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Original Article

Novel low-shrinkage-stress bioactive nanocomposite with anti-biofilm and remineralization capabilities to inhibit caries

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Background/purpose: A common reason for dental composite restoration failure is recurrent caries at the margins. Our objectives were to: (1) develop a novel low-shrinkage-stress, anti-bacterial and remineralizing resin composite; (2) evaluate the effects of dimethylaminohexadecyl methacrylate (DMAHDM) on mechanical properties, biofilm inhibition, calcium (Ca) and

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nanoparticles;
Dental
nanocomposite;
Low shrinkage stress;
Secondary caries

phosphate (P) ion release, degree of conversion, and shrinkage stress on the new low-shrinkage-stress resin composite for the first time.

Material and methods: The resin consisted of urethane dimethacrylate (UDMA) and triethylene glycol divinylbenzyl ether (TEG-DVBE) with high resistance to salivary hydrolytic degradation. Composites were made with 0%–8% of DMAHDM for antibacterial activity, and 20% of nanoparticles of amorphous calcium phosphate (NACP) for remineralization. Mechanical properties and *Streptococcus mutans* biofilm growth on composites were assessed. Ca and P ion releases, degree of conversion and shrinkage stress were evaluated.

Results: Adding 2–5% DMAHDM and 20% NACP into the low-shrinkage-stress composite did not compromise the mechanical properties ($p > 0.05$). The incorporation of DMAHDM greatly reduced *S. mutans* biofilm colony-forming units by 2–5 log and lactic acid production by 7 folds, compared to a commercial composite ($p < 0.05$). Adding 5% DMAHDM did not compromise the Ca and P ion release. The low-shrinkage-stress composite maintained a high degree of conversion of approximately 70%, while reducing the shrinkage stress by 37%, compared to a commercial control ($p < 0.05$).

Conclusion: The bioactive low-shrinkage-stress composite reduced the polymerization shrinkage stress, without compromising other properties. Increasing the DMAHDM content increased the antibacterial effect in a dose-dependent manner.

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Introduction

Dental caries is one of the most prevalent diseases worldwide. According to the World Health Organization (WHO) reports, 5–10% of the entire health care budget is spent on treating dental caries.¹ In some high-income countries, dental caries is among the main reasons for children's hospitalization.¹ Therefore, dental caries is a major challenge facing the entire society. Dental caries is a multifactorial disease that involves an interaction between acidogenic bacterial, dietary carbohydrates glycolysis, and demineralization of dental hard tissues. If this process is not reversed by the natural presence of calcium and phosphate ions in saliva, cavitation occurs and restorative treatment becomes necessary.²

Methacrylate-based resin composites continue to be the material of choice in daily dental practice for several reasons, including their good mechanical properties, conservative cavity design, and superior esthetics.³ However, the longevity of resin composite restorations ranges only 5–10 years.⁴ Recurrent caries and tooth fracture are the most common types of failure during the first 6 years of clinical service.⁴ These failures are often caused by the polymerization shrinkage of the methacrylate resin-based dental restorative materials.⁵ This polymerization shrinkage can lead to interfacial debonding, post-operative sensitivity, and recurrent caries.⁶

To address this problem, several strategies have been proposed to reduce the shrinkage stress. These methods include changing the monomer chemistry, for example, step growth thiolene resin system,⁷ epoxy resin system,⁸ and silorane resin system.⁹ Recent studies tested a modification of the resin composite filler by using epoxy oligomers or using polymeric nanogels fillers to alleviate the shrinkage stress.^{10,11} These studies focused on reducing the shrinkage stress without influencing the material

bioactivity. Recently, a unique low shrinkage resin was developed using urethane dimethacrylate (UDMA) and hydrolytically stable, ether-based triethylene glycol divinylbenzyl ether (TEG-DVBE) copolymers. This resin system has a unique polymerization kinetics that include a slower polymerization rate results in a delayed gel point. The longer time taken by the UDMA/TEG-DVBE composite to reach rigidity allowed easier stress relaxation and prevented excessive contraction stress accumulation.¹¹ In addition, the urethane group in UDMA can reduce the material degradation by improving the resistance to salivary hydrolysis.¹² TEG-DVBE is the diluent monomer with a proven stability against hydrolytic challenges and estrade degradation.¹²

Previous antibacterial monomers with various compositions of quaternary ammonium methacrylates (QAMs) were co-polymerized into dental materials and achieved a long-term antibacterial effect.¹³ A bonding agent contained 12-methacryloyloxydodecylpyridinium bromide (MDPB) was effective to eradicate residual bacteria from the dentinal tubules of the cavity preparation.¹⁴ Also, several other antibacterial formulations were incorporated in dental resin composites and bonding agents, including quaternary ammonium polyethylenimine (QPEI), methacryloxyethyl cetyl dimethyl ammonium chloride (DMAE-CB), and quaternary ammonium dimethacrylate (QADM) to reduce biofilm growth.^{14–16} Recently, dental resin composite contained dimethylaminohexadecyl methacrylate (DMAHDM) exhibited excellent long-term antimicrobial activities.¹⁷

Several previous studies suggested that increasing the mass fraction of QAM will increase its antibacterial function.¹⁸ For instance, increasing the mass fraction of DMAHDM from 0.75% to 3% in a traditional resin system increased the antibacterial potency.¹⁹ In a different report, a greater antibiofilm effect was obtained when increasing the DMAHDM concentration from 3% to 5%.²⁰ These studies

indicated the dose-dependent effect of DMAHDM when used with a traditional resin system.^{19,20}

However, to date, there has been no report investigating the effect of different mass fractions of DMAHDM in a low-shrinkage-stress resin composite. Therefore, the present study aimed to systematically determine the effects of DMAHDM mass fraction in a low-shrinkage-stress resin composite for the first time.

To further enhance the bioactivity of this low-shrinkage-stress resin composite, nanoparticles of amorphous calcium phosphate (NACP) were incorporated. Previous studies showed that calcium (Ca) and phosphate (P) ion release was not affected by the addition of 3% DMAHDM into the same low-shrinkage-stress resin composite.^{21,22} In addition, using NACP, enamel remineralization was achieved under a biofilm-induced demineralization treatment.²¹ To date, there has been no report evaluating the effect of DMAHDM mass fraction in a low-shrinkage-stress NACP-containing resin composite.

