Biological and Mechanical Evaluation of Novel Prototype Dental Composites

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Abstract

The breakdown of the polymeric component of contemporary composite dental restorative materials compromises their longevity, while leachable compounds from these materials have cellular consequences. Thus, a new generation of composite materials needed to be designed to have a longer service life and ensure that any leachable compounds are not harmful to appropriate cell lines. To accomplish this, we have developed concurrent thiol-ene–based polymerization and allyl sulfide–based addition-fragmentation chain transfer chemistries to afford cross-linked polymeric resins that demonstrate low shrinkage and low shrinkage stress. In the past, the filler used in dental composites mainly consisted of glass, which is biologically inert. In several of our prototype composites, we introduced fluorapatite (FA) crystals, which resemble enamel crystals and are bioactive. These novel prototype composites were benchmarked against similarly filled methacrylate-based bisphenol A diglycidyl ether dimethacrylate / triethylene glycol dimethacrylate (bisGMA/TEGDMA) composite for their cytotoxicity, mechanical properties, biofilm formation, and fluoride release. The leachables at pH 7 from all the composites were nontoxic to dental pulp stem cells. There was a trend toward an increase in total toughness of the glass-only-filled prototype composites as compared with the similarly filled bisGMA/TEGDMA composite. Other mechanical properties of the glass-only-filled prototype composites were comparable to the similarly filled bisGMA/TEGDMA composite. Incorporation of the FA reduced the mechanical properties of the prototype and bisGMA/TEGDMA composite. Biofilm mass and colony-forming units per milliliter were reduced on the glass-only-filled prototype composites as compared with the glass-only-filled bisGMA/TEGDMA composite and were significantly reduced by the addition of FA to all composites. Fluoride release at pH 7 was greatest after 24 h for the bisGMA/TEGDMA glass + FA composite as compared with the similarly filled prototypes, but overall the F⁻ release was marginal and not at a concentration to affect bacterial metabolism.

Keywords: composite materials, fluoride(s), resin(s), biofilm(s), biocompatibility, apatites

Introduction

Composite restorative materials have undergone many changes since their introduction in the 1960s, especially in the area of filler particle size and the percentage of particulate fill, which influence the composite's mechanical properties, degradation, and polymerization shrinkage (Wang et al. 2003; Ilie and Hickel 2009). The percentage of particle loading ranges between 53% and 87% by weight (Klymus et al. 2007). Furthermore, the size (20 nm to >1 μm) and shape (spherical to irregular) of the filler particles also influence the filler loading capacity and polishability of the composite (Skrtic et al. 1996; Dickens et al. 2003; Lu et al. 2006; Xu et al. 2006; Xu et al. 2011).

Notwithstanding the success of glass-filled composites from bisphenol A diglycidyl ether dimethacrylate / triethylene glycol dimethacrylate (bisGMA/TEGDMA), the hydrolytic degradation of these materials compromises their longevity, and leachable compounds from cured, methacrylate-based composites have cellular consequences (Hanks et al. 1991; Schweikl and Schmalz 1999; Darmani and Al-Hiyasat 2006; Mousavinasab 2011; Gupta et al. 2012). Consequently, a new generation of composite materials needed to be designed to counter these

deleterious consequences. To accomplish this, we developed concurrent thiol–ene–based polymerization (Scott 2008) and allyl sulfide–based addition-fragmentation chain transfer (Scott et al. 2005) chemistries to afford cross-linked polymeric matrices that demonstrate low shrinkage and low shrinkage stress.

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Unlike methacrylate, thiol–ene-based resins can be designed to be ester-free and inherently resistant to hydrolysis (Podgorski et al. 2015). Furthermore, norbornene-bearing monomers in thiol–ene-based resins were previously demonstrated to yield enhanced rates of polymerization (Cramer et al. 2010) and glass transition temperatures (Carioscia et al. 2007). Preoligomerization of these norbonyl monomers is required to be miscible with the allylic monomer, and preoligomerization was shown to reduce the shrinkage stress of resins (Carioscia et al. 2005) and to significantly reduce the malodor of thiol-based monomers (Reinelt et al. 2014). Through the Flory-Stockmayer theory, the oligomerization can be executed to the greatest extent before formation of an infinite network leads to gelation (see Appendix).

