Preparation and Properties of Novel Dentin Adhesives with Esterase Resistance

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ABSTRACT: A new methacrylate monomer, trimethylolpropane mono allyl ether dimethacrylate (TMPEDMA), was synthesized and evaluated. This branched methacrylate was designed to increase esterase-resistance when incorporated into conventional HEMA (2-hydroxyethyl methacrylate)/BisGMA (2,2-bis[4(2-hydroxy-3-methacryloyloxy-propyloxy)-phenyl] propane) dental adhesives. The new adhesives, HEMA/BisGMA/TMPEDMA in a 45/30/25 (w/w) ratio were formulated with H_2O at 0 (A0T) and 8 wt % water (A8T) and compared with control adhesives (HEMA/BisGMA, 45/55 (w/w), at 0 (A0) and 8 wt % (A8) water). Camphoroquinone (CQ), 2-(dimethylamino) ethyl methacrylate and diphenyliodonium hexafluorophosphate were used as photoinitiators. The new adhesives showed a degree of conversion comparable with the control and improved modulus and glass transition temperature (T_g). Exposure of photopolymerized discs to porcine liver esterase for up to eight days showed that the net cumulative methacrylic acid (MAA) release in adhesives formulated with the new monomer and 8% water (A8T: 182 μg/mL) was dramatically ($P < 0.05$) decreased in comparison to the control (A8: 361.6 μg/mL). The results demonstrate that adhesives made with the new monomer and cured in water to simulate wet bonding are more resistant to esterase than conventional HEMA/BisGMA adhesive. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 3588–3597, 2008

Key words: dentin adhesive; esterase resistance; methacrylate; biodegradation; properties

INTRODUCTION

Polymer resin composites have gained wide acceptance as an alternative to dental amalgam for dental restorations (e.g., fillings). Dental composites typically employ methacrylate materials that are photopolymerized *in situ*. An adhesive layer that binds to dentin is initially applied, followed by application and polymerization of the composite layer, which contains an inorganic filler (e.g., silicon) to improve mechanical properties. In comparison to dental amalgam, resin composites offer improved aesthetics without the associated concern of mercury release into the environment. However, failure rates are significantly higher for resin composites than for amalgam.[1,2] For example, results from a 2006 clinical study indicate that at 5 years after initial treatment, the need for additional restorative care was 50% greater in children treated with composite as compared to children treated with amalgam.[2]

The penetration of bacterial enzymes, oral fluids and bacteria into the spaces between the tooth and the polymer resin undermines the restoration and leads to recurrent caries, hypersensitivity, and pulpal inflammation.[3–6] The failed composite restoration must then be removed and replaced, causing additional damage to the underlying healthy tooth structure. There is an urgent need for improved dental materials with the desirable properties of methacrylates, but with greater resistance to the infiltration of oral fluids and to degradation by enzymes.

Human saliva contains a variety of enzymes which may participate in the degradation of the adhesive as well as the composite.[7–12] In particular, the susceptibility of acrylate dental composites to degradation by esterases is well established.[9–14] Yourtee et al., have demonstrated that dimethacylates containing aromatic functional groups or branched methacrylate linkages show greater resistance to degradation by esterases.[15] Previous studies have focused on determining the susceptibility of composite resins to breakdown upon exposure to salivary esterases, but there has been limited investigation of

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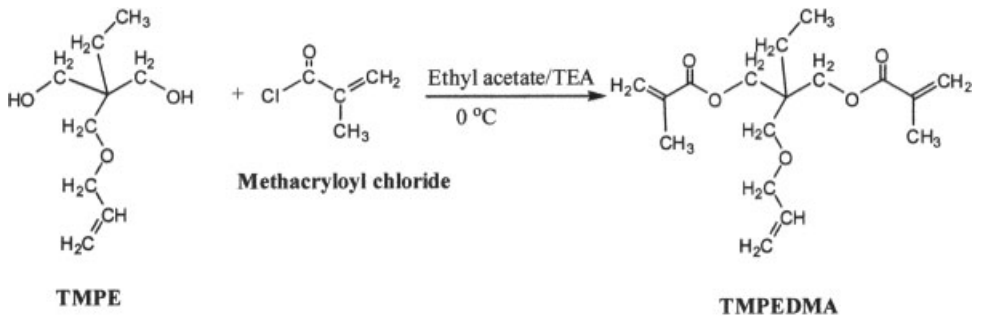


Scheme 1 Reaction scheme for new dimethacrylate monomer synthesis. TMPE, trimethylolpropane mono allyl ether; TMPEDMA, trimethylolpropane mono allyl ether dimethacrylate.

the effect of these enzymes on the underlying adhesive layer.[15–18] Under *in vivo* conditions, the adhesive layer and its bond to the underlying tooth structure can be the first defense against substances that may penetrate and ultimately undermine the composite restoration. Since water is always present in the oral cavity, adhesive performance depends on effective polymerization and bonding to dentin under wet conditions. Thus, the development of adhesives that are tolerant of the wet environment of the mouth and resistant to breakdown by esterases is central to the goal of producing durable composite restorations.

The aim of this study was to synthesize and characterize a new dimethacrylate monomer with a reactive branched side chain for use as a comonomer in dentin adhesives, and to evaluate the properties and enzymatic biodegradation of a new adhesive formulation that includes this monomer. The results demonstrate that the new adhesive has comparable mechanical properties and superior esterase resistance when compared to controls, particularly when polymerized in the presence of water. The new dimethacrylate therefore shows promise as a component of more durable dental materials.