Accordingly, the objectives of this study were to systematically investigate the effect of DMAHDM mass fraction in a low-shrinkage-stress resin composite and determine the optimal mechanical and anti-biofilm properties. The following hypotheses were tested: (1) incorporating different mass fractions of DMAHDM into a low-shrinkage-stress NACP-containing resin composite will not adversely affect the mechanical properties; (2) biofilm growth, acid production, and metabolic activity on the low-shrinkage-stress resin composite will monotonically decrease with increasing the DMAHDM mass fraction; (3) incorporating different mass fractions of DMAHDM into a low-shrinkage-stress NACP-containing resin composite will not negatively affect the ion release rate; and (4) the new resin composite would lower the polymerization shrinkage stress without compromising the degree of conversion.

Material and methods

Fabrication of resin composite containing dimethylaminohexadecyl methacrylate and nanoparticles of amorphous calcium phosphate

The low-shrinkage-stress resin consisted of 55.8% UDMA (Esstech, Essington, PA, USA) and 44.2% of TEG-DVBE (all mass %), following previous studies.^{21,22} This resin is denoted as “UV” resin. Briefly, TEG-DVBE was synthesized through dropwise addition of TEG in dimethylformamide (DMF) to a stirred suspension of NaH at 0–4 °C under an argon atmosphere for 30 min. After continuous stirring for 2 h, 4-vinylbenzyl chloride in DMF was added dropwise for 30 min, and the reaction mixture was stirred at room temperature for 18 h. Then, the reaction mixture was quenched by a slow add-on of a saturated NH₄Cl aqueous solution. The resultant solution was diluted with distilled water and extracted with ethyl acetate. The solvent was removed under reduced pressure producing a pale-yellow oil.^{21,22} The resin was photoactivated using 0.2 wt% camphorquinone (CQ, Aldrich, Saint Louis, MO, USA) and 0.8 wt% of 4-N, N-dimethylaminobenzoate (4EDMAB; Aldrich). The details of the synthesis and characterization of the monomers were explained in detail in a previous report.¹²

DMAHDM was synthesized via a modified Menshutkin reaction as described in a previous study.²³ DMAHDM was added to the UV resin at a final mass fraction of 0%, 2%, 3%, 4%, 5%, 6% and 8% into the final resin composite.

NACP with a mean particle size of 116 nm was synthesized via a spray-drying technique, as described previously.²⁴ The 20% NACP filler mass fraction was selected and added into the UV resin to maintain good mechanical properties of the resin composite and release a high level for Ca and P ions for remineralization.²¹ In addition, silanized barium borosilicate glass particles with a median size of 1.4 μm (Caulk/Dentsply, Milford, DE, USA) were used as co-filler to provide mechanical reinforcement to the resin composite. The total fillers in the resin composite were 65%, including 20% NACP and 45% glass. To produce a cohesive resin composite paste, a Speed-Mixer (DAC 150.1, FlackTek, Landrum, SC, USA) was used. In addition, Heliomolar (Ivoclar, Ontario, Canada) containing 66.7 wt% of nano-fillers of silica and ytterbium-trifluoride was used as a commercial comparative control. It was chosen in our study because of its fluoride releasing ability, following previous studies.^{20,21} The following nine composites were tested:

- 1) Heliomolar commercial control resin composite (denoted as “Commercial control”);
- 2) Experimental control resin composite: 35% UV + 65% glass (denoted as “UV control”);
- 3) 35% UV + 0% DMAHDM + 20% NACP + 45% glass (denoted as “0%DMAHDM + NACP”);
- 4) 33% UV + 2% DMAHDM + 20% NACP + 45% glass (denoted as “2%DMAHDM + NACP”);
- 5) 32% UV + 3% DMAHDM + 20% NACP + 45% glass (denoted as “3%DMAHDM + NACP”);
- 6) 31% UV + 4% DMAHDM + 20% NACP + 45% glass (denoted as “4%DMAHDM + NACP”);
- 7) 30% UV + 5% DMAHDM + 20% NACP + 45% glass (denoted as “5%DMAHDM + NACP”);
- 8) 29% UV + 6% DMAHDM + 20% NACP + 45% glass (denoted as “6%DMAHDM + NACP”);
- 9) 27% UV + 8% DMAHDM + 20% NACP + 45% glass (denoted as “8%DMAHDM + NACP”).

Flexural strength and elastic modulus

A stainless-steel mold of 2 × 2 × 25 mm³ was used to fabricate the resin composite specimens for mechanical testing.²² Mylar strips were used on both sides. Samples were cured using (Labolight, DUO, GC, Tokyo, Japan) for 1 min.²² The specimens were detached and stored dry at 37 °C for 24 h. Flexural strength and elastic modulus (n = 6) were measured using a three-point flexural test with a 10 mm span at a crosshead-speed of 1 mm/min on a computer-controlled Universal Testing Machine (5500R, MTS, Cary, NC, USA).^{24,25} Flexural strength was calculated using the following formula: $S = 3P_{\max}L/(2bh^2)$, where P_{\max} is the maximum fracture load, L is the span, b is specimen width and h is thickness. Elastic modulus was calculated using the following formula: $E = (P/d)(L^3/(4bh^3))$, where load P divided by displacement d is the slope in the linear elastic region of the load–displacement curve.²⁴

The flexural and elastic test results showed that the addition of DMAHDM in the mass fractions of 2%, 3%, 4%, and 5% had strengths within the recommendation of the ISO standards.²⁵ The two resin composites with 6% and 8% DMAHDM were excluded because their strengths were below the ISO standards.²⁵ Therefore, the following seven resin composites were used in subsequent experiments: Commercial control; UV control; 0%DMAHDM + NACP; 2% DMAHDM + NACP; 3%DMAHDM + NACP; 4%DMAHDM + NACP; and 5%DMAHDM + NACP.

