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Mechanical characterization and ion release of bioactive dental composites containing calcium phosphate particles

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ABSTRACT

Objective: to verify the effect of the addition of dicalcium phosphate dihydrate (DCPD) particles functionalized with di- or triethylene glycol dimethacrylate (DEGDMA or TEGDMA) on the degree of conversion (DC), post-gel shrinkage (PS), mechanical properties, and ion release of experimental composites.
Methods: Four composites were prepared containing a BisGMA/TEGDMA matrix and 60 vol% of fillers. The positive control contained only barium glass fillers, while in the other composites 15 vol% of the barium was replaced by DCPD. Besides the functionalized particles, non-functionalized DCPD was also tested. DC after 24 h (n = 3) was determined by FTIR spectroscopy. The strain gage method was used to obtain PS 5 min after photoactivation (n = 5). Flexural strength and modulus (n = 10) were calculated based on the biaxial flexural test results, after specimen storage for 24 h or 60 days in water. The same storage times were used for fracture toughness testing (FT, n = 10). Calcium and phosphate release up to 60 days was quantified by ICP-OES (n = 3). Data were analyzed by ANOVA/Tukey test (alpha: 5%).
Results: Composites containing functionalized DCPD presented higher DC than the control ($p < 0.001$). The material containing DEGDMA-functionalized particles showed higher PS than the other composites ($p < 0.001$). After 60 days, only the composite with DEGDMA-functionalized DCPD presented fracture strength similar to the control, while for flexural modulus only the composite with TEGDMA-functionalized particles was lower than the control ($p < 0.001$). FT of all composites containing DCPD was higher than the control after 60 days ($p < 0.005$). Calcium release was higher for the composite with non-functionalized DCPD at 15 days and no significant reductions were observed for composites with functionalized DCPD during the observation period ($p < 0.001$). For all the tested composites, phosphate release was higher at 15 days than in the subsequent periods, and no difference among them was recorded at 45 and 60 days ($p < 0.001$).
Conclusions: DCPD functionalization affected all the studied variables. The composite with DEGDMA-functionalized particles was the only material with strength similar to the control after 60 days in water; however, it also presented the highest shrinkage. The presence of DCPD improved FT, regardless of functionalization. DCPD functionalization reduced ion release only during the first 15 days.

1. Introduction

Resin-based composites containing calcium orthophosphate particles (CaP) are able to foster remineralization of enamel caries-like lesions *in vitro* (Langhorst et al., 2009), as well as inhibit the development of new lesions *in situ* (Melo et al., 2013a). Also, they have been tested as orthodontic cements (Liu et al., 2018), in atraumatic restorative treatment (ART) (Peters et al., 2010) and dentin bonding systems (Melo et al., 2013b). Unfortunately, the mechanical properties of these bioactive materials are usually lower than those presented by conventional composites due to the poor interaction between the CaP particles and the resin matrix, which make them behave as stress raisers and facilitate crack propagation within the composite (Xu and Moreau, 2010; Chiari et al., 2015).

The synthesis of dicalcium phosphate dihydrate particles (DCPD) functionalized with triethylene glycol dimethacrylate (TEGDMA) was described as a new strategy to improve their interfacial strength with the resin phase (Rodrigues et al., 2014). TEGDMA is a monomer

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ubiquitously found in commercial resin composite formulations and when added to one of the ion precursor solutions (*ab initio* functionalization) can establish ion-dipole bonds with calcium ions in the growing DCPD crystals *via* ethylene glycol groups (-O-CH_2-CH_2-) (Yoshida and Kihara, 1987; Morra, 1993). Additionally, functionalization would improve the wetting of the particles by the resin phase, contributing for a better mechanical interlocking. In fact, a dimethacrylate-based material containing TEGDMA-functionalized DCPD particles presented 32% higher fracture strength in comparison to a material containing non-functionalized particles (Rodrigues et al., 2016a).

The final TEGDMA content on the particle can be modulated by varying the amount of monomer added in the synthesis. Though higher TEGDMA contents improve the composite mechanical behavior (Rodrigues et al., 2018), it may affect other properties as well. For instance, composites containing DCPD particles with TEGDMA mass fractions higher than 14% (determined by elemental analysis) showed higher degree of conversion than the material containing non-functionalized particles. The molecular mobility of TEGDMA granted by its ether linkages increases the probability of polymer chain growth and, though this mobility is probably reduced by the fact these monomers are attached to the DCPD surface, the TEGDMA-rich domains created around the DCPD cores seem to contribute for the material's overall conversion (Alania et al., 2016). Considering the linear relationship between degree of conversion and polymerization shrinkage (Shah and Stansbury, 2014), it is possible that the use of TEGDMA-functionalized DCPD could result in an undesirable increase composite polymerization stress, jeopardizing the integrity of the tooth/restoration interface (Braga et al., 2005).

