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Effect of particle size of an amorphous calcium phosphate filler on the mechanical strength and ion release of polymeric composites

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Abstract

The random clustering of amorphous calcium phosphate (ACP) particles within resin matrices is thought to diminish the strength of their polymerized composites. The objective of this study was to elucidate the effect of ball-milling on the particle size distribution (PSD) of ACP fillers and assess if improved dispersion of milled ACP in methacrylate resin sufficiently enhanced filler/matrix interactions to result in improved biaxial flexure strength (BFS) without compromising the remineralizing potential of the composites. Un-milled and wet milled zirconia-hybridized ACP (Zr-ACP) fillers were characterized by PSD analysis, X-ray diffraction, thermogravimetric and chemical analysis, infrared spectroscopy and scanning electron microscopy. Composite specimens made from a photoactivated, ternary methacrylate resin admixed with a mass fraction of 40 % of un-milled or milled Zr-ACP were evaluated for the BFS (dry and wet) and for the release of calcium and phosphate ions into saline solutions. While having no apparent effect on the structure, composition and/or morphology/topology of the fillers, milling significantly reduced the average size of Zr-ACP particulates (median diameter, $d_m = 0.9 \mu\text{m} \pm 0.2 \mu\text{m}$) and the spread of their PSD. Better dispersion of milled Zr-ACP in the resins resulted in the improved BFS of the composites, even after aqueous soaking, and also gave a satisfactory ion release profile. The demonstrated improvement in the mechanical stability of anti-demineralizing/remineralizing ACP composites based on milled Zr-ACP filler may be beneficial in potentially extending their dental utility.

Keywords

bioactive material; calcium phosphate(s); composite/hard tissue; ion release; particle size distribution

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INTRODUCTION

Amorphous calcium phosphate (ACP), a plausible precursor in the formation of biological apatite, is a unique form of calcium phosphate that lacks the long-range, atomic scale order of crystalline calcium phosphates¹. The relatively high aqueous solubility of ACP and its ready conversion to apatitic calcium phosphates (Ap) make ACP suitable as a remineralizing agent. Recently, we investigated ACP as a bioactive filler in polymeric composites formulated for potential use as sealants and/or basing materials²⁻⁵. The physicochemical data collected so far indicate that ACP particulate fillers in polymeric matrices release calcium and phosphate ions in a sustained manner and create the desired state of supersaturation that may be conducive to Ap formation. Therefore, these composites offer a promising anti-demineralization/remineralization tool in not only preventing the formation of new lesions, but also in actively repairing existing or incipient lesions. However, the spontaneous, uncontrolled agglomeration of ACP particles during the synthesis results in their random clustering within the resin matrices of composites during formulation⁶. This hinders interfacial interactions with dental resins resulting in mechanically inferior composites compared to the more homogeneously dispersed particulate glass fillers in glass-reinforced materials. Consequently, the use of such composites may only be limited to low stress dental applications. Our attempts to reduce the size of ACP particles by introducing various surfactants and/or poly(ethyleneoxide) as potential dispersants “ab initio” during the ACP synthesis have proven unsuccessful (unpublished data).

The purpose of the present study was to establish if a simple treatment, such as ball milling of ACP prior to its utilization as filler phase in composites, may sufficiently reduce the average size of ACP by breaking up large aggregates into smaller agglomerates that will more intimately interact with the resin and, therefore, more homogeneously disperse in the composite. Relevant tasks were to physicochemically evaluate unmilled and milled ACP, formulate the composites with the same matrix resin and compare their mechanical behavior and the kinetics of mineral ion release. Such an assessment is deemed necessary in order to ensure that potential improvement in mechanical strength of composite is achieved without compromising its anti-demineralizing/remineralizing potential.

