dental resin. *Dent Mater* 19:226-231.
- Eick JD, Byerley TJ, Chappell RP, Chen GR, Bowles CQ, Chappelow CC (1993). Properties of expanding SOC/epoxy copolymers for dental use in dental composites. *Dent Mater* 9:123-127.
- Ferracane JL, Berge HX, Condon JR (1998). In vitro aging of dental composites in water—effect of degree of conversion, filler volume, and filler/matrix coupling. *J Biomed Mater Res* 42:465-472.
- Goldberg AJ, Burstone CJ, Hadjinikolaou I, Jancar J (1994). Screening of matrices and fibers for reinforced thermoplastics intended for dental applications. *J Biomed Mater Res* 28:167-173.
- ISO/FDIS 4049 (2000). Dentistry—polymer-based fillings, restorative and luting material. 3rd ed. Geneva, Switzerland: International Organization for Standardization.
- Loza-Herrero MA, Rueggeberg FA, Caughman WF, Schuster GS, Lefebvre CA, Gardner FM (1998). Effect of heating delay on conversion and strength of a post-cured resin composite. *J Dent Res* 77:426-431.
- O'Donnell JNR, Antonucci JM, Skrtic D (2006). Amorphous calcium phosphate composites with improved mechanical properties. *J Bioact Compat Polym* (in press).
- Sakaguchi RL (2005). Review of the current status and challenges for dental posterior restorative composites: clinical, chemistry, and physical behavior considerations. *Dent Mater* 21:3-6.
- Sarrett DC (2005). Clinical challenges and the relevance of materials testing for posterior composite restorations. *Dent Mater* 21:9-20.
- Skrtic D, Antonucci JM, Eanes ED (1996a). Improved properties of amorphous calcium phosphate fillers in remineralizing resin composites. *Dent Mater* 12:295-301.
- Skrtic D, Hailer AW, Takagi S, Antonucci JM, Eanes ED (1996b). Quantitative assessment of the efficacy of amorphous calcium phosphate/methacrylate composites in remineralizing caries-like lesions artificially produced in bovine enamel. *J Dent Res* 75:1679-1686.
- Skrtic D, Antonucci JM, Eanes ED, Eichmiller FC, Schumacher GE (2000). Physicochemical evaluation of bioactive polymeric composites based on hybrid amorphous calcium phosphates. *J Biomed Mater Res* 53:381-391.
- Söderholm KJ, Zigan M, Ragan M, Fischlschweiger W, Bergman M (1984). Hydrolytic degradation of dental composites. *J Dent Res* 63:1248-1254.
- Sutorik AC, Paras MS, Lawrence D, Kennedy A, Hinklin T (2003). Synthesis, characterization, and sintering behavior of calcium hydroxyapatite powders with average particle diameters of 150 nm. *Ceramic Trans* 147:73-82.
- Vogel GL, Chow LC, Brown WE (1983). A microanalytical procedure for the determination of calcium, phosphate and fluoride in enamel biopsy samples. *Caries Res* 17:23-31.
- Xu HH (2003). Long-term water aging of whisker-reinforced polymer-matrix composites. *J Dent Res* 82:48-52.
- Xu HH, Martin TA, Antonucci JM, Eichmiller FC (1999). Ceramic whisker reinforcement of dental composite resins. *J Dent Res* 78:706-712.
- Xu HH, Eichmiller FC, Smith DT, Schumacher GE, Giuseppetti AA, Antonucci JM (2002). Effect of thermal cycling on whisker-reinforced dental resin composites. *J Mater Sci Mater Med* 13:875-883.
- Xu HH, Quinn JB, Giuseppetti AA (2004a). Wear and mechanical properties of nano-silica-fused whisker composites. *J Dent Res* 83:930-935.
- Xu HH, Smith DT, Simon CG (2004b). Strong and bioactive composites containing nano-silica-fused whiskers for bone repair. *Biomaterials* 25:4615-4626.
- Zhang S, Gonsalves KE (1997). Preparation and characterization of thermally stable nanohydroxyapatite. *J Mater Sci Mater Med* 8:25-28.