Fillers used in dental composites have primarily consisted of glass, which is chemically stable and elicits a minimal biological response. In contrast, we have introduced fluorapatite (FA) crystals, which resemble enamel crystals in shape and composition (Chen, Sun, et al. 2006; Chen, Tang, et al. 2006) in several of our prototype composites. These FA crystals have desirable biological properties that make them ideally suited as a partial filler for composite restorative materials. They stimulate the growth, differentiation, and mineralization of dental pulp stem cells (Liu et al. 2010; Liu et al. 2011; Wang et al. 2012). This bioactivity would be an invaluable pulpal defense. They were shown to reduce carious lesion formation in an in vitro caries model (Clark et al. 2013) and to be antibacterial (Alhilou et al. 2016). However, these desirable biological properties have to be balanced against any decrease in the structural and mechanical properties of the dental composites.

This new generation of resins—formulated from preoligomerized tetramercaptoethyl silane–dinorbornyl ether bisphenol A / triallyl triazine trione (TMES-DNBPA/TATATO), tetramercaptoethyl silane–trinorbornyl triazine trione / triallyl triazine trione (TMES-TNTATO/TATATO), and their glass-filled composites with and without FA crystals—was benchmarked against glass-filled bisGMA/TEGDMA with and without FA crystals to compare their cytotoxicity, mechanical properties, and biofilm formation.

Materials and Methods

See Appendix Figures 1 to 6 for schemes of the monomers used and for monomer synthesis.

Synthesis of TMES

Tetravinylsilane (25 g, 0.183 mol), azobisisobutyronitrile (6 g, 0.037 mol), and thioacetic acid (75 mL, 1.066 mmol) were added to tetrahydrofuran (400 mL). The solution was purged with argon for 30 min, heated to 65 °C, and stirred overnight. A yellow oil was obtained after removal of the solvent under reduced pressure. The product was used without further purification.

To this product were added 100 mL dioxane and 100 mL methanol. Then, 100 mL concentrated hydrochloric acid was added as it was stirred. The flask was equipped with a reflux condenser, and the solution was stirred overnight at 80 °C. The solvent was then removed under reduced pressure, and 100 mL

water was added. The aqueous mixture was extracted 3 times with 100 mL methylene chloride. The combined organic phases were then subsequently washed with 2×100 mL saturated sodium bicarbonate solution, 100 mL water, and 100 mL brine. The organic phase was dried over sodium sulfate and filtered, and the solvent was removed under reduced pressure. The product obtained was a colorless malodorous oil after multiple distillations (200 °C, 0.3 Torr) with a Kugelrohr distillation apparatus.

Synthesis of TNTATO

To a 50 mL bomb flask were added dicyclopentadiene (8.75 g, 0.132 mol), TATATO (5.5 g, 0.022 mmol), and Q-1301 (14 mg). The flask was sealed and heated at 190 °C. After 2 d, the solution was cooled to room temperature and dissolved in 100 mL methylene chloride. The solution was filtered over a pad of Celite and the solvent removed under reduced pressure. The product was dissolved in a minimal amount of 2:1 hexanes:methylene chloride and loaded on a silica column. After being washed with hexanes, the product was separated with 2:1 hexanes:methylene chloride as the eluent. Removal of solvent under reduced pressure yielded the product as a white powder.

Synthesis of DNBPA

DNBPA was obtained as a white powder via the same synthetic and purification procedure as stated for TNTATO, starting from dicyclopentadiene (6.1 g, 0.092 mol), diallylether bisphenol A (7.2 g, 0.023 mol), and Q-1301 (13 mg).

TMES-TNTATO and TMES-DNBPA Oligomer Synthesis

The monomers were mixed in thiol:ene stoichiometric ratios 1:0.16 (TMES-TNTATO) and 1:0.32 (TMES-DNBPA) and formulated with 1 wt% Irgacure TPO, and approximately 5 g of the monomer mixture was dissolved in 50 mL THF. The solution was stirred overnight under irradiation with 405 nm at 15 mW/cm^2 with a Thorlabs M405L2 LED. After removal of the solvent under reduced pressure, a golden, mildly odorous oil was obtained. Complete consumption of the norbornyl moieties was confirmed through ^{1}H-NMR via the absence of the C=C double bonds in the 5.8 to 6.2 ppm region. The oligomers were formulated without further purification with TATATO to obtain a 1:1 thiol:ene stoichiometric ratio for the composite specimens.