Functionalization of DCPD particles with other ethylene glycol dimethacrylate (EGDMA) derivatives resulted in particles with different surface areas and size distribution, depending on the length of the spacer group (*i.e.,* number of ethylene glycol groups) (Natale et al., 2018). When added to one of the precursor solutions, ethylene glycol derivatives act as co-solvents, changing the surface tension of the reaction medium. That, in turn, affects crystal nucleation and, consequently, the characteristics of the final particle (Rodrigues et al., 2018). As an example, particles functionalized with diethylene glycol dimethacrylate (DEGDMA) showed the highest monomer content and surface area, and the lowest median particle size in relation to non-functionalized DCPD or those functionalized with EGDMA, TEGDMA or TETDMA (tetraethylene glycol dimethacrylate). Therefore, it is important to verify the effect of these DEGDMA-functionalized DCPD particles on the mechanical properties and polymerization shrinkage of experimental composites.

The effectiveness of remineralizing restorative materials relies upon a long-term sustained ion release. Unfortunately, most of the information available followed ion release for relatively short periods of time, usually 28 days or less (Xu and Moreau, 2010; Chiari et al., 2015; Alania et al., 2016; Marovic et al., 2014a; Xu et al., 2009; Skrtic et al., 1996). Still, it is possible to find studies that verified the occurrence of ion release in longer periods, of 56 days (Xu et al., 2007, 2010) and 70 days (L. Zhang et al., 2016). As ion release implies in particle surface dissolution, in theory, this long-term release could increase the water pathways through the particle-matrix interface, compromising the stability of CaP-containing composites.

Composite degradation is related to the presence of ester groups (-COO-) in the monomers structures, subjected to oxidation and hydrolysis when in contact with saliva and other fluids (Santerre et al., 2001). Matrix and resin-particle interfacial degradation reduce the composite elastic modulus (Ferracane et al., 1995), flexural strength (Arikawa et al., 1995; Drummond and Miescke, 1991) and fracture toughness (Ferracane et al., 1995; Truong and Tyas, 1988). Very few studies evaluated the degradation of CaP-containing composites. Materials containing 10% or 20% (by mass) of amorphous calcium phosphate (ACP) in a total inorganic content of 75% (ACP + reinforcing glass) did not differ from the control in terms of fracture strength after two years in water (Moreau et al., 2012). On the other hand, composites containing 10% or 20% (by volume) of non-functionalized DCPD and a total inorganic content of 60 vol% presented more severe reductions in flexural strength, flexural modulus, and fracture toughness after 28 days in water in relation to the control (without DCPD) (Chiari et al., 2015).

Based on the above, the present study aimed at evaluating the behavior of experimental composites containing functionalized DCPD particles in terms of mechanical properties, volumetric shrinkage, degree of conversion and 60-day ion release. Material degradation was indirectly accessed by the comparison of mechanical properties determined after 24 h and 60 days in water. The working hypotheses were (1) composite mechanical properties (namely, biaxial flexural strength, flexural modulus and fracture toughness) would improve with the use of functionalized DCPD particles in comparison to non-functionalized DCPD, (2) the presence of DCPD (functionalized or not) would not increase composite degradation after prolonged water storage 2) the higher in overall organic content in composites containing functionalized DCPD would increase degree of conversion and volumetric shrinkage in relation to the control and the composite with non-functionalized DCPD and 3) DCPD functionalization would not interfere with ion release from the composite.

2. Material and methods

2.1. Composite formulation

The resin phase of the experimental composites consisted of a BisGMA/TEGDMA mixture (1:1 in mols, all chemicals from Sigma Aldrich, St Louis MI, USA), plus camphorquinone and 2-(dimethylaminoethyl) methacrylate (0.5% by mass each) as photoinitiators. Four different composites were prepared, all of them with a total of 60% by volume of filler particles. Three of them contained 15 vol% of DCPD particles (functionalized with DEGDMA or TEGDMA, or non-functionalized) and 45 vol% of silanized barium glass (2 μm), while the control contained only barium glass particles. The synthesis of the DCPD particles are described in details elsewhere (Rodrigues et al., 2014). Briefly, a calcium nitrate solution (0.2 mol L^{-1}) was added drop-wise to an ammonium dihydrogen phosphate solution (0.2 mol L^{-1}) and kept under stirring for 24 h. Functionalization was achieved by adding the

Table 1
Characteristics of the dicalcium phosphate dihydrate particles used in the study.