EXPERIMENTAL

Materials

Adhesive formulations

The control formulation which is representative of current commercial dentin adhesives consisted of 2-hydroxyethylmethacrylate (HEMA, Acros Organics, NJ) and 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]-propane (BisGMA, Polysciences, Warrington, PA) with a mass ratio of 45/55. The new dimethacrylate monomer, TMPEDMA, was used as a comonomer in the experimental adhesive, HEMA/BisGMA/TMPEDMA = 45/30/25. TMPEDMA was selected for synthesis because the reactive branched side chain is expected to increase the extent of cross-linking in the adhesive, increasing esterase resistance. In addition, the estimated log *P* value (ChemDraw Ultra, CambridgeSoft, Cambridge, MA) of TMPEDMA of 3.36 is intermediate between those of BisGMA (log *P* = 5.09) and HEMA (log *P* = 0.47), suggesting compatibility with the BisGMA/HEMA system. TMPEDMA was synthesized from trimethylolpropane mono allyl ether (TMPE, Acros Organics, NJ) and methacryloyl chloride (Aldrich, Milwaukee, WI) (Scheme 1). Triethylamine (TEA), ethyl acetate and sodium hydroxide (NaOH) were used as received from Acros Organics (Fair Lawn, NJ). The following three-component visible light photoinitiators were used, all from Aldrich (Milwaukee, WI): camphorquinone (CQ, 0.5 wt %) as photoinitiator, 2-(dimethylamino) ethyl methacrylate (DMAEMA, 0.5 wt %), and diphenyliodonium hexafluorophosphate (DPIHP, 1.0 wt %) as coinitiators with respect to the total amount of monomer. Porcine liver esterase (PLE, EC 3.1.1.1) was obtained from Sigma Chemical, St. Louis, MO. All other chemicals were reagent grade and used without further purification.

Monomer synthesis (TMPEDMA)

TMPEDMA was synthesized by the widely-used condensation reaction between methacryloyl chloride and the appropriate alcohol, here TMPE (Scheme 1).[19–22]

To a three-neck flask containing TMPE (10 g, 0.057 mol), TEA (20.1 mL, 0.143 mol), and dry ethyl acetate (72 mL) under N_2 atmosphere, a solution of methacryloyl chloride (14 mL, 0.143 mol) in dry ethyl acetate was added dropwise with stirring at 0°C. An ice bath was used to maintain 0°C during the addition of the methacryloyl chloride solution. Following complete addition of the reagents, the reaction was allowed to continue at room temperature for another 22 h. After the reaction was completed, the mixture was purified by filtering out the triethylamine salts and repeated washing with 5% aqueous NaOH and brine until the solution was clear. The solution was then washed twice with distilled water. After drying over anhydrous $MgSO_4$, 0.05 wt % 2,6-di-tert-butyl-4-methylphenol (BHT) was

added and the solvent was removed with a rotary evaporator at 35–40°C. The yield of TMPEDMA, as light yellow colored oil, was in the range of 60–70%. The synthesized dimethacrylate monomer was identified using a Perkin-Elmer Spectrum One Fourier transform infrared spectrophotometer (FTIR) and nuclear magnetic resonance (NMR; FT-400 MHz Bruker Spectrometer, $CDCl_3$ as solvent).

Polymerization and degree of conversion

Control adhesive formulations in the presence of 0% (A0) and 8% (A8) water consisted of HEMA and BisGMA with a mass ratio of 45/55. The experimental adhesive formulations, HEMA/BisGMA/TMPEDMA = 45/30/25 (w/w) ratio, were formulated with 0% (A0T) and 8% (A8T) water. The control and experimental adhesives were irradiated for 20 s at room temperature with a commercial visible-light-curing unit, (Spectrum® 800, Dentsply, Milford, DE) at an intensity of 550 mW cm^{-2}, according to techniques published previously.[23,24] In brief, the photo-polymerization of the adhesives during irradiation was monitored *in situ* using a Perkin-Elmer Spectrum One Fourier transform infrared spectrophotometer (FTIR) with a resolution of 4 cm^{-1} in the ATR sampling mode. The extent of polymerization is related to the intensity of the peaks at 1637 cm^{-1}, which corresponds to the C=C stretching of the unreacted methacrylate monomers. In both adhesives, the peak at 1608 cm^{-1} assigned to the aromatic C=C bond was used as an internal standard. The change of the band height ratio [i.e., band height at 1637cm^{-1} (C=C): band height at 1608 cm^{-1} (phenyl)] in the cured (C) and uncured (U) states was monitored. The degree of conversion (DC)[23,25] was calculated by using the following equation based on the time-dependent decrease in the absorption intensity band ratios before and after light curing:

$$DC(\%) = [1 - (C/U)] \times 100$$

Mechanical properties

Rectangular beam specimens ($1 \times 1 \times 11$ mm^3) were cured in a glass-tubing mold for 20 s at a distance of 1 mm using a visible-light intensity of 550 mW cm^{-2} and used for the determination of mechanical properties. The tensile properties were determined for all samples after either: (i) 24 h dry storage at room temperature or (ii) storage for 24 h in distilled deionized water. Specimens were attached to the upper and lower grips using cyanoacrylate cement (Zapit, Dental Ventures of America, Corona, CA) and were loaded at a cross-head speed of 0.5 mm/min using an SSTM-5000 mechanical tester (United Calibration Corporation, CA) with a 150 lb load cell. The ultimate tensile strength (UTS, MPa) of each specimen was calculated as the maximum force at the point of failure divided by the specimen cross-sectional area. The elastic modulus (E, GPa) was measured as the slope of the linear portion of the stress-strain curve between 5 and 15% strain for all samples. Percent elongation (EL, %) was calculated as the value at the point of failure divided by the original specimen gauge length. Specimen toughness (T, m MN m^{-3}) was calculated as the area under the stress–strain curves. Four to eight specimens were evaluated per test condition.