FA Synthesis

The patented process for synthesizing the FA crystals was described by Chen et al. (Chen, Sun, et al. 2006; Chen, Tang, et al. 2006). Briefly, a solution containing 0.25M EDTA-Ca-Na$_2$, 0.15M NaH$_2$PO$_4$.H$_2$O, and 0.05M NaF was subjected to mild hydrothermal conditions (121 °C, 2 atm) for 10 h to dissociate

the Ca^{2+} and allow its slow release from EDTA-Ca complex. The resultant crystals were washed 3 times in distilled water. They were then ground with a SpeedMixer (FlackTek Inc.) resulting in a crystal length of approximately 600 nm to 1 μm. Even dispersion and wetting of the FA crystals in the resins were carried out with a SpeedMixer.

Composite Specimen Composition for all Tests

Composites made from TMES-TNTATO/TATATO and TMES-DNBPA/TATATO were tested and benchmarked against bisGMA (70 wt%) and TEGDMA (30 wt%) composites. The resins contained 1% Irgacure TPO as the photoinitiator and were cured at 405 nm with Thorlabs M405L2 LED at an intensity of 10 mW/cm^2 for 30 min. The samples reached 70% conversion. Each resin was filled with either 1) 50% w/w silanated glass (hybrid of 40% 700 nm barium borosilicate glass filler [BBAS] + 60% 2 μm BBAS) or 2) 50% w/w glass + FA (hybrid of 30% 700 nm BBAS + 40% 2 μm BBAS + 30% 600 nm–1 μm FA).

Material Cytotoxicity

Following ISO 10993-12 and ISO 10993-5 protocol, human dental pulp stem cells (Liu et al. 2011; Wang et al. 2012) were used to evaluate the toxicity of the synthesized polymers (TMES-TNTATO/TATATO, TMES-DNBPA/TATATO, and bisGMA/TEGDMA) and their glass- or glass + FA–filled composites. The leachables were extracted from 4.5 specimens (5 × 25 × <0.5 mm each) after their incubation in 1-mL Dulbecco's modified Eagle's medium (DMEM) with the addition of 10% fetal bovine serum at pH 7.4 for 24 h. After 5 × 10^4 dental pulp stem cells were seeded into 96-well plates, they were cultured for 24 h and then treated with serially diluted leachables from the composites for 24 h. XTT solutions, or 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetra-zolium-5-carboxanilide, were added to each test well, and the plate was incubated for 5 h. Optical density (OD) values were measured at 450 nm. Statistical analysis by 2-way analysis of variance (ANOVA) was based on GraphPad Prism 6, and significance was considered at $P < 0.05$.

Mechanical Property Evaluation

Composite beams (25 × 2 × 2 mm) were fabricated in stainless-steel split molds and specimens light cured in 5 overlapping sections for maximum conversion on the top and bottom surfaces, according to ISO 4049 protocol. Flexural tests ($n = 5$ per condition) were conducted on an Admet eXpert 450 Universal Testing Machine at 0.01-mm/s until failure. During each test, load and deflection were recorded, and stress and strain were calculated from standard 3-point beam-bending equations: preyield, postyield, and total toughness were calculated per the area under the elastic, plastic, and total stress-strain curve at fracture, respectively. Due to the brittle nature of the composites, postyield toughness was negligible, and preyield toughness was equal to total toughness and therefore not reported.