METHODS

Synthesis of ACP filler

Zr-ACP precipitated instantaneously in a closed system at 23 °C upon rapidly mixing equal volumes of a 800 mmol/L $\text{Ca}(\text{NO}_3)_2$ solution, a 536 mmol/L Na_2HPO_4 solution that contained a molar fraction of 2 % $\text{Na}_4\text{P}_2\text{O}_7$ as a stabilizing component for ACP, and an appropriate volume of a 250 mmol/L ZrOCl_2 solution (mole fraction of 10 % ZrOCl_2 based on Ca reactant). The reaction pH varied between 8.6 and 9.0. The suspension was filtered, the solid phase washed subsequently with ice-cold ammoniated water and acetone, and then lyophilized.

Milling of the filler

Approximately 50 g of Zr-ACP solid was mixed with 1 kg very high density ZrO_2 balls (2 mm in diameter; Glen Mills Inc., Clifton, NJ, USA). Isopropanol was added in the amount sufficient to cover the Zr-ACP/ ZrO_2 mixture. Wet milling (ball-milling machine, Dayton Electric MFG Co., Chicago, IL, USA) was performed at 57 rad/s for 2.5 h. Milled Zr-ACP solid was separated from the ZrO_2 balls by sieving. Isopropanol was then rotary-evaporated (2.7 kPa; approximately 2 h at 50 °C) and finally the milled filler was dried in vacuum-oven at 40 °C for 24 h.

Physicochemical characterization of the fillers

The amorphous state of unmilled (control) Zr-ACP as well as the milled filler was verified by powder X-ray diffraction (XRD: Rigaku X-ray diffractometer, Rigaku/USA Inc., Danvers, MA, USA) and Fourier-transform spectroscopy (FTIR: Nicolet Magna-IR FTIR System 550 spectrophotometer, Nicolet Instrument Corporation, Madison, WI, USA). The standard uncertainty of measuring the d-spacing values was 0.0013, and the measured d-values were within 0.05 % of the reported values of NIST SRM® 640 (silicon powder, $2\theta = 28.442$, $d = 3.1355$). The wavelength accuracy of FTIR measurements was $\leq 0.01 \text{ cm}^{-1}$ at 2000 cm^{-1} . Morphological/topological features of the fillers, after the specimens were sputter-coated with gold, were examined by scanning electron microscopy (SEM; JEOL 35C instrument, JEOL Inc., Peabody, MA, USA). Particle size distribution (PSD) of the fillers was determined by gravitational and centrifugal sedimentation analysis (SA-CP3 particle size analyzer, Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) following dispersion of the solids in isopropanol and 10 min ultrasonication of the mixture. Water content of the fillers was determined by thermogravimetric analysis (TGA; Perkin Elmer 7 Series Thermal Analysis System, Norwalk, CT, USA). The TGA (3 separate runs) were performed by heating (5 to 10) mg of the filler at the rate of $20 \text{ }^{\circ}\text{C}/\text{min}$ over a temperature range of (30 to 600) $^{\circ}\text{C}$ in air. The surface or loosely bound water was attributed to the mass loss that occurred from $23 \text{ }^{\circ}\text{C}$ to $125 \text{ }^{\circ}\text{C}$. Structural or more tightly bound water was attributed to the mass loss that occurred from $150 \text{ }^{\circ}\text{C}$ to $600 \text{ }^{\circ}\text{C}$. Ca/PO₄ ratio of the solids after dissolution in HCl was calculated from solution Ca²⁺ and PO₄ values (UV/VIS Carey Model 219 spectrophotometer, Varian Analytical Instruments, Palo Alto, CA, USA)^{7,8}.

Formulation of methacrylate resins

The experimental resins were formulated from the commercially available dental monomers and photoactivated for visible light polymerization by addition of the components of the photoinitiator system [Table 1, Fig. 1.] The indicated acronyms (Table 1) will be used throughout this manuscript. EBPADMA/TEGDMA/HEMA, the termonomer resin (mass fraction of 29.7 %, 44.6 % and 24.7 %, respectively) was photoactivated by the inclusion of CQ (mass fraction of 0.2 %) and 4EDMAB (mass fraction of 0.8 %) as photo-oxidant and photo-reductant, respectively.