	Organic content (wt%)[a]	Density (g/cm^3)[b]	Surface area (m^2/g)[c]	Equivalent spherical diameter (D_{50}, in μm, D_{10}-D_{90} in parentheses)[d]
Non-functionalized DCPD (NF)	N/A	2.4	28	25 (13–50)
DEGDMA-functionalized DCPD (DG)	34.4	1.7	40	12 (6–23)
TEGDMA-functionalized DCPD (TG)	14.1	2.2	16	19 (10–37)

[a] Determined by elemental analysis.
[b] Determined by helium picnometry.
[c] Calculated based on nitrogen adsorption isotherms using the BET (Brunauer, Emmet and Teller).
[d] determined by dynamic laser scattering. For details on the characterization methods, please refer to (Natale et al., 2018).

monomer to the ammonium dihydrogen phosphate solution at a molar ratio of 1:1. Reaction byproducts and excess monomer were removed by filtration under vacuum and the resulting gel was freeze-dried. The characteristics of the DCPD particles are detailed in Table 1. The resin mixture and the particles were mechanically mixed under vacuum (Speedmixer DAC150.1 FVZ-K, FlackTek Inc., Landrum, SC, EUA) and kept under refrigeration until two hours prior to use.

2.2. Degree of conversion

Degree of conversion (DC, n = 3) was determined using near-infrared spectroscopy (Vertex 70, Bruker Optics, Germany). The uncured material was inserted in a cylindrical silicone mold (7 × 1 mm), pressed between two glass slides and positioned in the spectrometer sample holder. The spectrum of the non-polymerized material was obtained by the co-addition of 32 scans, under 4 cm^{-1} resolution. The material was light-cured through the glass slide (Bluephase, Ivoclar-Vivadent, Schaan, Liechtenstein, 1200 mW/cm^2 for 20 s, effective diameter of the light guide: 8 mm) and dry-stored for 24 h at 37 °C before a new spectrum was obtained. DC was calculated according to the ratio of the absorption bands corresponding to the aliphatic =C-H group located at 6165 cm^{-1} according to the formula:

$$DC = \left(1 - \frac{polymerized}{non - polymerized}\right) x100$$

2.3. Post-gel shrinkage

Post-gel shrinkage was determined according a previously described strain-gage method (Sakaguchi et al., 1997). A standardized volume of composite was pressed against the strain gage grid and shaped as a semi-sphere with a 1.5 mm height. The strain gage terminals were connected to the interface of a universal testing machine (model 5565, Instron Corp., Canton, MA, USA). Microstrain data acquisition (10 data points per second) used the testing machine software (Blue Hill, Instron). Monitoring started 10 s before photoactivation (20 s, Bluephase, Ivoclar-Vivadent) was triggered and continued for 5 min. Dimensionless microstrain values were converted to percentage and volumetric shrinkage was calculated multiplying these values by three.

2.4. Biaxial flexural test

Disk-shaped specimens (12 × 1 mm, n = 20) were made using a stainless steel split mold. Composite was photoactivated from the top surface, 20 s per quadrant (24 J/cm^2, Bluephase, Ivoclar Vivadent), with the light guide in contact with a mylar strip placed on top of the composite. Half of the specimens were stored in water at 37 °C for 24 h, while the other half was stored for 60 days. Specimens were fractured on a "piston on three spheres" testing jig positioned under the crosshead of a universal testing machine (Instron model 5565, Instron Corp, Canton MA, USA). The center of the specimen was loaded at a crosshead speed of 0.5 mm/min and specimen deflection was monitored by a contact transducer (model W-E401-E, Instron). Biaxial flexural strength (BFS, in MPa) was calculated using the equations below:

$$\sigma_{BI} = \frac{-0,2387P \quad (X \quad - \quad Y)}{b^2}$$

$$X = (1 + \upsilon) In\left(\frac{r_2}{r_3}\right)^2 + \left[\left(\frac{1 - \upsilon}{2}\right)\right]\left(\frac{r_2}{r_3}\right)^2$$

$$Y = (1 + \quad \upsilon)\left[1 \quad + In\left(\frac{r_1}{r_3}\right)^2\right] + (1 - \quad \upsilon)\left(\frac{r_1}{r_3}\right)^2$$

where P is the failure load (in Newtons); b is the specimen thickness (in mm); is the specimen Poisson's ratio (0.3); r_1 is the radius of the circle where the spheres were positioned (5.0 mm); r_2 is the loading piston radius (0.6 mm); r_3 is the specimen radius (in mm).