Hardness of resin composites

The hardness of the resin composites was measured using Vickers indentation (HMV II; Shimadzu Corporation, Kyoto, Japan) at an indentation load of 50 g and a dwell time of 15 s.²¹ The results represented the average of four repetitive measurements for each of three resin composite bars, with a total of 12 indents per resin composite.

Resin composite samples for biofilm testing

Resin composite disks with a diameter of 9 mm and thickness of 2 mm were light-cured using (Labolight) for 1 min on each side.²² To remove the initial burst of uncured monomer, the cured disks were immersed in distilled water and magnetically stirred at 100 rpm for 1 h following previous studies.²⁶ The disks ($n = 6$) for each group were then sterilized with ethylene oxide (Anprolene AN 74i, Andersen, Haw River, NC, USA) and degassed for 7 days, following the manufacturer's instructions.

Bacteria inoculation and biofilm formation

The use of bacterial species was approved by the University of * Institutional Review Board. *Streptococcus mutans* (*S. mutans*) (UA159) was selected as it is one of the most commonly-associated organisms in dental caries.²⁷ For all the biofilm assays, *S. mutans* was cultured overnight in brain heart infusion broth (BHI, Difco, Sparks, MD, USA) at 37 °C with 5% CO₂.²² The inoculum was adjusted using a spectrophotometer (Genesys 10S, Thermo Scientific, Waltham, MA, USA) to 10⁷ colony-forming unit counts CFU/mL based on the standard curve of OD_{600 nm} versus the CFU/mL graph.²⁸ The culture medium (BHI) with 2% sucrose (wt/vol) was distributed into each well of 24-well plates and incubated for 24 h. After 24 h of culture, the resin composite disks were transferred to a new 24-well, filled with fresh medium with sucrose, and incubated for another 24 h. This totaled 48 h of culture, which was previously shown to be sufficient to form relatively mature biofilms on resin composite disks.¹⁷

Biofilm colony-forming units counts

The 2-day biofilms on resin composite disks were harvested in phosphate buffered saline (PBS) by scraping and sonication/vortexing (FS-30, Fisher, Pittsburg, PA, USA).²¹ The suspensions were serially diluted and spread on BHI agar plates. After 48 h of incubation at 37 °C in 5% CO₂, the

colony number was counted and the biofilm colony-forming units (CFU) counts were determined.¹⁷

Lactic acid production by biofilms

The 2-day biofilms on resin composite disks were immersed in buffered peptone water (BPW, Sigma–Aldrich) supplemented with 0.2% sucrose and incubated at 37 °C in 5% CO₂ for 3 h.^{23,38} The lactate concentrations in BPW were determined using a lactate dehydrogenase enzymatic method by measuring OD_{340nm} using a microplate reader (SpectraMax) as previously described.²⁶

Metabolic activity of biofilms

Metabolic activity of biofilms was measured by a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay.²⁹ MTT is a colorimetric assay that measures the enzymatic reduction of a yellow tetrazole to formazan.²⁹ The resin composite disks were transferred to a new 24-well plate filled with 1 mL of MTT dye (0.5 mg/mL MTT in PBS) and then incubated at 37 °C in 5% CO₂.²⁹ After 1 h, the disks were transferred into a new 24-well plate with 1 mL dimethyl sulfoxide (DMSO) in each well and incubated at room temperature for 20 min to dissolve the formazan crystals. Then, 200 µL of the DMSO solution was obtained from each specimen and transferred to a 96-well plate, and the absorbance was measured at 540 nm by a microplate reader (SpectraMax).²⁹ A higher absorbance is correlated to a higher formazan concentration, which indicates a greater metabolic activity in the biofilm on the disk.²⁹

Measurement of calcium and phosphate ion release

To measure the effect of adding DMAHDM on the ion release, two groups were tested: 0% DMAHDM + NACP resin composite and 5%DMAHDM + NACP resin composite. The solution was prepared using a sodium chloride (NaCl) solution (133 mmol/L) buffered to pH = 4 with 50 mmol/L lactic acid.²⁶ Three specimens of approximately 2 × 2 × 12 mm³ were immersed in 50 mL of the solution to yield a specimen volume/solution of 2.9 mm³/mL, similar to that in a previous study.²⁶ The concentrations of Ca and P ions released from the specimens were measured at 1, 3, 5, 7, 14, 21, 28, and 35 days.²¹ At each time, aliquots of 0.5 mL were removed and replaced by the fresh solution. The aliquots ($n = 4$) were analyzed for Ca and P ions using a spectrophotometric method (SpectraMax M5, Molecular Devices, Sunnyvale, CA, USA) with known standards and calibration curves, following a previous study.²⁶

Polymerization shrinkage stress and degree of conversion

Helimolar was not included in the polymerization stress and degree of conversion measurement because it yielded a low degree of conversion, which made Helimolar resin composite unsuitable for comparison.²¹ Therefore, to have a baseline in evaluating the polymerization stress and degree of conversion of the low-shrinkage-stress resin

composite, a traditional resin composite, consisting of bisphenol A glycidyl dimethacrylate (BisGMA, Esstech) and triethylene glycol dimethacrylate (TEGDMA, Esstech), were mixed at a mass ratio of 1:1 (referred to as BT resin). BT resin was included as a comparative resin composite control. The following six groups were tested for the polymerization stress and degree of conversion:

- 1) Experimental control resin composite: 35% BT + 65% glass (denoted as "BT control");
- 2) 35% BT + 0% DMAHDM + 20% NACP + 45% glass (denoted as "BT+0%DMAHDM + NACP");
- 3) 30% BT + 5% DMAHDM + 20% NACP + 45% glass (denoted as "BT+5%DMAHDM + NACP");
- 4) Experimental control resin composite: 35% UV + 65% glass (denoted as "UV control");
- 5) 35% UV + 0% DMAHDM + 20% NACP + 45% glass (denoted as "UV+0%DMAHDM + NACP");
- 6) 30% UV + 5% DMAHDM + 20% NACP + 45% glass (denoted as "UV+5%DMAHDM + NACP").