Preparation of composites

Composite pastes were made from mixing the EBPADMA/TEGDMA/HEMA resin (mass fraction 60 %) and either un-milled or milled Zr-ACP filler (mass fraction 40 %) by hand spatulation. The homogenized pastes were kept under a moderate vacuum (2.7 kPa) overnight to eliminate the air entrained during mixing. The pastes were molded into disks (14.9 mm to 15.3 mm in diameter and 1.31 mm to 1.53 mm in thickness) by filling the circular openings of flat Teflon molds, covering each side of the mold with a Mylar film plus a glass slide, and then clamping the assembly together with spring clips. The disks were photo-polymerized by irradiating sequentially each face of the mold assembly for 120 s with visible light (Triad 2000, Dentsply International, York, PA, USA).

Evaluation of composites

Dispersion of the ACP fillers on the surface of composite specimens was evaluated by optical microscopy (Leica optical microscope; Leica Heerburg AG, Heerburg, Switzerland).

Biaxial flexure strength (BFS) of dry and wet (after 528h of immersion in HEPES-buffered, pH = 7.40, saline solutions at $23 \text{ }^{\circ}\text{C}$) composite disk specimens (three or more specimen per group) was determined by using a computer-controlled Universal Testing Machine (Instron 5500R, Instron Corp., Canton, MA, USA; crosshead speed: 0.5 mm/min) operated by

Testworks4 software. BFS values were calculated according to mathematical expressions given in ASTM F394–78⁹.

Mineral Ion release from each individual composite disk specimen in a continuously stirred, HEPES-buffered (pH = 7.40) 240 mOsm/kg saline solution was examined at 23 °C. Ca²⁺ and PO₄ levels were determined utilizing the spectrophotometric analytical methods^{7,8}. Ion release data were corrected for variations in the total area of surface disk exposed to the immersion solution using a simple relation for a given surface area (in mm²), A: normalized value = (measured value) × (500/A).

The thermodynamic stability of immersion solutions containing the maximum levels of mineral (calcium and phosphate) ions released from composites, taken as a quantitative measure of the anti-demineralizing/remineralizing potential of the composites, was calculated using the Gibbs free-energy expression:

$$\Delta G^0 = -2.303 (RT/n) \ln (IAP/K_{sp}) \quad (1)$$

where IAP is the ionic activity product for the hydroxyapatite (Ca₁₀(OH)₂(PO₄)₆; HAP), K_{sp} is the HAP's thermodynamic solubility product, R is the ideal gas constant, T is the absolute temperature and n is the number of ions in the IAP (n = 18). Chemist software provided by MicroMath Research, St. Louis, MO, USA, was used for the IAP calculations.

Statistical data analysis

One standard deviation (SD) is given in this paper for comparative purposes as the estimated standard uncertainty of the measurements. Experimental data were analyzed by ANOVA ($\alpha = 0.05$). Significant differences between specific groups were determined by all pair-wise multiple comparisons.

RESULTS

Physicochemical characteristics of ACP fillers

Both the un-milled as well as milled Zr-ACP filler employed in this study showed no discrete XRD peaks; their XRD patterns consisted of two diffuse, broad bands resembling XRD spectra of noncrystalline substances such as glasses and certain polymers [Fig. 2a]. A corresponding FTIR spectrum [Fig. 2b] showed only two wide bands typical for phosphate stretching and phosphate bending of noncrystalline calcium phosphate in the region of (1200 to 900) cm⁻¹ and (630 to 500) cm⁻¹, respectively. Gravitational/sedimentation analysis [Fig. 3a, b; Table 2] revealed highly heterogeneous PSD for the unmilled Zr-ACP filler with particles ranging from sub-micrometer values up to 80 µm in size. The median diameter (d_m) and the specific surface area (SSA) of the unmilled Zr-ACP filler calculated from the 3 independent PSD measurements were: d_m = (5.9 ± 0.7) µm and SSA = (0.5 ± 0.1) m²/g. By contrast, milled Zr-ACP had quite narrow PSD (spanning from 0.2 µm to 3.0 µm) with d_m = (0.9 ± 0.2) µm and SSA = (3.8 ± 1.0) m²/g. Dramatic reduction in the particle sizes of milled vs. unmilled Zr-ACP filler is also reflected in the SEM results [Fig. 4a, b]. Total water content of the fillers and their Ca/PO₄ ratios were unaffected by milling [Table 2]. TGA data also indicated that there was no change in the ratio of the surface-bound (mobile water)/structurally incorporated water (approx. ratio of 2.5).