Flexural modulus (E, in GPa) was calculated according to the following equation:

$$E = \frac{\beta Pa^2}{\omega h^3} x0,001$$

where E is the flexural modulus (in GPa), β is a constant related to the deflection at the center of the disk (0.509), P is the load (in Newtons), a is the disk radius (in mm), ω is the deflection corresponding to P and h is the disk thickness (in mm). Specimens fracture surface were observed under the scanning electron microscope (Quanta 650 FEG, Thermo Fischer Scientific, Hillsboro, OR, USA).

2.5. Fracture toughness

Composite fracture toughness was determined using the "single-edge notched beam" method. Beam-shaped specimens (25 mm x 5 mm x 2 mm, n = 20) with a 2.5-mm central notch were made by inserting the composite into a stainless steel mold with a razor blade. Photoactivation was performed by overlapping four 10-second exposures on one of the surfaces, with the light guide in contact with a mylar strip placed on top of the composite (Bluephase, Ivoclar Vivadent). Specimens were stored in water at 37 °C for 24 h or 60 days and loaded under three-point bending, with a 20-mm span between the supporting rods and a crosshead speed of 0.5 mm/min. After the test, notch length was determined using a stereomicroscope. Fracture toughness (K_{1c}, in MPa $m^{0,5}$) was calculated using the following equations:

$$K_{ic} = [(PXS)/bxw^{1,5})]xf(a/w)$$

$$f(a/w) = 3\sqrt{(a/w)}\left\{\frac{1,99 - (a/w)\,x\,[1 - (a/w)]\,x\,[2,15 - 3,93(a/w) + 2,7(a/w)^2}{2x[1 + 2(a/w)]\,x\,[1 - (a/w)^{3/2}}\right\}$$

where, "a" is the notch length, w is the specimen width, b is the specimen height (all in millimeters), P is the failure load (in Newtons) and S is the distance between the supporting rods (in mm). Only the specimens showing a/w ratio between 0.45 and 0.55 were used for calculating K_{1C}.

2.6. Ion release

Disc-shaped specimens (5 × 1 mm, n = 3) were prepared using a silicone mold. A single 20-second irradiation was used to photoactivate the material, with the light guide in contact with a mylar strip placed on top of the composite (24 J/cm^2, Bluephase, Ivoclar Vivadent). After 24-h storage at 37 °C under 100% relative humidity, specimens were individually immersed in 5 mL of NaCl solution (133 mmol/L) buffered to pH 7 using HEPES solution (50 mmol/L) (Xu and Moreau, 2010). The immersion medium was replaced by fresh solution every two weeks, for two months. Calcium and phosphorous concentrations in the immersion solutions were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES 700, Agilent Technologies, Santa Clara, CA, USA). Before the analysis, the solution was filtered (pore size: 3 μm) and acidified with 10% nitric acid (1:1 volume ratio).

2.7. Statistical analysis

After verification of normality (Levene test) and homoscedasticity (Kolmogorov-Smirnov test), data were analyzed by one-way ANOVA (degree of conversion and post-gel shrinkage) or two-way ANOVA (mechanical properties and ion release). Tukey test was used for multiple comparisons. A significance level of 5% was adopted in all of the statistical procedures.

Table 2
Means and standard deviations for degree of conversion (in %) and post-gel volumetric shrinkage (in %) of experimental composites as a function of presence/type of DCPD particles. Values followed by the same letter are not statistically different (one-way ANOVA/Tukey test, $p > 0.05$).

	Degree of conversion (%)	Post-gel shrinkage (%)
Barium glass only (control)	75.9 (0.2 C	1.2 (0.2) B
DEGDMA-functionalized DCPD (DG)	77.1 (0.7) B	2.2 (0.3) A
TEGDMA-functionalized DCPD (TG)	78.9 (0.5) A	1.5 (0.4) B
Non-functionalized DCPD (NF)	76.5 (0.1) BC	1.6 (0.4) B

3. Results

The type of DCPD particle significantly affected composite DC (Table 2, $p < 0.001$). TG presented statistically higher DC than the other materials, followed by DG. NF reached DC similar to both DG and the control composite. The highest post-gel shrinkage was developed by DG, while the other composites reached statistically similar values (Table 2, $p < 0.001$).