Glass-transition temperature

The thermal behavior in the glass transition temperature (T_g) region was measured with a TA Instruments model Q100 modulated differential scanning calorimeter (MDSC, New Castle, DE) equipped with a refrigerated cooling system (RCS) using a protocol published previously.[23] In brief, following one-month *in vacuo* storage, the specimens were weighed (5–15 mg) in the aluminum DSC pans. The DSC cell was purged with nitrogen and the specimens were heated under nitrogen purge from −80°C to 200°C at 3°C/min, with a modulation period of 60 s and amplitude of ±2°C. Only the first cycle of heating was taken into account, and the results are shown as differential reversing heat flow versus temperature. The T_g values were reported as the temperatures of the peaks, i.e., inflection points of the heat flow curves.

Viscosity

Viscosities of the liquid resin formulated with/without water were measured with a TA Instruments AR2000 rheometer (New Castle, DE) in the controlled-rate mode. Measurements were made over a shear rate range of 10/s to 100/s, at 10 points per decade. At each shear rate, shear was applied for 60 s before the viscosity measurement, which was collected in the last 10-second sample period. The 10 viscosity measurements over the shear rate range were averaged. Measurements were made at 25°C with a cone and plate configuration, with 40 mm diameter and 2° cone angle.

Adhesive penetration into dentin

SEM studies were conducted to evaluate *in vitro* penetration of experimental adhesives into the dentin substrate and integrity at the interface between adhesive and dentin. The preparation of dentin specimens has been described previously.[5,26] Extracted unerupted human third molars stored at 4°C

in 0.9% wt/vol NaCl containing 0.002% sodium azide were used in this work. The teeth were collected under a protocol approved by the University Adult Health Sciences IRB. In brief, the sample preparation protocol was as follows: the occlusal one third of the crown was sectioned perpendicular to the long axis of the tooth using a water-cooled low-speed diamond saw (Buehler, Lake Bluff, IL). A smear layer was created by abrading the exposed dentin surface with 600 grit silicon carbide under water. The prepared dentin specimens were treated with control and experimental adhesives according to the following protocol[27]: the dentin is etched with 35% phosphoric acid gel for 15 s and rinsed with water; excess water is removed by gently air drying the surface with an air-water syringe, a procedure that allows the dentin surface to remain visibly moist. The adhesive is applied and polymerized for 20 s as described previously. The prepared specimens were stored for a minimum of 24 h in water at 25°C before sectioning. The treated dentin surfaces were sectioned perpendicular and parallel to the bonded surfaces using a water-cooled low-speed diamond saw. The specimens were prepared for scanning electron microscopic analysis using techniques published previously.[28,29] In brief, the sectioned specimens were treated with 5*N* HCl for 15 s, 5% NaOCl for 30 min and rinsed thoroughly with distilled water. The specimens were then dehydrated using a graded series of ethyl alcohol solutions, fixed in hexamethyldisilazane (HMDS) for 10 min and air dried in a fume hood overnight. Following drying, the specimens were mounted, gold-palladium coated, and examined at a variety of magnifications using a Field Emission Philips XL30 ESEM-FEG 515 (Philips Electron Optics, Hillsboro, OR) at 10 kV.

In vitro enzymatic degradation

PLE was used for biodegradation of the adhesives. Each resin formulation was polymerized as discs (4 mm diameter × 1 mm thickness) with visible light at 550 mW cm^{-2} light intensity. In sterile bottles, five discs with a total surface area of 2.0–2.4 cm^2 mL^{-1} (ISO 7405) were prewashed in sterile 0.05*M* phosphate buffer saline (PBS, pH 7.4) for three days to remove unreacted monomer. Following the prewash, the adhesive discs were incubated in 0.2*M* phosphate buffer (PB) with 30 U/mL PLE or in an identical control solution without enzyme at 37°C for up to eight days with shaking. Enzyme activity was maintained at >95% using the substrates and procedures recommended by the manufacturer. The solution was removed daily and replaced with fresh enzyme solution. The samples removed each day were centrifuged and the supernatant collected and analyzed for methacrylic acid (MAA) content by HPLC with UV-detection at 208 nm,[15] as described later. An enzyme-free solution at pH 7 and 37°C served as a negative control and a measure of the nonenzymatic hydrolysis of each material. The esterase resistance of experimental adhesives containing the new monomer (A0T and A8T) was compared to that of control adhesives (A0 and A8).

High performance liquid chromatography[15]

MAA release during enzyme-catalyzed hydrolysis of dentin adhesives was assayed by reverse phase HPLC using a 600E system controller, a 717 plus autosampler, a 484 tunable wavelength UV (208 nm) detector from Waters (Milford, MA). A Phenomenex Luna 5 μm C_{18} 4.6 × 250 mm (Phenomenex, Torrance, CA) column and security guard cartridge were used to isolate the products. The mobile phase was CH_3CN: 10 mM potassium phosphate buffer (80:20, v/v) at a flow rate of 1.0 mL/min. Under these conditions, MAA elution occurred at 2.2 min. MAA concentrations were determined by comparing peak areas with a calibration curve prepared using MAA standards of known concentration.

RESULTS AND DISCUSSION

Characterization of new monomer, TMPEDMA

The structure of the newly synthesized dimethacrylate monomer, TMPEDMA, was confirmed using FTIR, ^{1}H-NMR and ^{13}C-NMR spectroscopy (Figs. 1–3).