Mechanical strength of composites

The results of the BFS testing of dry (before immersion) and wet (after 528h immersion in saline solutions) unmilled and milled Zr-ACP composite specimens are summarized in [Fig. 5.] The average BFS values of dry unmilled and milled Zr-ACP composites ((46.7 ± 7.5) MPa and (49.3 ± 5.4) MPa, respectively) showed no statistically significant difference (Tukey test

at 95 % confidence interval). Upon exposure to aqueous environment the mechanical strength of unmilled ACP-based composites deteriorated to approximately 2/3 of the dry strength values (31.4 ± 7.3 MPa). However, milled ACP-based composites not only maintained their strength upon immersion but the average wet BFS value of (59.3 ± 7.6) MPa was approximately 20 % higher than the average BFS of dry samples. The observed increase was found statistically significant ($p < 0.05$; Tukey test).

Maximum concentrations of calcium and phosphate ions released from the EBPADMA/TEGDMA/HEMA resin composites filled with the unmilled and milled Zr-ACP attained after 528 h of immersion in buffered saline are presented in [Fig. 6]. Calcium and phosphate solution levels obtained from composites utilizing milled Zr-ACP filler were reduced for 28 % and 21 %, respectively, compared to the composites based on unmilled filler. The thermodynamic calculations for the immersion solutions containing the maximum concentrations of calcium and phosphate ions released from both types of composite disk specimens [Table 3] revealed that the overall level of supersaturation with respect to hydroxyapatite (the measure of the remineralizing capacity) of the milled Zr-ACP composites has been reduced by 10.4 % compared to composites utilizing the unmilled filler.

DISCUSSION

It is hypothesized that to attain the desired chemical and/or mechanical properties of ACP-filled composites, it is essential to achieve a fairly uniform distribution of ACP particulates in the polymer matrix, i.e., minimize the uneven formation of filler-rich and filler-depleted areas within the composites⁶ and, in turn, achieve more stable, interactive filler/matrix interface. The latter has been indicated as the major factor affecting both physicochemical as well as the thermal properties of polymer composites^{10,11}. Formation of voids or non-bonding spaces in the filler/matrix interfaces is generally controlled by the nature of the filler phase, the method of polymerization and/or the type and the degree of interaction of the filler phase with the polymeric matrix phase. As proven by the XRD, FTIR, TGA and chemical analysis, ball milling had no adverse effect on either structure or the composition of Zr-ACP filler [Fig. 2, Table 2]. Large ACP agglomerates [Figs. 3a, 4a], frequently detected in the un-milled filler, have been efficiently broken down by milling resulting in relatively homogeneous PSD of milled ACP and 85 % reduction in the median particle diameter [Figs. 3b, 4b]. Whether the number of agglomerates decreased upon milling or the extremely fine particles formed during the grinding formed new, but much smaller agglomerates remains an open question. Nevertheless, the milled, more uniform Zr-ACP (particle sizes $< 3 \mu\text{m}$) admixed easily with the EBPADMA/TEGDMA/HEMA resin. Moreover, the surfaces of light-cured, milled-ACP composite disk specimens [Fig. 7b] showed very little heterogeneity, a feature typically seen in unmilled ACP composites [Fig. 7a], leading to the conclusion that improved dispersion of milled ACP filler is being achieved in this experimental dental resin.