Biofilm Evaluation

Biofilms were formed with a monospecies biofilm model containing a luciferase-expressing *Streptococcus mutans* (Zajdowicz et al. 2018). Briefly, composite discs 7 mm in diameter and 1 mm thickness were sterilized in 70% alcohol containing 0.5% triclosan for 30 min; 3 experiments were conducted with 3 discs for each sample, yielding a total 9 discs per sample. Following sterilization, discs were rinsed in sterile saline and brain-heart infusion (BHI) broth for at least 1 h. *S. mutans* firefly was grown in BHI broth overnight at 37 °C with 5% CO_2. Following incubation, the OD at 600 nm (OD_{600}) was measured, and cultures were diluted to an OD_{600} of 0.05 into fresh BHI broth supplemented with 1% sucrose. Discs were transferred to sterile white opaque 24-well plates (Fisher Scientific); 1 mL of the bacterial dilution was added to each well; and the plates were incubated with static conditions at 37 °C with 5% CO_2 for 24 h to allow for mature biofilm formation. Controls were also prepared (no bacteria added), where only BHI + 1% sucrose were added to the wells containing discs. After incubation, all discs were transferred to fresh BHI + 1% sucrose and incubated for 1 h to allow for repletion of ATP. To measure luciferase activity, 2mM D-luciferin (Fisher Scientific) was added, and luminescence was measured at 2-min intervals up to 10 min (FilterMax F5; Molecular Devices). Statistical analysis was performed with a Student's *t* test and ANOVA.

After luciferase activity was measured, biofilms on each resin were disrupted through sonication with a water bath sonicator (Ultrasonic Bath 1.9L; Thermo Fisher Scientific) for 10 min, followed by agitation by vortexing for 3 min. The bioburden on discs was enumerated as colony-forming units per milliliter (CFU/mL), and statistical analysis was performed with a Student's *t* test and ANOVA.

Fluoride Release

Composite discs were prepared and cured as described. One surface and the edges of the cured discs were sealed with varnish, leaving an area of 0.38 cm^2 exposed to 5 mL distilled H_2O for 24 h at pH 7.0: the time and pH used for biofilm formation. Each composite had 5 replicates for fluoride measurements. Fluoride release was also measured at pH 4 for 24 h. Fluoride measurements were conducted with an ORION 370 fluoride electrode (Thermo Scientific). The results were analyzed with 1-way ANOVA/Tukey's multiple comparison.

Contact Angle

Eighteen resin discs, with and without FA crystals, were polished and cleaned with UV-Ozone (model 144AX; Jetlight Company Inc.). The static contact angle of a 3 μL droplet of water was measured on a Ramé-Hart 200-F1 goniometer with 18.1 MΩ cm water. Results were analyzed within each composite group, with and without FA, with 1-way ANOVA/Tukey's multiple comparison.
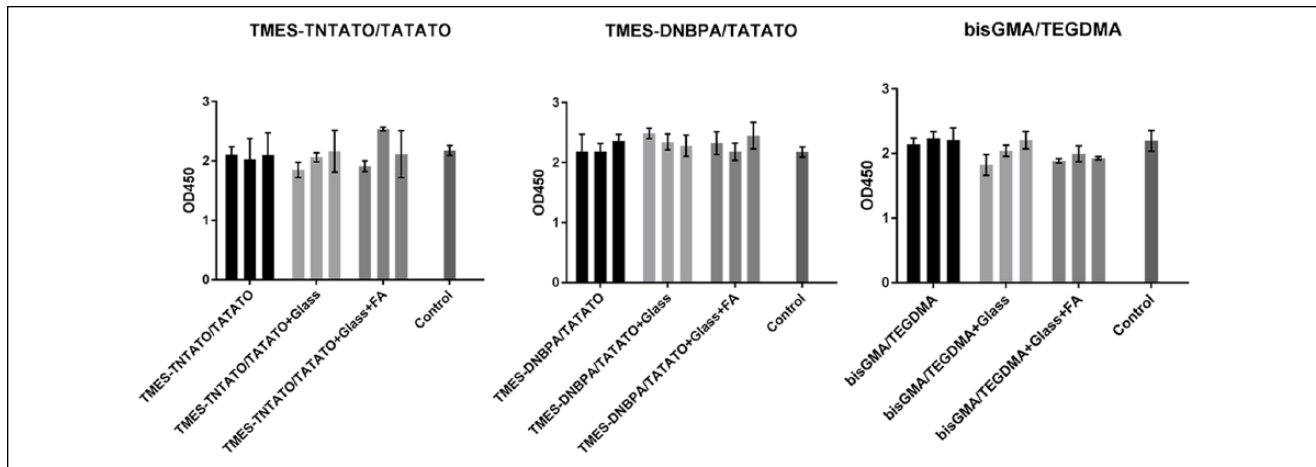
Figure 1. Composite toxicity evaluation. Viability by XTT assay of human dental pulp stem cells. The columns from the left to right in each group indicate dilutions of the original leachables: 10×, 100×, and 1,000×, respectively. Values are presented as mean (SD). bisGMA/TEGDMA, bisphenol A diglycidyl ether dimethacrylate / triethylene glycol dimethacrylate; FA, fluorapatite; OD, optical density; TMES-DNBPA/TATATO, tetramercaptoethyl silane–dinorbornyl ether bisphenol A / triallyl triazine trione; TMES-TNTATO/TATATO, tetramercaptoethyl silane–trinorbornyl triazine trione / triallyl triazine trione.