The characteristic FTIR peaks for the new methacrylate monomer are: 1718.5 cm^{-1} (C=O, strong, stretching), 1637.9 cm^{-1} (C=C, medium, stretching), 1158.4 cm^{-1} (C—O, strong stretching), 816 cm^{-1} (C=C, medium, twisting) (Fig. 1). Disappearance of the hydroxyl group at 3680–3100 cm^{-1} and appearance of the C=C stretching band at 1637.9 cm^{-1} confirmed the formation of the new methacrylate monomer. In the ^{1}H-NMR spectrum (Fig. 2), the chemical shifts of the TMPEDMA were (ppm): f and i, 6.0 and 5.5 (2H, —C*H*$_2$=C(CH_3)COO—); a, 5.8–5.7 (1H, CH_2=C*H*—CH_2O—); b, 5.2–5.0 (2H, C*H*$_2$=CH—CH_2O—); d, 4.1 (2H, —CH_2=C(CH_3)COOC*H*$_2$—); c, 3.9 (2H, CH_2=CH—C*H*$_2$O—); e, 3.3 (2H, CH_2=CH—CH_2OC*H*$_2$—); j, 1.9 (3H, —CH_2=C(C*H*$_3$)COO—); g, 1.4—1.5 (2H, CH_3C*H*$_2$C—); h, 0.8–0.9 (3H, C*H*$_3$$CH_2$C—). In ^{13}C NMR spectrum (Fig. 3), the chemical shifts of the TMPEDMA were (ppm): i, 166.9 (—CH_2=C(CH_3)*C*OO—); j, 136.2 (—CH_2=*C*(CH_3)COO—); a, 134.6 (CH_2=*C*H—CH_2O—); k, 125.5 (—*C*H$_2$=C(CH_3)COO—); b, 116.7 (CH_2=CH—CH_2O—); c, 72.3 (CH_2=CH—*C*H$_2$O—); d, 69.9 (CH_2=CH—CH_2O*C*H$_2$—); e, 64.7 (—CH_2=C(CH_3)COO*C*H$_2$—); f, 41.8 (CH_3CH_2*C*—); g, 23.2 (CH_3

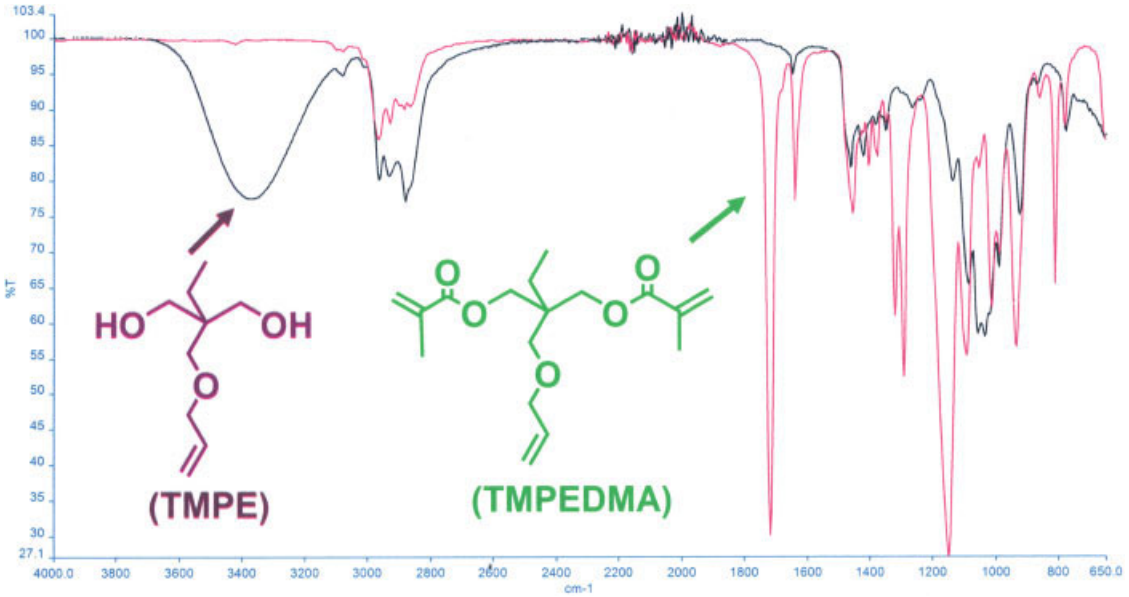


Figure 1 FTIR spectra of TMPE and TMPEDMA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