For BFS, only the presence/type of DCPD had a statistically significant effect on the results ($p < 0.001$, Table 3). Initial BFS was significantly reduced by the replacement of barium glass by DCPD, but this reduction was less severe for DG. After prolonged storage none of the tested composites presented significant changes in BFS and all the composites with DCPD showed similar RFB, but only DG was also similar to the control. FM was influenced by the presence/type of DCPD and storage time ($p < 0.001$ for both, Table 3), with no statistically significant interaction between factors ($p > 0.005$). NF had higher initial FM than both DG and TG, while after 60 days the only difference was between TG and the control. All composites presented numerical reductions in FM after storage, between 8% and 26%, but only for NF the reduction was statistically significant.

Fig. 1 shows examples of fracture surfaces from biaxial flexural test specimens. It is possible to observe several sharp-edged, plate-like particles. The ragged edges in the 24-h specimens (B and C) are suggestive of particle cohesive fracture. After 60 days in water, empty slots indicate the previous locations of the DCPD particles that were dislodged during crack propagation (D).

Initial K_{1c} was not affected by DCPD functionalization and only TG was statistically higher than the control. After 60 days in water, the control showed a statistically lower K_{1c} compared to the DCPD-containing composites and only TG showed a statistically significant reduction in K_{1c} (Table 4, $p < 0.05$).

Composites containing functionalized DCPD showed a similar behavior in terms of calcium release. No statistically significant differences were observed among immersion times for a given composite, or between the two composites at any given time. Compared to the other two composites, NF showed a statistically higher release after the first 15 days only. Also, calcium concentration detected in the solution after 15 days was statistically higher than at 45 and 60 days (Table 5, $p < 0.001$).

NF also showed higher phosphate release than the other two materials after 15 days (Table 6, $p < 0.001$). After 30 days, only TG released less phosphate than NF. Statistically significant reductions in phosphate release were observed up to 45 days (NF and DG) or between 15 and 30 days only (TG).

4. Discussion

Resin-based materials containing ion-releasing fillers, such as bioactive glass, calcium silicates and calcium phosphates, are being investigated by several research groups as a strategy to remineralize the hybrid layer (Tay and Pashley, 2009), prevent hydrolytic/enzymatic degradation of the bonded interface (Tezvergil-Mutluay et al., 2017), and reduce enamel lesions around existing composite restorations and orthodontic brackets (Melo et al., 2013a; N. Zhang et al., 2016). The proposed mechanism is that calcium and phosphate ions released from these particles would create a supersaturated environment, leading to apatite deposition (Marovic et al., 2014b). Another important role attributed to calcium is to enhance fluoride retention in the biofilm, therefore increasing fluoride availability during pH drop events (Vogel et al., 2008).

DCDP, or brushite, has been used as osteoconductive scaffolds (*i.e.*, bone cements) due to its metastability under physiological conditions, which makes it more rapidly resorbed than hydroxyapatite (Cama et al., 2009). This behavior also translates into a higher solubility in relation to other calcium orthophosphates (Dorozhkin, 2013) and, therefore, a potential ion-releasing filler for use in dentistry. Another aspect of particular interest in the formulation of photoactivated resin-based materials is that DCPD refractive index (1.54–1.55) is very close to that of dimethacrylates used in matrix formulation (1.50–1.55). Consequently, light transmittance through the material is not compromised (Rodrigues et al., 2016b).

Early attempts of incorporating CaP particles into resin matrices revealed that, differently from silanated glass fillers, they do not reinforce the material; in fact, the material's fracture strength was severely reduced (Skrtic et al., 1996). The poor interaction between CaP and the resin phase, either chemical or micromechanical, makes these particles behave as flaws and facilitate crack initiation under relatively low stress levels. Also, relatively large particles as those used in the present study would even further increase the likelihood of crack initiation. In order to improve the interaction between both phases, the surface of the particles can be modified by organic compounds such as silanes (Xu et al., 2007), carboxylic acids (Arcis et al., 2002) and TEGDMA (Rodrigues et al., 2016b).