Table. Mechanical Properties of Novel FA Composites Baselined against Glass Composites.

Composite	Flexural Modulus, GPa[a,b]	Yield Strength, MPa[a]	Flexural Strength, MPa[a,b]	Postyield Toughness, MPa	Total Toughness, MPa[a,b]
bisGMA/TEGDMA					
50% glass	5.94 (1.35)	73.34 (16.13)	73.34 (16.13)	0.00 (0.00)	0.53 (0.26)
50% glass + FA	4.22 (1.08)	51.21 (12.87)[d]	51.21 (12.87)[d]	0.00 (0.00)	0.37 (0.22)
TMES-DNBPA/TATATO					
50% glass	3.56 (0.85)	68.60 (16.75)	68.60 (16.75)	0.00 (0.00)	0.73 (0.28)
50% glass + FA	3.44 (0.16)	59.22 (2.71)	59.22 (2.71)	0.00 (0.00)	0.58 (0.09)
TMES-TNTATO/ TATATO					
50% glass	3.24 (0.61)	67.11 (15.14)	67.11 (15.14)	0.00 (0.00)	0.81 (0.33)
50% glass + FA	3.26 (0.56)	46.17 (7.17)[c]	46.17 (7.17)[c]	0.00 (0.00)	0.36 (0.09)[c]

Values are presented as mean (SD). Significance was considered at $P < 0.05$.
bisGMA/TEGDMA, bisphenol A diglycidyl ether dimethacrylate / triethylene glycol dimethacrylate; FA, fluorapatite; TMES-DNBPA/TATATO, tetramercaptoethyl silane–dinorbornyl ether bisphenol A / triallyl triazine trione; TMES-TNTATO/TATATO, tetramercaptoethyl silane–trinorbornyl triazine trione / triallyl triazine trione.
[a]Significant effect of glass composite type, 1-way analysis of variance.
[b]Significant effect of glass + FA composite type, 1-way analysis of variance.
[c]Significant difference with glass composites having the same resin, *t* test.

Results

Material Cytotoxicity

At the highest leachable concentrations (10× dilution), the 2 prototype resins (TMES-DNBPA/TATATO, TMES-TNTATO/ TATATO), the bisGMA/TEGDMA control, and their composites filled with glass only or glass + FA showed no toxic effects when extracted under the DMEM culture media conditions (Fig. 1).

Mechanical Property Evaluation

Data passed tests of normality and homogeneity. Each group of 3 resins (the 2 novel prototypes and the bisGMA/TEGDMA control), having the same filler type (glass or glass + FA), was compared via 1-way ANOVA. Within each class of resin, glass-filled versus glass + FA–filled composites were compared via *t* tests. Flexural modulus and strength are reported in the Table, with yield strength, postyield toughness, and total toughness. The latter 2 properties are indicative of the ductility of the new resins. Postyield toughness was negligible but reported to indicate that total toughness was dominated by the preyield toughness.

The properties of the glass-only-filled composites fall within the expected range of properties for microfill composites and composites having low (50% to 60%) weight fraction of filler (Lu et al. 2006; Ilie and Hickel 2009). There was a significant effect of glass composite type on modulus, yield strength, flexural strength, and total toughness (see note a in the Table). There was a significant effect of glass + FA composite type on yield strength, flexural strength, and total

toughness (see note b in the Table). The major effect of the new glass-filled resin composites was seen in the toughness, where TMES-DNBPA/TATATO + glass and TMES-TNTATO/TATATO + glass trended toward a greater total toughness than that of bisGMA/TEGDMA + glass. Yield strength and flexural strength were significantly less in bisGMA/TEGDMA glass + FA composites versus bisGMA/TEGDMA glass composites. Yield strength, flexural strength and total toughness were significantly less in TMES-TNTATO/TATATO glass + FA composites versus TMES-TNTATO/TATATO glass composites (see note c in the Table).