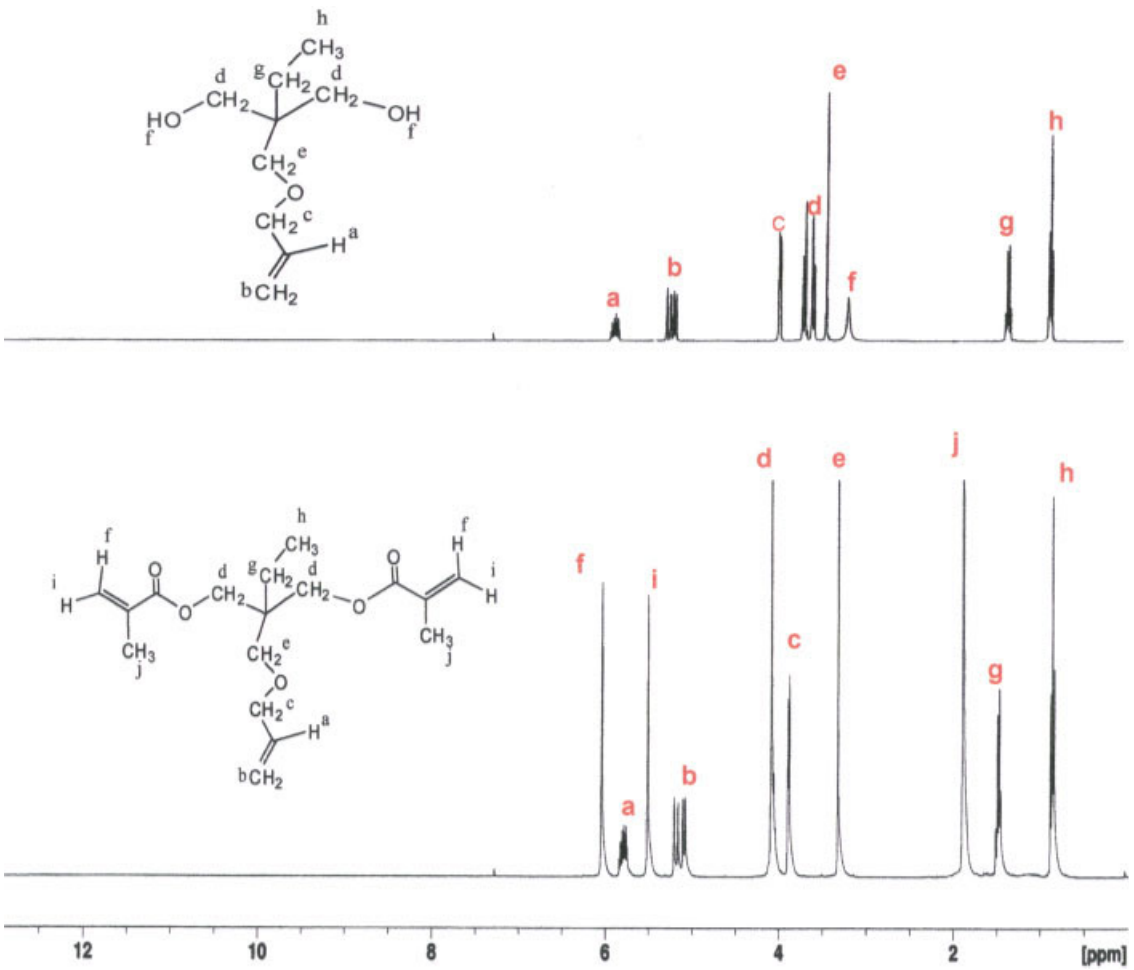


Figure 2 ^{1}H-NMR spectra of TMPE and TMPEDMA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

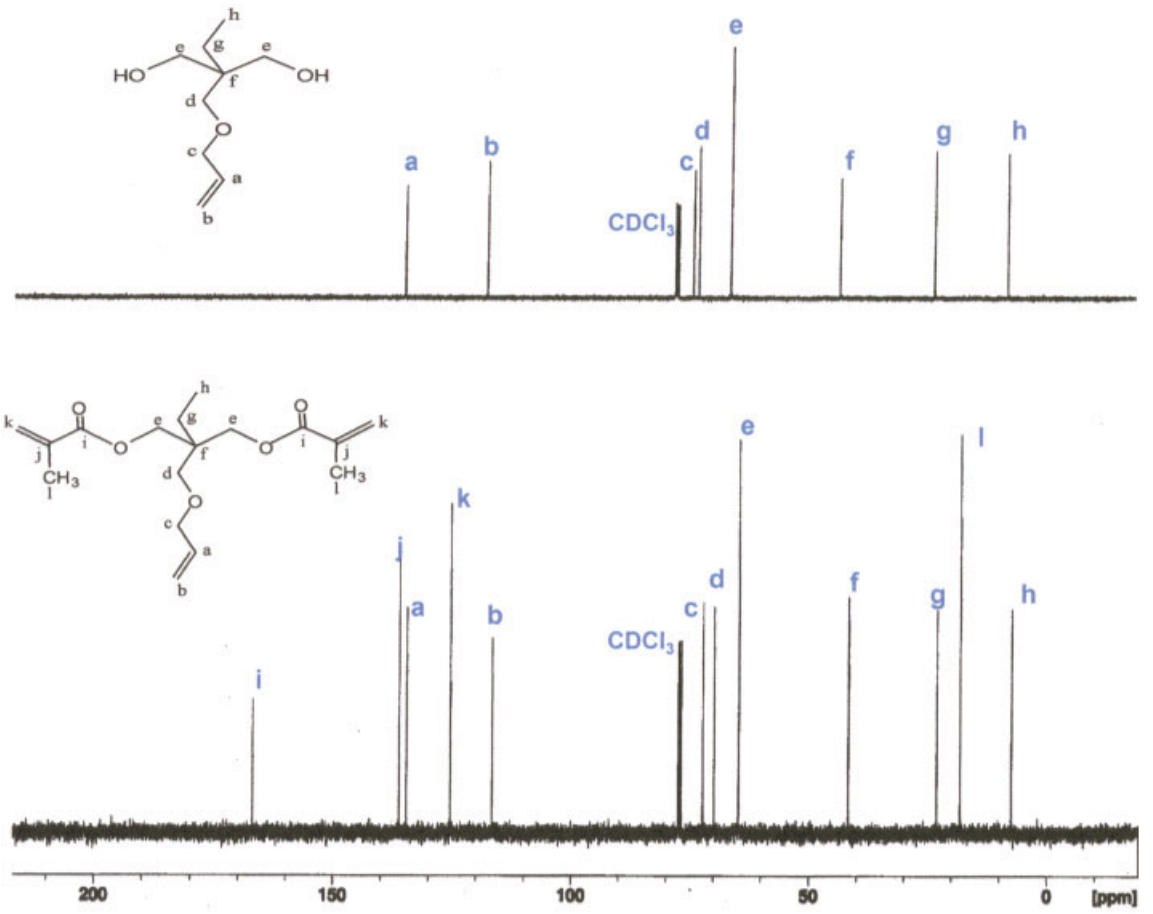


Figure 3 ^{13}C-NMR spectra of TMPE and TMPEDMA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CH_2C—); l, 18.2 (—CH_2=C(CH_3)COO—); h, 7.5 (CH_3CH_2C—). The methacrylate groups are supported by the presence of two singlets (δ = 6.0 and 5.5 ppm) on the ^{1}H-NMR spectrum and by the peaks at 136.2 and 125.5 ppm in the ^{13}C-NMR assignable to the double bond of a methacrylate group.

Polymerization and degree of conversion of adhesives cured in the presence of 0 and 8% water

Polymerization conversion was measured as a function of time for the control (A0 and A8) and experimental (A0T and A8T) adhesives using FTIR. Both control and experimental adhesives showed a final degree of conversion (DC) of 79–86% (Table I).