The mechanical strength of dry composite specimens was unaffected by the type of the filler utilized. However, upon exposure of composite specimens to aqueous environment, unmilled and milled ACP composites behaved differently. The observed 32.8 % decrease in strength upon immersion of unmilled ACP EBPADMA/TEGDMA/HEMA composites compares well with, on average, 30.6 % deterioration in BFS upon immersion of the composites utilizing unmilled ACP filler in a similar resin system comprising EBPADMA/TEGDMA/HEMA and a mass fraction of 2.5 % methacryloxyethyl phthalate (unpublished results). This reduction in mechanical strength is attributed to either reduction in ACP's intactness and rigidity at the filler/matrix interface due to spatial changes that may have occurred during the calcium/phosphate ion efflux, internal ACP to apatite conversion, or excessive water absorption.

The finding that milled ACP composites have not deteriorated when exposed to aqueous milieu, but have even modestly improved in their BFS values [Fig. 5] is especially encouraging in our continuing efforts to improve the mechanical properties of bioactive ACP composites. So far, we have shown that hybridization with zirconia and to a lesser extent with silica makes these hybrid fillers more resistant to internal conversion to Ap^{12} and makes their composites less susceptible to softening after water exposures¹³. However, attempts to improve the strength of ACP composites by surface-modifying hybrid ACP fillers with a silane coupling agent proved unsuccessful^{5,6}. Although water sorption by dental composites is generally controlled by the resin matrix¹⁴, the hydrophilic ACP filler in the composites can make a significant contribution – proportional to its mass fraction in the composite – to the amount of water absorbed¹⁵. The differences in the strength of wet unmilled (coarse filler) and milled (fine filler) ACP composite specimens possibly may be explained by the different levels of interaction that developed at the ACP/resin interface resulting in accelerated water diffusion and enhanced hydration of unmilled ACP surfaces while these processes were inhibited or reduced in composites with milled ACP filler. Several studies¹⁶⁻¹⁸ have addressed the changes in mechanical properties of Bis-GMA-based resin composites after prolonged exposure to aqueous media. It was found that their fracture toughness, elastic modulus, hardness or flexural strength were affected. Water sorption/desorption studies and extensive mechanical testing may be required in the future to establish if the above hypotheses are indeed pertinent to EBPADMA/TEGDMA/HEMA resin composites. Preliminary studies performed with the similar ACP fillers and EBPADMA/TEGDMA/HEMA/methacryloxyethyl phthalate matrices indicated 20 % less water sorption in composites based on milled ACP filler (a mass fraction of 1.89 %) compared to the unmilled ACP composites (a mass fraction of 2.36 %)¹⁹.

We have previously shown that ion release from bioactive, un-milled ACP composites may be dramatically affected by the chemical structure and the composition of the monomer system and, to much lesser extent, by the type of ACP filler^{4-6,20}. It was found that, in general, the anti-demineralizing/remineralizing potential of these composites may be advanced by introducing EBPADMA as a base monomer or including high levels of HEMA in the resin formulation. Higher ion releases obtained with these particular monomers were attributed to EBPADMA's tendency to form more open cross-linked polymeric networks and the hydrophilic HEMA's potential for promoting water uptake and mineral saturation. Despite the moderate reduction in the attained levels of remineralizing calcium and phosphate ions released from the milled-ACP EBPADMA/TEGDMA/HEMA composites, their remineralizing potential ($\Delta G^\circ = (-5.07 \pm 0.31) \text{ kJ/mol}$; [Table 3]) remained higher than the supersaturation levels achieved with unmilled ACP EBPADMA/TEGDMA/HEMA/methacryloxyethyl phthalate composites ($(\Delta G^\circ = (-4.11 \pm 0.20) \text{ kJ/mol})^{19}$) or un-milled ACP Bis-GMA/TEGDMA/HEMA composites ($\Delta G^\circ = (-4.43 \pm 0.49) \text{ kJ/mol}$; unpublished results).