Biofilm Evaluation

Luciferase measurements indicated a significant reduction ($P < 0.01$) in the luminescence observed on bisGMA/TEGDMA + glass + FA, TMES-DNBPA/TATATO + glass + FA, and TMES-TNTATO/TATATO + glass + FA composites when compared with bisGMA/TEGDMA + glass, indicating a reduction in bioburden associated with the biofilms grown on these composites (Fig. 2A). These samples had 2-, 10-, and 4-fold reduction, respectively, in comparison with the bisGMA/TEGDMA + glass.

After luciferase activity was measured, biofilms on each composite were disrupted through sonication and agitation; the number of CFU/mL within the biofilm was enumerated. Statistical analysis was performed, and a significant reduction ($P < 0.01$) in the number of bacteria recovered from biofilms developed on each novel resin was observed on bisGMA/TEGDMA + glass + FA, TMES-DNBPA/TATATO + glass + FA, and TMES-TNTATO/TATATO + glass + FA when compared with biofilms associated with bisGMA/TEGDMA + glass (Fig. 2B); these samples had between a 2- and 4-log reduction in comparison with the bisGMA/TEGDMA + glass sample.

Fluoride Release

Fluoride release (Fig. 3) was greater at pH 4 versus pH 7 for all 6 composites with and without FA. At pH 4 and pH 7, the fluoride release from the TMES-DNBPA/TATATO + glass and the TMES-TNTATO/TATATO + glass was statistically greater than that from the bisGMA/TEGDMA + glass. The fluoride release from the TMES-DNBPA/TATATO + glass + FA composite at pH 7 was statistically greater than that of the bisGMA/TEGDMA + glass + FA composite. The fluoride release from the TMES-DNBPA/TATATO + glass + FA composite at pH 4 and the TMES-TNTATO/TATATO + glass + FA composite at pH 4 and pH 7 was not statistically different from that of the bisGMA/TEGDMA + glass + FA.

Contact Angle

The contact angles (Fig. 4) for all the composites with and without FA are relatively low. The addition of the FA reduced the contact angle for the 3 composites, but this was statistically significant ($P \leq 0.0001$) for only bisGMA/TEGDMA + glass
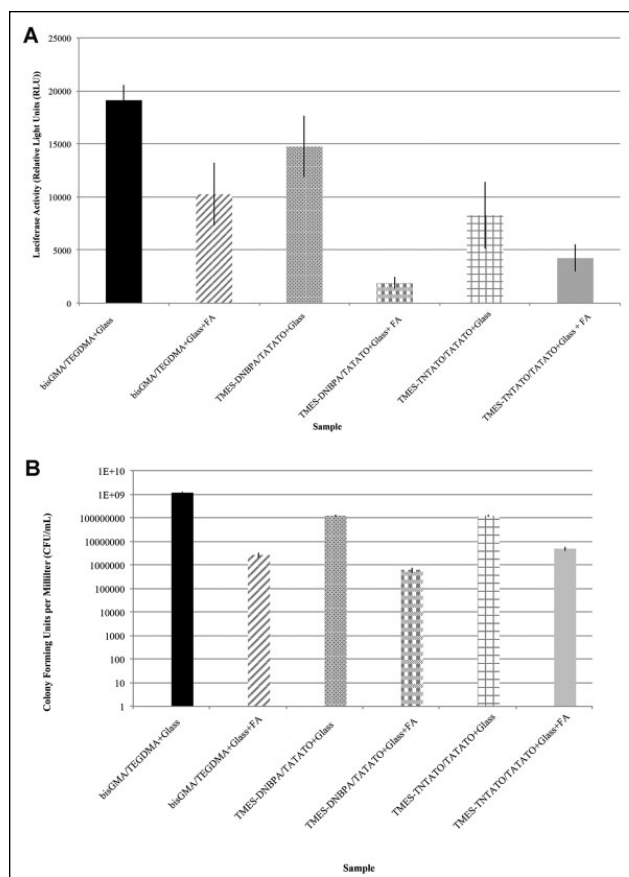

Figure 2. *Streptococcus mutans* biofilm grown on novel and bisGMA/TEGDMA composite discs: (**A**) luciferase evaluation and (**B**) CFU/mL determination. Values are presented as mean (SD). bisGMA/TEGDMA, bisphenol A diglycidyl ether dimethacrylate / triethylene glycol dimethacrylate; CFU/mL, colony-forming units per milliliter; FA, fluorapatite; TMES-DNBPA/TATATO, tetramercaptoethyl silane–dinorbornyl ether bisphenol A / triallyl triazine trione; TMES-TNTATO/TATATO, tetramercaptoethyl silane–trinorbornyl triazine trione / triallyl triazine trione.