The dimethacrylates used as functionalizing agents, DEGDMA and TEGDMA, differ in the size of the spacer group. DEGDMA has a central oxygen atom shared by both ethylene glycol groups, which seems to facilitate the interaction with calcium during the synthesis. TEGDMA, on the other hand, has one ethylene glycol group at the center of the spacer group. Due to steric hindrance, it is unlikely that both oxygen atoms will establish bonds with calcium. Also, the longer spacer group allows for more complex spacial configurations eventually hindering the interaction with calcium. These aspects are reflected in the higher

Table 3
Means and standard deviations for biaxial flexural strength (in MPa) and flexural modulus (in GPa) of experimental composites as a function of presence/type of DCPD particles and storage time. Values followed by the same letter are not statistically different (upper-case letters: columns, lower-case letters: rows, two-way ANOVA/Tukey test, $p > 0.05$).

	Biaxial flexural strength (MPa)		Flexural modulus (GPa)	
	24 h	60 days	24 h	60 days
Barium glass only (control)	142.8 (26.5) Aa	125.8 (30.6) Aa	16.6 (3.1) ABa	15.3 (1.1) Aa
DEGDMA-functionalized DCPD (DG)	113.6 (24.0) Ba	100.2 (21.3) ABa	13.1 (1.8) Ba	11.8 (3.6) ABa
TEGDMA-functionalized DCPD (TG)	91.4 (10.1) Ca	92.5 (15.8) Ba	13.6 (3.8) Ba	10.2 (2.1) Ba
Non-functionalized DCPD (NF)	95.3 (12.3) Ca	96.0 (13.0) Ba	17.9 (2.9) Aa	13.3 (1.3) ABb