The polymerization shrinkage stress of each resin composite paste was measured using a cantilever beam-based tensometer.³⁰ The setup consisted of the tensometer with a built-in high-speed NIR spectrometer,³¹ which permits simultaneous monitoring of the real-time double-bond conversion in transmission. An uncured, disk-shaped specimen (2 mm in height and 2.5 mm in diameter) of each resin composite paste ($n = 3$) was placed between two flat methacrylate-silane-treated quartz rods.³¹

A curing light (LZ1-10DB0, LED Engin High Powder LEDs, Mouser Electronics, Mansfield, TX; 60 s irradiation, 1000 mW/cm² intensity) was used.³¹ The calculation for the polymerization shrinkage stress was done using the beam formula:³¹ $\sigma = F/A = 6\delta EI/\pi r^2 a^2 (3l - a)$, where σ is the polymerization shrinkage stress, F is the force exerted by the sample shrinkage, A is the cross-sectional area, r is the radius of the sample, δ is the beam deflection at the free end, E is Young's modulus, I is the moment of inertia of the beam, and l and a are the length of the beam and the distance between the sample position and the clamped edge of the beam, respectively.³⁰

The double-bond (6165 cm⁻¹) conversion was measured by guiding the NIR signal through the specimen's sides using optical fiber cables (1 mm in diameter).³¹ The degree of conversion was calculated:³² Degree of conversion (%) = $100 \times (1 - \text{Area}_{\text{polymer}} / \text{Area}_{\text{monomer}})$, in which the $\text{Area}_{\text{monomer}}$ is the peak area of the sample before the beginning of irradiation, while $\text{Area}_{\text{polymer}}$ is the peak area of the sample at each time point during the polymerization process. For 5 min, the concomitant polymerization stress/degree of conversion was continuously recorded.³¹

Statistical analysis

All statistical analyses were performed using SPSS, version 25.0 (SPSS, Chicago, IL, USA). One-way ANOVA with Tukey's multiple comparison tests were performed to detect significant differences between groups. T-test was performed for the Ca and P ion release. Results were considered statistically significant at a p-value of less than 0.05.

Results

Flexural strength and elastic modulus

The flexural strengths and modulus of elasticity of the resin composites are plotted in Fig. 1 (Mean \pm sd; $n = 6$): (A) Flexural strength, and (B) elastic modulus. The resin composites containing 2%, 3%, 4%, and 5% DMAHDM with 20% NACP had flexural strengths comparable to commercial control. The UV control had the highest flexural strength ($p < 0.05$). These results demonstrate that increasing the DMAHDM concentration up to 5% DMAHDM compared to the commercial resin composite did not significantly reduce the flexural strength of the resin composite ($p > 0.05$).

The elastic modulus values of the resin composites varied between 5 GPa and 9 GPa. The incorporation of 2% or 3% DMAHDM and 20% NACP resulted in higher elastic modulus

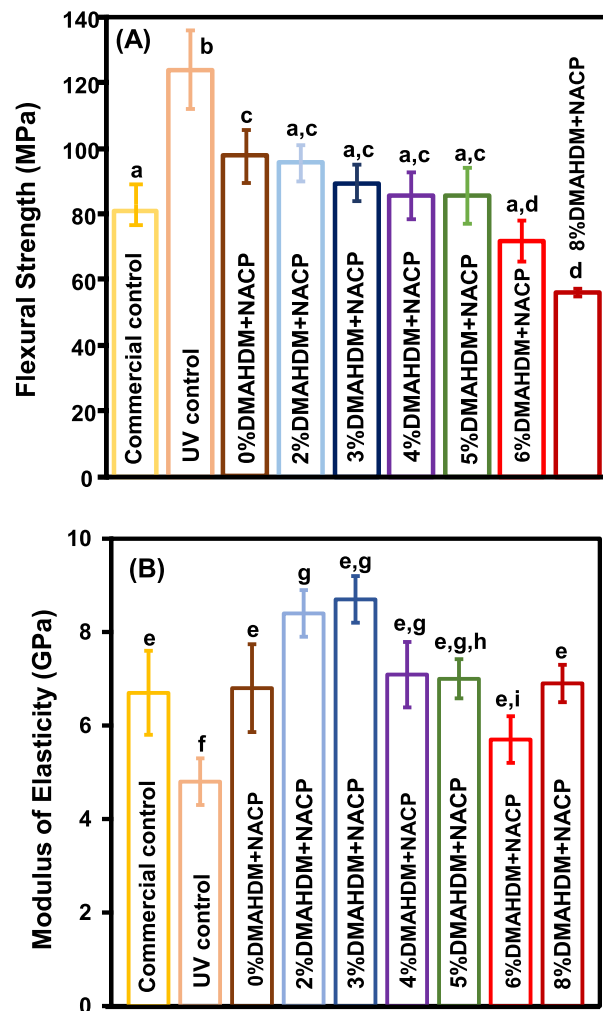


Figure 1 Mechanical properties of resin composites (A) Flexural strength and (B) elastic modulus (mean \pm SD; $n = 6$). The incorporation of up to 5% DMAHDM into the NACP resin composite did not compromise the strength and elastic modulus, which were comparable to commercial control. Dissimilar letters indicate values that are significantly different from each other ($p < 0.05$).

values when compared to UV control resin composite ($p < 0.05$). However, the incorporation of 4% and 5% DMAHDM with 20% NACP resulted in comparable elastic modulus values to the commercial control ($p > 0.05$).

Hardness of resin composites

The resin composite hardness data are presented in Fig. 2 (mean \pm SD; $n = 3$). The hardness values ranged between 0.25 GPa and 0.37 GPa. In general, there was an increase in hardness with increasing the mass fraction of DMAHDM. The highest hardness was observed in the 5%DMAHDM + NACP resin composite. Both UV control and 0% DMAHDM + NACP had the lowest hardness.

Biofilm colony-forming units counts

The CFU results of the *S. mutans* biofilm are shown in Fig. 3 (mean \pm SD; $n = 6$). The biofilm CFU reduction was 5–5.5 log by the 4%DMAHDM + NACP resin composite, compared to the commercial control ($p < 0.05$). The greatest CFU reduction was observed in the 5%DMAHDM + NACP group, with 7–7.5 log reduction, compared to UV control ($p < 0.05$). The incorporation of 2% or 3% DMAHDM in resin composites significantly reduced the CFU count for *S. mutans* compared to the UV control ($p < 0.05$). However, in the 0%DMAHDM + NACP group, *S. mutans* CFU were comparable to the UV control ($p > 0.05$). These results showed that increasing DMAHDM concentration from 2%, 3%, 4%–5% demonstrated a monotonically increased antibacterial potency.