+ FA versus its non-FA control. All experimental composites had statistically smaller contact angles than those of biaGMA/TEGDMA with or without FA.

Discussion

When extracted under the DMEM culture media conditions at the highest leachable concentrations (10× dilution) and the more diluted concentrations (100× and 1,000× dilutions), all 3 resins—TMES-TNTATO/TATATO, TMES-DNBPA/TATATO, and bisGMA/TEGDMA—and their composites filled with glass, with and without FA crystals, did not show toxic effects when compared with the cell-only controls. In the future, we will test the cellular response on the enzymatic leachables from the composites.

Composites fabricated from TMES-DNBPA/TATATO and TMES-TNTATO/TATATO exhibited a trend toward greater total toughness than that from the bisGMA/TEGDMA composite. The increase in toughness did not come at the expense of strength or stiffness, as is typical, as these properties were
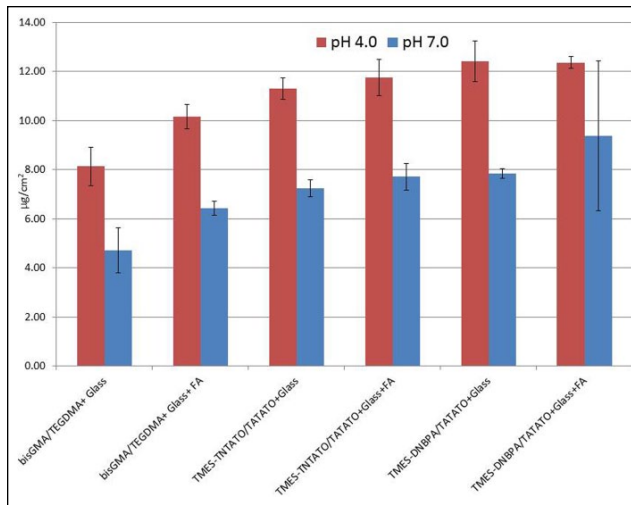
Figure 3. One-day F⁻ release of bisGMA/TEGDMA + glass, bisGMA/ TEGDMA + glass + FA, TMES-TNTATO/TATATO + glass, TMES- TNTATO/TATATO + glass + FA, TMES-DNBPA/TATATO + glass, and TMES-DNBPA/TATATO + glass + FA at pH 4.0 and 7.0. Values are presented as mean (SD). bisGMA/TEGDMA, bisphenol A diglycidyl ether dimethacrylate / triethylene glycol dimethacrylate; FA, fluorapatite; TMES-DNBPA/TATATO, tetramercaptoethyl silane–dinorbornyl ether bisphenol A / triallyl triazine trione; TMES-TNTATO/TATATO, tetramercaptoethyl silane–trinorbornyl triazine trione / triallyl triazine trione.

Figure 4. Contact angle measurements on novel and bisGMA/ TEGDMA composite discs. Values are presented as mean (SD). *$P <$ 0.05. bisGMA/TEGDMA, bisphenol A diglycidyl ether dimethacrylate / triethylene glycol dimethacrylate; FA, fluorapatite; TMES-DNBPA/ TATATO, tetramercaptoethyl silane–dinorbornyl ether bisphenol A / triallyl triazine trione; TMES-TNTATO/TATATO, tetramercaptoethyl silane–trinorbornyl triazine trione / triallyl triazine trione.

not significantly different from those of the bisGMA/TEGDMA composite. Introduction of FA reduced the strength but not the modulus when compared with the same resin filled with glass only. This is expected, given the nonsilanated nature of the FA/ resin coupling and the resultant inferior bonding of FA to the matrix being compared, as well as the greater dependence of strength on the resin/filler bond; however, the magnitude of these properties exceeds that of bisGMA/TEGDMA composite containing FA fillers.