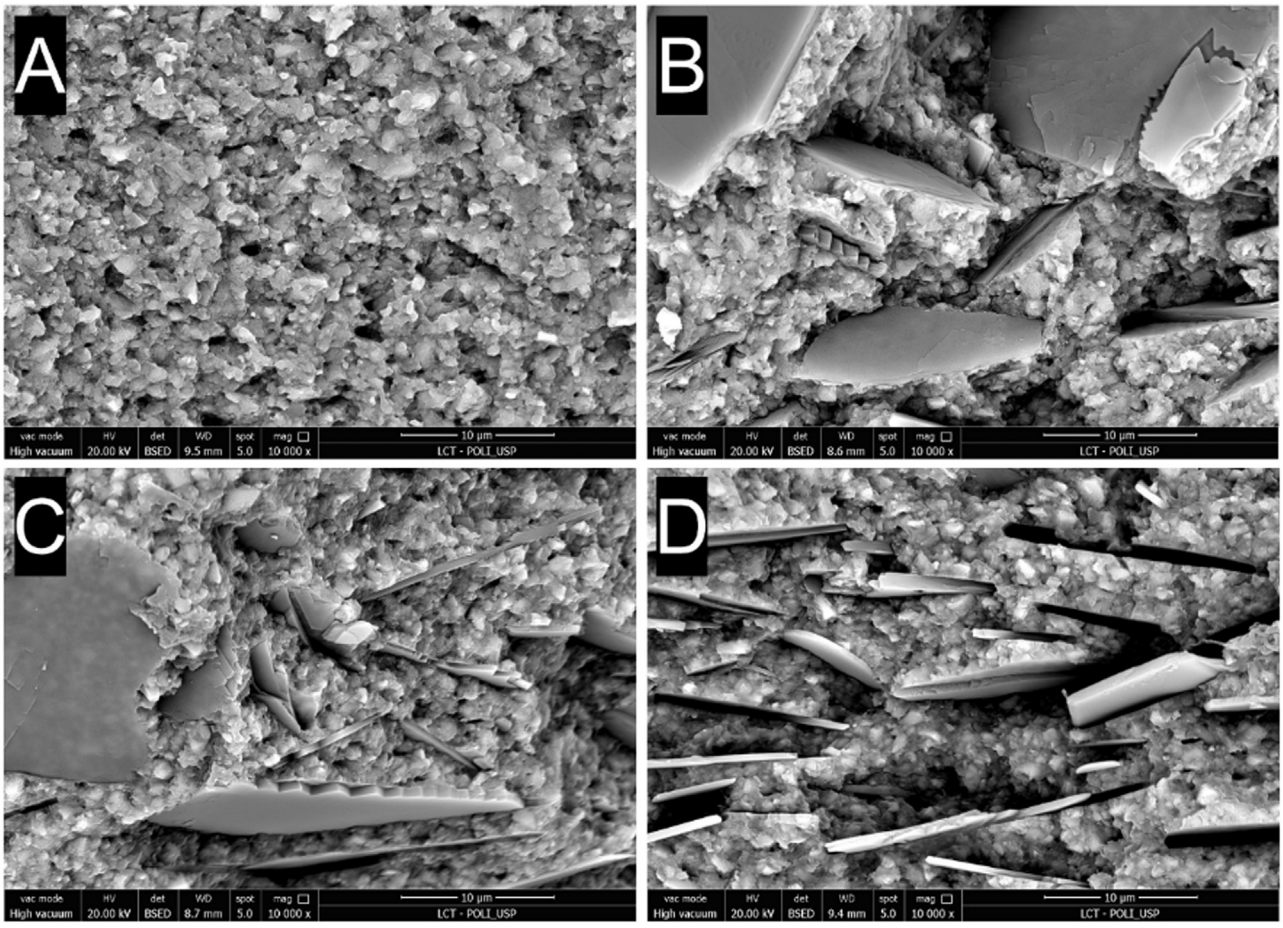

Fig. 1. Scanning electron microscopy images of fracture surfaces from biaxial flexural strength specimens. A: control, 60-day storage; B: non-functionalized DCPD, 24-h storage; C: DEGDMA-functionalized DCPD, 24-h storage; D: DEGDMA-functionalized DCPD, 60-day storage.

Table 4
Fracture toughness (K_{1c}) of experimental composites (means and standard deviations, in MPa.m$^{0.5}$) as a function of presence/type of DCPD particles and storage time. Values followed by the same letter are not statistically different (upper-case letters: columns, lower-case letters: rows, two-way ANOVA/Tukey test, $p > 0.05$).

	24 h	60 days
Barium glass only (control)	1.23 (0.15) Ba	1.00 (0.11) Ba
DEGDMA-functionalized DCPD (DG)	1.50 (0.34) ABa	1.63 (0.35) Aa
TEGDMA-functionalized DCPD (TG)	1.71 (0.19) Aa	1.35 (0.2) Ab
Non-functionalized DCPD (NF)	1.52 (0.33) ABa	1.42 (0.21) Aa

Table 5
Means and standard deviations for calcium release (in parts per million) from experimental composites as a function of DCPD type and storage time. Values followed by the same letter are not statistically different (upper-case letters: columns, lower-case letters: rows, two-way ANOVA/Tukey test, $p > 0.05$).

	Non-functionalized DCPD (NF)	DEGDMA-functionalized DCPD (DG)	TEGDMA-functionalized DCPD (TG)
15 days	3.94 (0.80) Aa	1.50 (0.56) Ab	1.74 (0.59) Ab
30 days	2.59 (0.04) ABa	2.43 (0.48) Aa	2.27 (0.63) Aa
45 days	1.15 (0.39) Ba	1.74 (0.79) Aa	0.80 (0.49) Aa
60 days	1.77 (0.60) Ba	1.34 (0.69) Aa	1.50 (0.45) Aa

organic content in the DEGDMA-functionalized particles. The high monomer content, in turn, would explain the lower density and the higher surface area of these particles in relation to the non-functionalized and the TEGDMA-functionalized DCPD. The slightly smaller particle sizes found in the functionalized particles may be due to the fact that ethylene glycol derivatives act as co-solvents in water-based systems, reducing surface tension and favoring crystal nucleation and, consequently, smaller particles (Metha et al., 2009).

Table 6
Means and standard deviations for phosphate release (in parts per million) from experimental composites as a function of DCPD type and storage time. Values followed by the same letter are not statistically different (upper-case letters: columns, lower-case letters: rows, two-way ANOVA/Tukey test, $p > 0.05$).

	Non-functionalized DCPD (NF)	DEGDMA-functionalized DCPD (DG)	TEGDMA-functionalized DCPD (TG)
15 days	0.71 (0.06) Aa	0.38 (0.07) Ac	0.56 (0.05) Ab
30 days	0.30 (0.07) Ba	0.20 (0.03) Bab	0.14 (0.01) Bb
45 days	0.13 (0.03) Ca	0.09 (0.05) Ca	0.12 (0.04) Ba
60 days	0.17 (0.01) BCa	0.10 (0.02) Ca	0.20 (0.04) Ba