Samples polymerized in the presence of 8% water (A8 and A8T) showed a higher degree of conversion (85–86%) than those polymerized without water (A0 and A0T, ~ 79%), perhaps because of enhanced mobility of reactive species with lower viscosity. The results demonstrate that resins containing the new monomer can reach a degree of conversion comparable to the control adhesives in the presence and absence of water.

TABLE I
Modulus, Viscosity, and DC of Adhesive Resins

		Control adhesive		Experimental adhesive	
		A0	A8	A0T	A8T
Moduli (GPa)	Dry	1.68 (0.16)	1.57 (0.16)	2.03 (0.08)	1.85 (0.21)
	Wet	1.03 (0.11)	0.99 (0.10)	1.43 (0.17)	1.01 (0.12)
Viscosity (cP)[a]		195.2 (0.57)	84.3 (0.18)	35.9 (0.13)	28.9 (0.18)
DC (%)		79.3 (1.11)	86.2 (1.01)	79.4 (0.98)	85.3 (0.91)

[a] The viscosity of liquid resin prior to polymerization.

TABLE II
Mechanical Properties of Adhesive Resins as a Function of Concentration of Water

Sample	Adhesive formulation	Water content (wt %)	Storage and test condition	Toughness (MN/m^2)	Elongation (%)	Ultimate tensile strength (MPa)
A0	HEMA/BisGMA = 45/55	0	In air	5.73 (1.75)	0.12 (0.03)	70.78 (1.37)
			In water	3.61 (0.29)	0.13 (0.01)	41.43 (2.03)
A8	HEMA/BisGMA = 45/55 + 8% H_2O	8	In air	6.63 (1.75)	0.15 (0.02)	62.00 (3.47)
			In water	3.98 (0.86)	0.13 (0.02)	37.78 (1.80)
A0T	HEMA/BisGMA/TMPEDMA = 45/30/25	0	In air	10.32 (2.94)	0.17 (0.06)	69.93 (3.61)
			In water	6.25 (1.51)	0.19 (0.05)	40.79 (3.40)
A8T	HEMA/BisGMA/TMPEDMA = 45/30/25 + 8% H_2O	8	In air	7.41 (0.71)	0.20 (0.02)	62.21 (1.48)
			In water	4.30 (0.60)	0.16 (0.01)	36.29 (1.38)

Physical properties of control and experimental adhesives

The mechanical properties of the control (HEMA/BisGMA) and experimental (HEMA/BisGMA/TMPEDMA) adhesives were comparable (Table II).

The ultimate tensile strength (UTS) values for samples prepared without water (A0, A0T) were 71 ± 1 MPa and 70 ± 4 MPa, respectively, indicating that the control and experimental resins have identical tensile strength under dry conditions. The UTS decreased in samples cured in the presence of water i.e., the UTS values of control and experimental resins polymerized with 8 wt % water (samples A8, A8T) were 62 ± 3 MPa and 62± 2 MPa, respectively. UTS values for both control and experimental adhesives stored in water for 24 h were significantly lower ($P < 0.05$) than samples stored in air (Table II). The results were analyzed statistically using analysis of variance (ANOVA), together with Turkey's test at $\alpha = 0.05$. Elongation of the resins was not affected by type of storage or sample water content during polymerization (Table II). For the samples containing the new monomer (A0T and A8T), there is a minor increase in elongation relative to control (A0 and A8), and samples stored in water showed lower toughness than those stored in air. Toughness values for the experimental adhesives (A0T and A8T) under both wet and dry storage were somewhat greater than those of the controls (A0 and A8). This difference was significant ($P < 0.05$) for samples cured in the absence of water (A0T vs. A0). Moduli of the specimens are in the range of 1.01–2.03 MPa (Table I). For both adhesives, specimens stored in water exhibited lower moduli than dry samples stored in air, following the trend observed in the UTS and toughness tests. The experimental adhesives (A0T, A8T) stored under dry and wet conditions showed significantly greater moduli ($P < 0.05$) than those of control (A0, A8), with the exception of the sample cured at 8% water and stored in water (A8T). Dry experimental specimens cured with 0% water (A0T) showed the highest modulus (2.03 ± 0.08 MPa) of all the groups. Since material properties such as modulus and glass transition temperature are directly related to the number of crosslinks,[30] the higher moduli of the newly formulated adhesive (A0T and A8T) in this work may be attributed to: (i) the trifunctional nature of the new monomer, which provides more polymerization sites than either HEMA or BisGMA on a molar basis and may contribute to increased crosslink density and (ii) the lower molecular weight of the new monomer, which provides a higher concentration of cross-linking monomer per gram than BisGMA, again increasing crosslink density.

The results suggest that inclusion of the new monomer, a branched dimethacrylate containing a reactive vinyl group, leads to a more highly crosslinked network when copolymerized with HEMA and BisGMA. It is well known that free radical polymerization of multifunctional monomers produces pendant double bonds on the growing polymer chains. These pendant double bonds can react with propagating radicals by three different reaction mechanisms[31,32]: primary cyclization (in which the macroradical attacks the pendant double bond in the same chain), secondary cyclization (in which the radical attacks pendant double bonds on other chains already incorporated in the network), and intermolecular crosslinking. Primary cyclization causes small loops to be formed in the network, resulting in microgels and heterogeneity in the cured polymer. These cyclization reactions do not contribute significantly to the crosslinked net-