Filler content has a major effect on the mechanical properties of dental composites. Notably, the prototype resin fill was standardized at 50% w/w, an amount that is at the low end of the fill range of the commercially available composites. The percentage fill was chosen to ensure complete wetting and homogeneous mixing of the glass beads and FA crystals with the resins. Because these novel glass-only-filled composites show a promising trend in toughness without the loss of other desirable mechanical characteristics, the glass fill will be increased to approximately 70% w/w, which should increase the mechanical properties as shown by Podgorski et al. (2015). Similarly, increasing the fill of the glass + FA composites should also result in an increase of their mechanical properties.

In this short-term monospecies biofilm model, the new resins and composites showed marked differences in the *S. mutans* biofilm formation as compared with the conventional bisGMA/ TEGDMA samples. The results indicate that the new resins and composites show overall less *S. mutans* biofilm biomass formation and fewer CFU/mL, which are substantially decreased again by the addition of the FA crystals. Based on our preliminary data (Fig. 3), although the fluoride release was greater at pH 7 for the 3 glass + FA filled composites, the
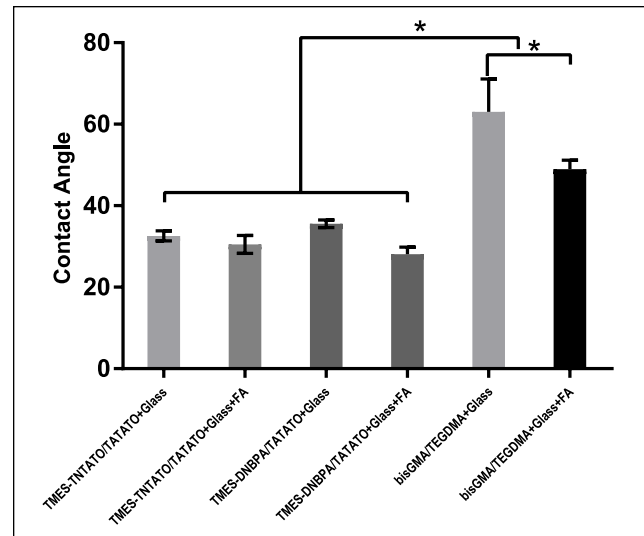
effect on biofilm formation is unlikely to be the result of this fluoride release, because it was not at a concentration that might affect bacterial viability (Hamilton and Bowden 1988) but could affect remineralization.

The contact angles for the composites are low, and the addition of the FA further reduced the contact angle, which may have influenced biofilm formation. Certainly, the increased hydrophilicity of dentin bonding agents and coating hydroxyapatite and rat teeth with a hydrophilic compound resulted in reduced biofilm formation (Shimotoyodome et al. 2007; Brambilla et al. 2014). However, this effect may be a combination of the physicochemical surface properties of the composites, such as hydrophilicity, charge, and surface roughness, which were reported to affect bacterial attachment

Further evaluation is necessary, but these results are intriguing. If they are replicated in a longer-term multispecies biofilm model, then this biological effect may extend the longevity of these new composites.

It was not surprising to see the fluoride release increasing at pH 4 from the glass + FA–filled composites, but it was surprising to see this occurring in the glass-only-filled composites. This may be due to the release of fluoride inherent in the composites, perhaps from the glass beads. This fluoride release is relatively small, but 0.024- to 0.054-ppm amounts of fluoride were shown to prevent carious lesion cavitation in an in vitro study (Margolis et al. 1986).

Author Contributions

H.L. Van der Laan, B.J. Bielajew, T.A. Davidson, J. Gardinier, S. Chahal, contributed to data acquisition, drafted the manuscript; S.L. Zajdowicz, D.H. Kohn, J. Liu, contributed to conception,

design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; K. Kuroda, contributed to conception and design, drafted the manuscript; S. Chang, contributed to data acquisition and analysis, drafted the manuscript; J. Gerszberg, contributed to data acquisition, analysis, and interpretation, critically revised the manuscript; T.F. Scott, contributed to conception, design, data acquisition, analysis, and interpretation, critically revised the manuscript; B.H. Clarkson, contributed to conception and design, drafted and critically the revised manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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