Previous studies testing composites containing 10 vol% and 20 vol% of DCPD showed a trade-off between mechanical properties and ion release (Chiari et al., 2015; Alania et al., 2016). Therefore, in the present study the CaP fraction was fixed in 15 vol% as an attempt to balance both characteristics. The incorporation of functionalized DCPD in the composite increased its degree of conversion in relation to the control. This is an evidence that the functionalizing monomers were incorporated into the polymer network and, even in relatively low concentrations, are capable of lowering the viscosity of the system and increase conversion (Floyd and Dickens, 2006). In the present study the post-gel shrinkage (*i.e.,* the fraction of total shrinkage taking place after polymer gelation) was measured because it is more closely related to

polymerization stress development (Boaro et al., 2010). Though a linear relationship exists between conversion and total shrinkage (Shah and Stansbury, 2014), the lower molecular weight of DEGDMA (242 g/mol) in relation to TEGDMA (286 g/mol) and also the fact that DG particles have a higher monomer mass fraction (34.4%, *versus* 14.1% for TG particles) explain the higher shrinkage of the DG composite, in spite of the otherwise similar composition between these materials.

The initial flexural strength of DG was higher than that of the other DCPD-containing composites. This finding can be ascribed to its high functionalizing agent content (Alania et al., 2016). Also, its lower particle size in relation to the other DCPD particles evaluated may have contributed for this result, as along with a better DCPD/resin interface, smaller particles mean smaller potential "defects" that could act as crack initiation sites. Hydrolytic effects on composite properties are well-known (Ferracane, 2006). However, in the present study the 60-day water storage was not long enough to cause reductions in flexural strength. Still, it is noteworthy that after 60 days only the composite with DEGDMA-functionalized particles remained statistically similar to the control.

No difference in initial FM was observed between NF and the control, in agreement with previous studies (Chiari et al., 2015; Rodrigues et al., 2016b). However, it was higher for NF in comparison to DG and TG, probably because the organic content in the functionalized particles reduces the particles' stiffness. After 60 days, only NF presented a statistically significant reduction in FM possibly because the lack of a stronger interface between the particle and the resin matrix facilitated water transit at the particle-matrix interface, increasing the softening of the resin phase.

DCPD functionalization did not influence composite fracture toughness, either after 24 h or 60 days of immersion. Similar to flexural modulus, only TG presented a statistically significant reduction in K_{1c} after prolonged storage. The presence of large, plate-like DCPD particles had a positive effect on this property in relation to the control, also confirming previous findings (Chiari et al., 2015). DCPD particles cannot be considered reinforcing fillers, as confirmed by SEM images showing evidences of particle fracture. Still, the results suggest that during crack propagation, both the fracture of DCPD particles and the deflection of the crack front reduce the stress intensity at the crack tip and postpones its unstable growth, contributing to toughening (Cesar et al., 2006).

Functionalization reduced calcium release only in the first 15-day period. The higher concentration released by NF contributed for its higher cumulative release after 60 days (9.45 ppm), in comparison to the other composites (TG: 6.31 ppm, DG: 7.01 ppm). The overall lower release by composites with functionalized DCPD can be ascribed to the lower DCPD content of their particles, as part of their DCPD volume fraction is actually occupied by the functionalizing monomer. Phosphate release showed statistically significant reductions overtime, but no differences among composites at 45 and 60 days. The lower phosphate release is due to its structural role in crystalline CaP phases. Also, oxygen atoms form H-bonds with structural water, inhibiting its release (Arsic et al., 2004). It is important to point out that ion release, particularly calcium, was detected up to 60 days. Though it is difficult to estimate if these concentrations will be effective to reduce the mineral loss, it is important to point out that ion release is increased under acidic conditions (Xu et al., 2009). Also, an *in situ* study verified caries inhibition around restorations using a composite containing ACP particles, which showed a cumulative calcium release of 3 ppm under neutral pH over a 28-day period (Melo et al., 2013a).

In conclusion, the present findings showed that composites containing 15 vol% of DCPD particles and 45 vol% of reinforcing fillers presented higher fracture toughness than the control material with 60 vol% of reinforcing fillers. Overall, the use of DEGDMA-functionalized DCPD particles was advantageous over TEGDMA-functionalized and/or non-functionalized DCPD in terms of mechanical properties after 60 days of immersion. However, the use of DEGDMA-functionalized DCPD increased composite post-gel shrinkage. Water storage led to significant reductions in flexural modulus for NF and fracture toughness for TG. Ion release was higher for the composite with non-functionalized DCPD at 15 days only. Therefore, the three null hypotheses can be rejected.

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