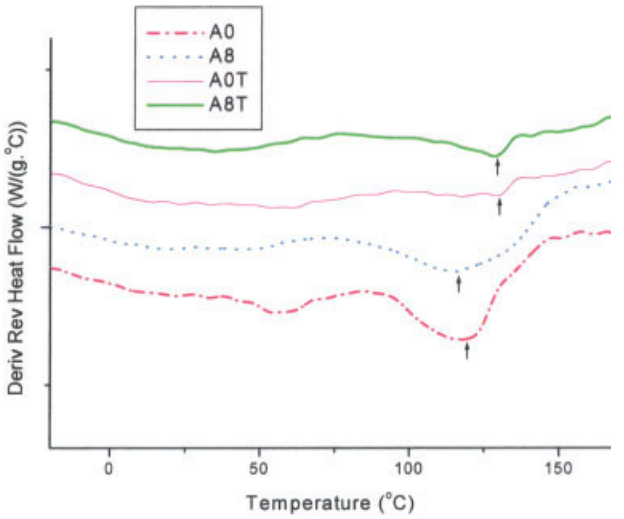


Figure 4 Representative T_g curves of control (A0, A8) and experimental (A0T, A8T) dentin adhesives cured in the presence of 0% (A0, A0T) and 8% (A8, A8T) water. Arrows indicate assignment of the T_g value as the peak in the derivative of reversible heat flow. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

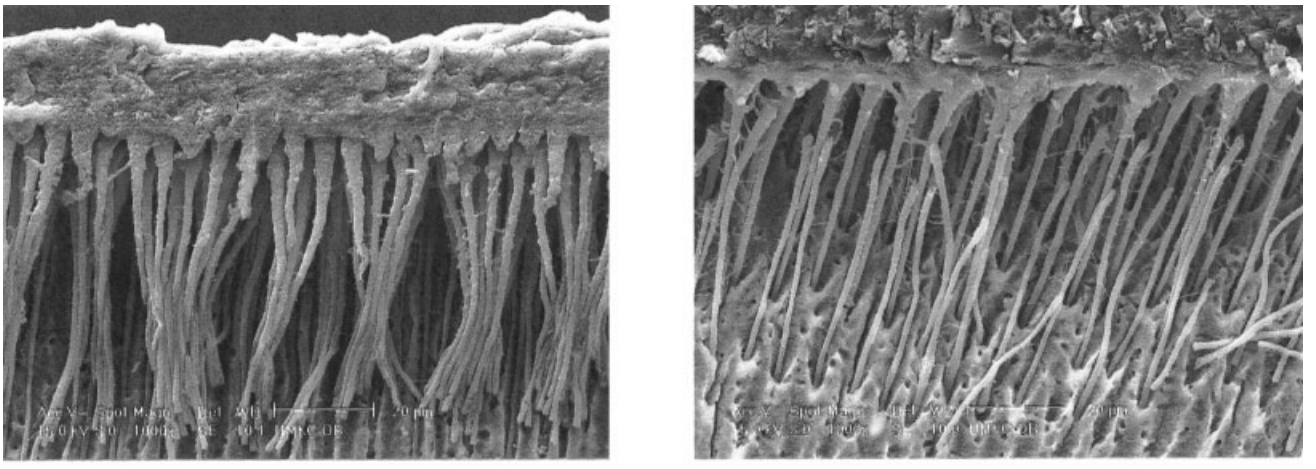
work. In contrast, intermolecular crosslinking reactions, which are responsible for the aggregation of the microgels, lead to the formation of the so-called ''macrogel'' and contribute to the overall strength of the network. Adding a solvent like water to the adhesive formulation may increase the probability of primary cyclization because of the dilution of the monomer. Primary cyclization may also be favored by the reduced rate of polymerization, which causes the radical to remain in close proximity to pendant double bonds on the same chain for longer times.

Thermal, viscous, and microscopic evaluation of control and experimental adhesives

T_g values were clearly observed for control resins cured in the absence (A0) and presence (A8) of water at 118.7°C and 115.7°C, respectively (Fig. 4). Adhesives containing the new monomer (A0T and A8T) showed somewhat greater T_g values of 130.8°C and 128.9°C, respectively. The higher T_g values of the experimental adhesives as compared to the controls are consistent with the formation of a more highly crosslinked network, as discussed earlier.

Viscosity is of practical importance for dental adhesives, since less viscous formulations are generally more desirable because of their ease of application and their ability to infiltrate the demineralized dentin matrix. The viscosity of monomer solutions decreased in the order (cp): A0 (195.2) > A8 (84.3) > A0T (35.9) > A8T (28.9) (Table I). As expected, the viscosities of monomer solutions diluted with 8% water were less than those without water. The viscosities of solutions corresponding to control resins (A0 and A8) were greater than those for monomer solutions of the experimental resins (A0T and A8T). The higher viscosities of the controls can be attributed to strong hydrogen bonding because of the two pendant hydroxyl groups of BisGMA, which is present in greater concentration in the control formulations.

It is generally accepted that the primary factors critical in determining an adequate adhesive/dentin bond are wetting of the dentin substrate by components of the adhesive system and micromechanical interlocking via resin infiltration and entanglement of exposed collagen fibrils in the demineralized dentin.[33–35] Both control and experimental adhesives showed numerous resin tags formed by the polymerization of monomers that penetrated into the dentinal tubules, indicative of good resin penetration (Fig. 5).



(a) (b)

Figure 5 Representative SEM micrographs of the dentin interfaces with experimental (a) and control (b) adhesives.