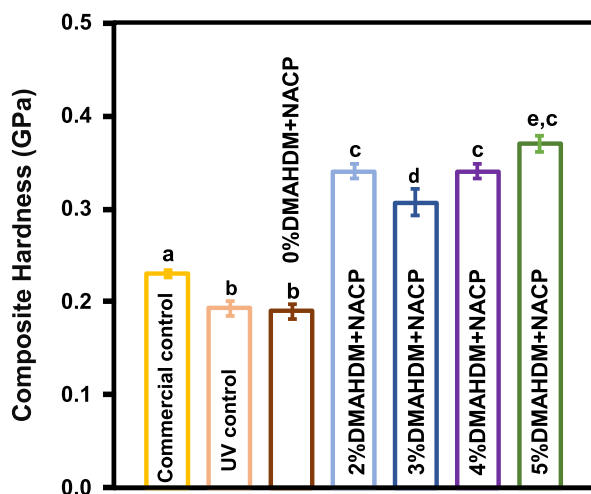


Figure 2 Hardness of resin composites (mean \pm SD; $n = 3$). The addition of DMAHDM up to 5% with 20% NACP resulted in higher hardness, compared to the commercial control ($p < 0.05$). The highest value was reported for 5% DMAHDM + NACP resin composite and the lowest value was reported for UV control and 0%DMAHDM + NACP resin composites. Values with dissimilar letters are significantly different from each other ($p < 0.05$).

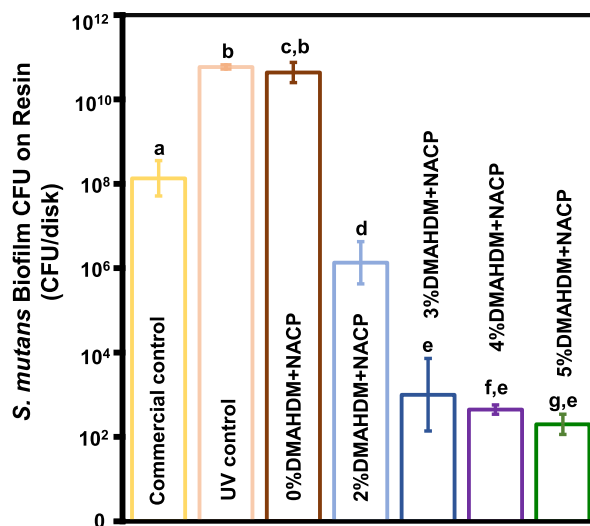


Figure 3 The CFU counts of biofilms on resin composite disks (mean \pm SD; $n = 6$). The UV control and 0%DMAHDM + NACP had similar CFU results. The gradual increase in the DMAHDM mass fractions up to 5% helped to reduce the *S. mutans* growth by 4–7 orders of magnitude less than those on the UV control. Dissimilar letters indicate values that are significantly different from each other ($p < 0.05$).

Lactic acid production by biofilms

Lactic acid production on the resin composite disks is plotted in Fig. 4 (mean \pm SD; $n = 6$). The amount of acid productions of biofilms on the commercial control and the UV control group were comparable ($p > 0.5$). In contrast, the lactic acid production of biofilms was substantially reduced by increasing the DMAHDM mass fraction in the low-shrinkage-stress resin composite ($p < 0.05$).

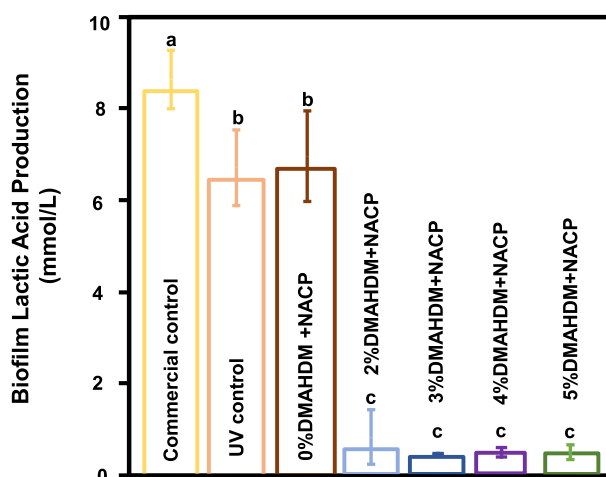


Figure 4 Lactic Acid production by biofilms on resin composites (mean \pm SD; $n = 6$). UV control and 0%DMAHDM + NACP had similar lactic acid. Resin composites with different mass fractions of DMAHDM reduced lactic acid with a similar pattern ($p > 0.05$). Dissimilar letters indicate values that are significantly different from each other ($p < 0.05$).

Metabolic activity of biofilms

The MTT metabolic activity of biofilms on resin composites is illustrated in Fig. 5 (mean \pm SD; $n = 6$). The metabolic activity of commercial control resembled that of the UV control ($p > 0.05$). A great decrease in biofilm metabolic activity was observed with increasing the DMAHDM mass fraction in the low-shrinkage-stress resin composite ($p < 0.05$).

Measurement of calcium and phosphate ion release

The release of Ca and P ions from the composites is plotted in Fig. 6 (A) Ca ions and (B) P ions (mean \pm SD; $n = 4$). After one month of ion release, 5%DMAHDM + NACP resin composite had Ca ion release of 4.50 ± 0.81 mmol/L, comparable to the Ca ion released by 0%DMAHDM + NACP resin composite 5.34 ± 0.054 mmol/L ($p > 0.05$). Therefore, the incorporation of 5% DMAHDM to the remineralizing low-shrinkage stress resin composite did not compromise the ion release ability ($p > 0.05$). The P ion release showed a similar trend.