In summary, milled ACP-based, light-polymerized EBPADMA/TEGDMA/HEMA composites exhibited improved mechanical strength upon aqueous exposure with only a minimal reduction in their remineralizing capability. The enhanced mechanical stability is attributed primarily to a more intimate contact of filler particles with the resin and their better dispersion in the matrix. As a result of this study, it would appear beneficial to utilize milled ACP fillers in formulating anti-demineralizing/remineralizing composites for a wider spectrum of potential dental applications.

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EBPADMA

TEGDMA

HEMA

CQ

4EDMAB

Fig. 1.
Chemical structure of the monomers and photo-curing agents used in the study.

Fig. 2.
XRD patterns (a) and FTIR spectra (b) of the unmilled and milled Zr-ACP fillers utilized in the study.

Fig. 3. Particle size distribution (PSD; mean $\pm$ SD (indicated by bars)) of the unmilled (a) and milled (b) Zr-ACP filler obtained by gravitational and sedimentation analysis.

Fig. 4. Scanning electron microscopy images of the unmilled and milled Zr-ACP fillers utilized in the study.

Fig. 5. Biaxial flexure strength (BFS; mean $\pm$ SD (indicated by bars)) of dry and wet EBPADMA/TEGDMA/HEMA resin composites filled with the unmilled and milled Zr-ACP, respectively. The number of runs in each experimental group $n \geq 5$. Standard deviation (SD) is taken as a measure of the standard uncertainty.

Fig. 6. Maximum concentration of the mineral ions (mean $\pm$ SD (indicated by bars)) released from the EBPADMA/TEGDMA/HEMA resin composites filled with the un-milled and milled Zr-ACP, respectively, attained after 528 h of immersion in buffered saline. The number of runs in each experimental group $n = 3$. The SD is taken as a measure of the standard uncertainty.

Fig. 7.
Optical photomicrographs of the EBPADMA/TEGDMA/HEMA composite disk specimens made with the unmilled (a) and milled ACP (b).

Table 1

Monomers and photoinitiators employed in resin formulations.

Chemical name	Acronym	Manufacturer
ethoxylated bisphenol A dimethacrylate	EBPADMA	Esstech, PA, USA
triethylene glycol dimethacrylate	TEGDMA	Esstech, PA, USA
2-hydroxyethyl methacrylate	HEMA	Esstech, PA, USA
camphorquinone	CQ	Aldrich, WI, USA
ethyl-4-N,N-dimethylaminobenzoate	4EDMAB	Aldrich, WI, USA

Table 2

Physicochemical characterization of the ACP fillers utilized in the study.

Parameter	Unmilled Zr-ACP	Milled Zr-ACP
Particle size range (μm)	0.3 – 80.0	0.2 – 3.0
Median particle diameter, d_m (μm)	5.9 (0.7)	0.9 (0.2)
Specific surface area, SSA (m^2/g)	0.5 (0.1)	3.8 (1.0)
Water content (mass fraction, %)	17.3 (1.6)	16.9 (1.4)
Calcium/phosphate ratio	1.91 (0.09)	1.86*

Standard deviation of values is indicated in parentheses.

* single experiment.

Table 3

Comparison of the ion activity product (IAP; mean $\pm$ SD) and the thermodynamic stability* (ΔG^0 , mean $\pm$ SD) of the solutions containing the maximum concentrations of calcium and phosphate ions released from Zr-ACP - EBPADMA/TEGDMA/HEMA composites. Negative ΔG^0 value indicates solution supersaturated with respect to stoichiometric hydroxyapatite. Standard deviation of values is indicated in parentheses.

Type of Zr-ACP filler	IAP	ΔG^0 (kJ/mol)
Unmilled	99.26 (0.68)	-5.66 (0.21)
Milled	101.21 (1.02)	-5.07 (0.31)