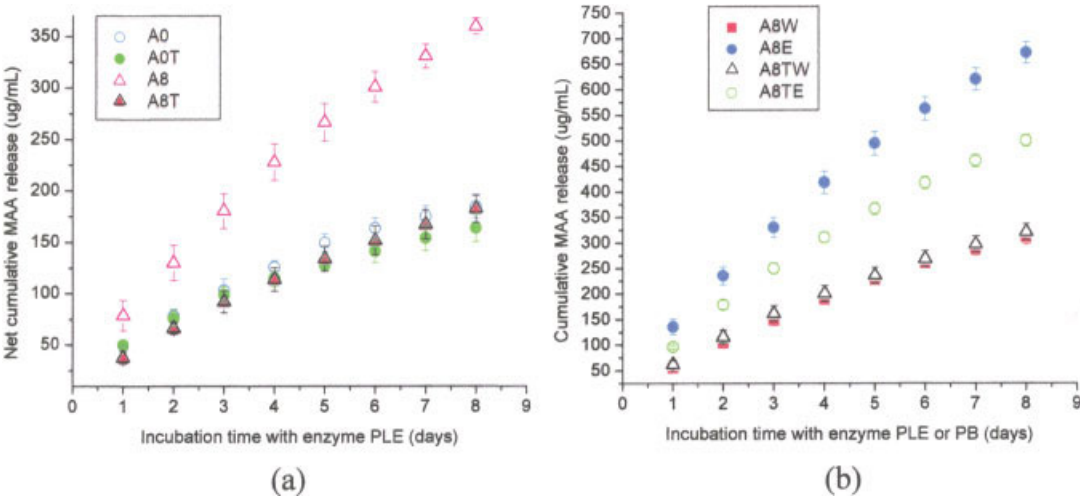


Figure 6 Net cumulative MAA (a) and cumulative MAA (b) release from dentin adhesives as a function of incubation time on exposure to esterase for control formulations and experimental formulations containing TMPEDMA. Symbols: A0, control formulation polymerized at 0% water; A0T, experimental (''test'') formulation polymerized at 0% water; A8, control formulation polymerized at 8% water; A8T, experimental (''test'') formulation polymerized at 8% water; A8W, adhesive A8 incubated in water; A8E, adhesive A8 incubated in enzyme solution; A8TW, adhesive A8T incubated in water; A8TE, adhesive A8T incubated in enzyme solution. $N = 3 \pm$ S.D. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

There were no marked differences in the control and experimental adhesives on SEM evaluation.

Enzymatic biodegradation of adhesive formulations

Methacrylate dental adhesives contain numerous ester bonds that are subject to chemical and/or enzymatic hydrolysis. Previous investigations have demonstrated that human saliva contains sufficient esterase activity to catalyze the degradation of methacrylate monomers.[36] While the enzymatic breakdown of dental composites has been investigated,[14] to our knowledge this is the first study to examine the stability of adhesives under esterase challenge. This study tested the effect of enzyme-exposure on the release of MAA from adhesives formulated in water to simulate wet bonding conditions. MAA release from the control adhesive was compared to the adhesive containing a new monomer, TMPEDMA (25 wt %). Figure 6(a) shows the net cumulative MAA release [$MAA_{(in\ PLE)} - MAA_{(in\ PB)}$] from adhesives after incubation with PLE for 8 days. The net cumulative release of MAA was similar for A0, and A0T adhesives [Fig. 6(a)].

As shown in Figure 6(a), the net cumulative MAA release in adhesives formulated with the new monomer and 8% water (A8T: 182 μg/mL) was dramatically ($P < 0.05$) decreased and approximately one-half the amount released from the control (A8: 361.6 μg/mL). Furthermore, after 8-days PLE incubation, the adhesive formulated with the new monomer shows similar MAA release irrespective of whether the material is cured in the presence (8%) or absence of water. As shown in Figure 6(b), the cumulative MAA release (A8E = 675.2 μg/mL; A8TE = 503.6 μg/mL) following 8 days of exposure to the enzyme was greater than in PB (A8W = 321.1 μg/mL; A8TW = 313.5 μg/mL), indicating that degradation of adhesive is facilitated by enzyme. These results suggest that adhesives formulated with the new monomer and cured in water to simulate wet bonding are more resistant to esterase. The improved esterase resistance of the new adhesive may be attributable to the increased crosslink density and to the steric hindrance of branched alkyl side chain of new monomer. BisGMA has a relatively unhindered ester bond as compared to the new monomer. BisGMA has two pendant hydroxyl groups, which are responsible for the high water sorption, and may increase its susceptibility to hydrolytic degradation. In contrast, the branched alkyl side chain of new monomer could mask the ester groups against water and enzyme. Although the new adhesive showed a degree of conversion similar to control, the physical properties of light-cured polymers made from HEMA, BisGMA and TMPEDMA showed that the inclusion of TMPEDMA seemed to create a denser polymer network with higher modulus and T_g. The new monomer has a trivinyl group that can contribute to the cross-linking reaction, and adhesives containing this monomer have a higher concentration of active double bonds than the control formulation. One way to measure the cross-linking density is to determine the molecular weight between cross-links, M_c [eq. (1)]. The number-average molecular weight between cross-links is defined as the density, ρ (total weight

of polymer/volume), divided by the concentration of cross-linked chains, ν[37]:

$$M_c = \rho/\nu \quad (1)$$

The theoretical M_c can be calculated for an ideal crosslinked polymer with complete conversion and no cyclization. As shown in Eq. (2), the theoretical concentration of cross-linked chains equals the number of double bonds (nd) of the crosslinking agent times the concentration of crosslinking molecules, $[M_x]$.[37]

$$\nu = nd[M_x] \quad (2)$$

The new monomer used in this work has a higher number of double bonds. It also has a higher concentration of crosslinking molecules because the molecular weight between double bonds on the crosslinking molecule, TMPEDMA, is less than that of BisGMA. Thus, adhesives containing the new monomer may produce more highly crosslinked networks as compared to the control.

CONCLUSIONS

A new dimethacrylate monomer with a branched side chain was synthesized and used as a comonomer in dentin adhesives. The new experimental adhesives showed a degree of double bond conversion comparable with the control and improved modulus of elasticity and glass transition temperature. In addition, the new adhesive showed good penetration into the dentin surface and a uniform hybrid layer. On exposure to porcine liver esterase, the net cumulative MAA release from the new adhesive polymerized in the presence of 8% water (A8T) was dramatically decreased. This improved esterase resistance may be due to a reduction of enzymatic hydrolysis due to steric hindrance of the branched alkyl side chain and/or increased crosslink density of the experimental resin.

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