Polymerization shrinkage stress and degree of conversion

The degree of conversion is presented in Fig. 7 ($n = 3$). Overall, all the groups showed a gradual increase in the degree of conversion with polymerization time, followed by a plateau. The BT control resin composite had the lowest degree of conversion (69%). The UV groups showed a delayed arrival to the peak, compared to BT. At 5 min of starting the photo-curing, the UV resin composite with 5% DMAHDM reached a degree of conversion of approximability

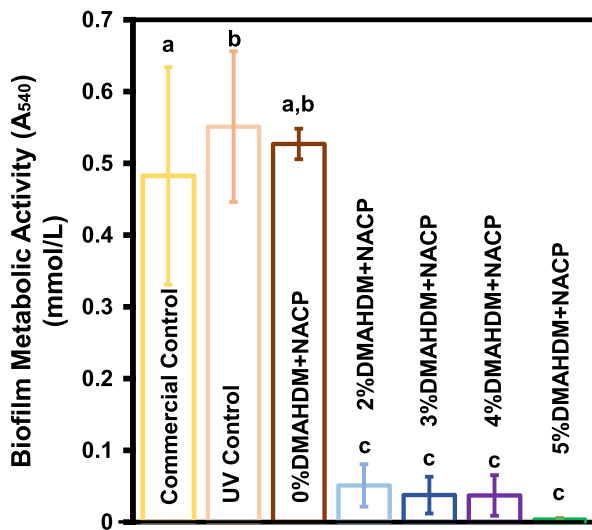


Figure 5 Metabolic activity of biofilm MTT (mean \pm SD; $n = 6$). The incorporation of DMAHDM up to 5% was more effective in reducing metabolic activity than NACP alone ($p < 0.05$). Dissimilar letters indicate values that are significantly different from each other ($p < 0.05$).

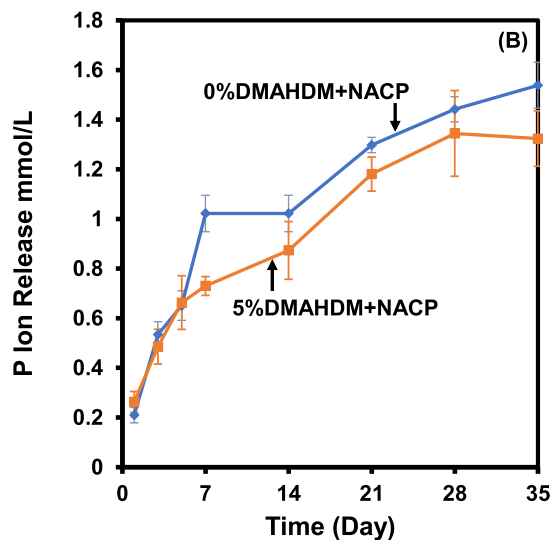
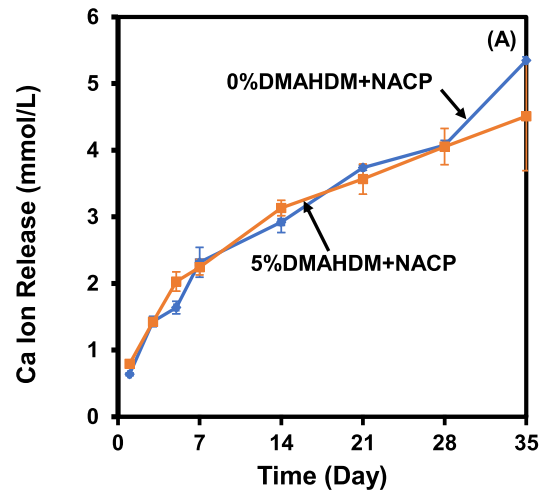


Figure 6 Calcium (Ca) and phosphate (P) ion release from resin composites (A) Calcium ion release, and (B) phosphate ion release (mean \pm SD; $n = 4$). Both groups have high Ca and P ion releases over 35 days. Increasing the DMAHDM percentage to 5% did not compromise Ca and P ion release ($p < 0.05$).

$\approx 70\%$, while the UV+0%DMAHDM + NACP resin composite reached 77% degree of conversion ($p < 0.05$).

The polymerization shrinkage stress vs. polymerization time is plotted in Fig. 8 ($n = 3$). Higher polymerization shrinkage stress was recorded for BT resin composite groups, compared to UV groups ($p < 0.05$). In addition, UV groups displayed a delayed development of polymerization stress. The BT+5%DMAHDM + NACP resin composite had a polymerization shrinkage stress of 1.69 ± 0.08 MPa. The UV+5%DMAHDM + NACP resin composite had a polymerization shrinkage stress of 1.05 ± 0.12 MPa.

Discussion

The present study systematically investigated the effects of DMAHDM mass fraction in a low-shrinkage-stress and

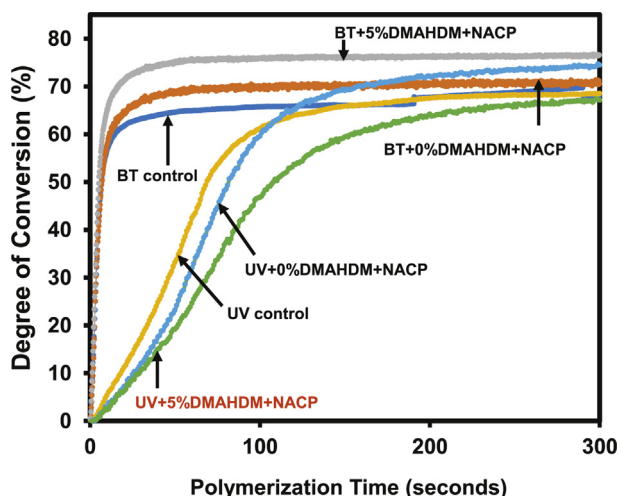


Figure 7 The degree of conversion vs. polymerization time ($n = 3$). The UV+5%DMAHDM + NACP resin composite had the slowest rate of polymerization upon curing. However, all the groups had a degree of conversion within the clinically acceptable level.

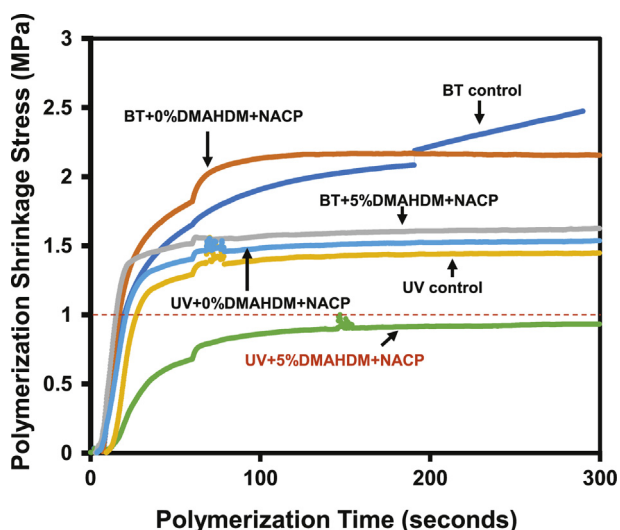


Figure 8 Polymerization shrinkage stress vs. polymerization time ($n = 3$). The UV resin composite groups showed delayed development of polymerization stress and lower shrinkage stresses ($p < 0.05$). The BT+5%DMAHDM + NACP resin composite had a polymerization shrinkage stress of 1.69 MPa. In contrast, the UV+5%DMAHDM + NACP resin composite dropped the stress level to 1.05 MPa.

remineralizing NACP resin composite for the first time. "The first hypothesis was partially accepted as the incorporation of no more than 5% DMAHDM and NACP into the low-shrinkage-stress resin composite did adversely affect the mechanical properties. However, the further hypotheses were proven that the *S. mutans* biofilm growth was reduced, Lactic acid production and metabolic activity monotonically decreased as DMAHDM mass fraction increased." Moreover, the new low-shrinkage-stress resin composite containing the 5% DMAHDM and NACP provided

high levels of Ca and P ion releases for remineralization. Additionally, the 5%DMAHDM + NACP resin composite significantly reduced the shrinkage stress by 37% when compared to a traditional resin composite, without compromising the degree of conversion ($\approx 70\%$).

In the past decades, extensive improvements in the mechanical properties, esthetic of the resin composite restoration have been achieved. However, volumetric shrinkage and subsequent shrinkage stress arising during the polymerization reaction are still major drawbacks.⁵ Polymerization shrinkage stresses can affect the integrity of the tooth structure and can compromise the tooth-resin composite interface, leading to gap formation and recurrent caries.³³ Therefore, it would be highly desirable to develop a low-shrinkage-stress resin composite to increase the survival rate of the restoration and minimize the stresses on the tooth structure. A previous study used a thiol-ene system to reduce polymerization stress from 2.8 MPa in a Bis-GMA/TEGDMA resin composite to 0.30 MPa.³⁴ However, it had a low mechanical property.³⁴ Another group investigated the substitution of TEGDMA with tricyclo decanedimethanol acrylate (SR833s) and isobornyl acrylate (IBOA).³⁵ The results showed a stress reduction; however, the degree of conversion was also decreased.³⁵ In addition, a meta-analysis revealed that the 12-month follow-up assessment of the marginal retention of a silorane-based resin composite was not significantly better than traditional methacrylate's-based composite resins.³⁶ Recently, the use of UDMA and TEGDVB monomers to develop a low-shrinkage-stress resin composite achieved low polymerization stress, adequate mechanical strength, and strong antibacterial effect.²¹ However, the antibacterial mass fraction was limited to a single mass fraction of 3% DMAHDM.²²

In the present study, we systematically investigated the effects of DMAHDM mass fraction from 0% to 8% in the low-shrinkage-stress resin composite. This experimental design enabled the determination of the maximum amount of antibacterial monomer that can be added to this low-shrinkage-stress resin composite without compromising its mechanical and physical properties. The present study showed that increasing the DMAHDM mass fraction to above 5% led to a viscous resin and a less cohesive resin composite paste. The flexural strength and elastic modulus for the 6% or 8% DMAHDM groups were less than the ISO recommendations.²⁵ Therefore, we focused on the DMAHDM mass fractions of 2%–5%. These formulations achieved good flexural strength and modulus of elasticity similar to the commercial resin composite. This can be attributed to the small particle size of NACP that leaves room for the reinforcement of glass fillers (45%). In addition, comparable mechanical properties were obtained between BT and UV resin composites containing 3% or 5% DMAHDM in addition to 20% NACP.²⁰ Meanwhile, the novel low-shrinkage-stress resin composite substantially reduced the polymerization shrinkage stress 37% lower than that of the BT resin composite.

Furthermore, all study groups showed clinically acceptable hardness values of between 0.25 GPa to 0.37 GPa.³⁷ Indeed, the hardness of the new low-shrinkage-stress resin composite with DMAHDM and NACP are comparable to commercial dental resin composites such as Tetric N-

Ceram with a hardness of 0.33 GPa and IPS Empress Direct with a hardness of 0.29 GPa.³⁸

Dental resin composites lack antibacterial properties and tend to accumulate more plaque than amalgam restorations.³⁹ This accumulation can contribute to cariogenic bacterial attachment, hydrolytic enzyme production, material degradation, and recurrent caries.¹⁶ Therefore, efforts were made to change the concept of dental treatment from drill and fill to a smart bioactive material that can interact with the oral environment.⁴⁰ Different antibacterial agents were used, including chlorhexidine. A short-term antibacterial effect was observed with the addition of chlorhexidine in a low-shrinkage-stress resin composite.⁴¹ Nanoparticles such as silver and zinc oxide were also added to dental materials as antibacterial fillers.⁴² However, the mechanical properties of the material were compromised due to the loss of a large amount of the agent in a short period of time, leading to increased surface porosity.⁴² To overcome this critical drawback, copolymerized quaternary ammonium methacrylates were used to covalently bond to dental resins, leading to long-term antibacterial effects.¹⁹

DMAHDM has presented a potent effect on the reduction and modulation of cariogenic species.²⁰ Studies have shown that increasing the mass fraction of DMAHDM in BisGMA/TEGDMA resin-based composite would increase its antibacterial function.^{18,20} The suppression of the biofilm with increased DMAHDM mass fraction can be attributed to increasing its charge density. Increasing the positive charge of DMAHDM helps to interact with more of the negatively charged bacterial membrane, which can lead to disruption of the membrane functions, essential ions unbalance (i.e., K^+ , Na^+ , Ca^{2+} , and Mg^{2+}), followed by bacterium lysis.²¹ Therefore, the different resin composite formulations with different mass fractions of DMAHDM could be used according to the clinical needs of the patient. For example, in a young patient with normal salivary flow and stronger chewing forces, resin composite with 2% or 3% DMAHDM +20% NACP will have an adequate antibacterial effect ranging between 2 to 4 logs of reduction in *S. mutans* biofilm growth and flexural strength that varies between 89–96 MPa. In older individuals with impaired physical performance, low salivary flow, and weaker muscle function, resin composites with 4% or 5% mass fraction of DMAHDM +20% NACP with 4–5 logs reduction in *S. mutans* biofilm growth and acceptable mechanical strength around 86 MPa will be of a better choice.

NACP particles were added to the low-shrinkage-stress antibacterial resin composite to make the resin composite a smart material that can interact with the oral environment.²⁴ This NACP resin composite rapidly raises the pH and modifies the microenvironment around the dental plaque.⁴³ In this study, the ion release data showed a similar ion release reported in previous studies using the same resin system with 3%DMAHDM+20%NACP.^{21,22} Therefore, the 5% DMAHDM+20%NACP resin composite, developed in this study, is also expected to continue the ion release for 90 days with close value to the previous report.^{21,22} Another potential advantage of NACP containing resin composite, that it has a Ca and P recharge capability to prolong the ion release.²⁹ A previous study conducted 12 recharge cycles, each recharged cycle was followed by 14 days of continuous ion

release without further recharge.²⁶ Therefore, it would be highly desirable for future studies to consider the low-shrinkage-stress resin composite to have the potential to be rechargeable for a longer ion release.

The polymerization degree of conversion is an important property that determines the physical and biological properties and clinical longevity of dental resin composites.⁴⁴ Previous reports indicate that most dimethacrylate monomers in dental materials have a degree of conversion that ranges from 55% to 75%.⁴⁵ The present study achieved relatively high degrees of conversion, ranging from 69% to 76% for the BT resin composite, and from $\approx 70\%$ to 77% for the UV resin composite. In general, UV groups showed a delayed arrival to the peak, compared to BT groups. For the UV control resin composite, the degree of conversion reached 72%. However, with the addition of 20% NACP, the polymerization rate accelerated and reached 77%. This trend was consistent with a previous study, which speculated that the small particle size of NACP could surround its surface by a thin layer of resin. This thin surface of resin likely enabled more light penetration during curing, and thus increased the degree of conversion.¹⁷ However, with the addition of 5% DMAHDM to the resin composite, we noticed a lower rate of conversion. This decrease could be related to the presence of DMAHDM covering NACP particles and slightly decreasing the light penetration during the curing. Future investigations are needed to fully understand the mechanism behind the degree of conversion in the new resin composite.

Optimizing the resin composite marginal seal is a key factor for the longevity of the restoration.^{46,47} Previous studies showed a relation between polymerization shrinkage stress and sealing ability.⁴⁸ Therefore, it is practically important to demonstrate that using low-shrinkage-stress UV resin composite helped to reduce the polymerization shrinkage stress 37% lower than the BT resin composite. Consequently, the low-shrinkage-stress resin composite can maintain a good marginal seal. Even with the addition of NACP particles and a 5% DMAHDM monomer, the shrinkage stress reduction was maintained at 1.05 MPa for UV compared to 1.69 MPa for BT resin composite. This can be related to the difference in the polymerization rate achieved by both resins.¹⁴ The longer the polymerization rate taken by the UV resin composite to reach rigidity allowed easier stress relaxation and prevented excessive contraction stress accumulation.¹² Furthermore, a previous study showed a direct relationship between the polymerization stress and microleakage in class V cavities restored with three different commercial resin composites.⁴⁹ Another study showed that using one layer of unfilled resin will reduce the stress between 13 and 24%.⁵⁰ However, when the unfilled resin composite layer was added, the material radiolucency affected the radiographic diagnosis, and the fluidity of the material affecting its handling and application.⁴⁹ It is valuable to know that our low-shrinkage-stress resin composite will be beneficial in reducing the clinical steps when restoring class V restorations by using only one type of resin composite. In addition, this low-shrinkage-stress resin composite has an antibacterial property that will help suppress acidogenic bacteria, as well as with NACP particles that can neutralize the acids and remineralize the lesions to protect the tooth structures.

In summary, in the present study, we developed a bioactive low-shrinkage-stress NACP-containing resin composite and systematically investigated the effects of DMAHDM mass fraction for the first time. The incorporation of NACP and 2%–5% DMAHDM into the low-shrinkage-stress resin composite did not adversely affect the mechanical properties. The resin composites had a dose-dependent reduction in the *S. mutans* biofilm with increasing mass fraction of DMAHDM from 2% to 5%. Biofilm CFU counts were reduced by 2–5 orders of magnitude, compared to commercial resin composite. Ca and P ion releases were not compromised with increasing mass fractions of DMAHDM in the low-shrinkage-stress composite. The 5% DMAHDM + NACP composite maintained a high conversion ($\approx 70\%$) and reduced the polymerization shrinkage stress by 37%, compared to a traditional composite. Therefore, the low-shrinkage-stress composite with antibacterial and remineralizing properties is promising to overcome secondary caries and increase the restoration longevity. Further studies are needed to investigate the antibacterial effects and mechanical properties of the low-shrinkage-stress resin composite in long-term experiments. In addition, future study is warranted to compare the mechanical properties of this new low-shrinkage-stress resin composite against commercial bulk-fill nano-hybrid resin composites and other low-shrinkage resin composites available on the market which do not possess antibacterial or remineralizing capabilities. Further studies are also needed to investigate the degradation-resistant and low-shrinking UV monomer in a wide range of applications, including composites, bonding agents, sealants and cements to lower the shrinkage stresses and increase the longevity.

Declaration of competing interest

The authors declare no conflict of interest.

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