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Dynamic mechanical analysis and esterase degradation of dentin adhesives containing a branched methacrylate

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Abstract

A study of the dynamic mechanical properties and the enzymatic degradation of new dentin adhesives containing a multifunctional methacrylate are described. Adhesives contained 2-hydroxyethyl methacrylate (HEMA), 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]-propane (BisGMA), and a new multifunctional methacrylate with a branched side chain-trimethylolpropane mono allyl ether dimethacrylate (TMPEDMA). Adhesives were photopolymerized in the presence of 0, 8 and 16 wt% water to simulate wet bonding conditions in the mouth and compared to control adhesives. The degree of conversion as a function of irradiation time was comparable for experimental and control adhesives. In dynamic mechanical analysis (DMA), broad $\tan \delta$ peaks were obtained for all samples, indicating that the polymerized networks are heterogeneous; comparison of the full-width-at-half-maximum values obtained from the $\tan \delta$ curves indicated increased heterogeneity for samples cured in the presence of water and/or containing TMPEDMA. The experimental adhesive showed higher T_g and higher rubbery modulus indicating increased crosslink density as compared to the control. The improvement in esterase resistance afforded by adhesives containing the TMPEDMA is greater when this material is photopolymerized in the presence of water, suggesting better performance in the moist environment of the mouth. The improved esterase resistance of the new adhesive could be explained in terms of the densely crosslinked network structure and/or the steric hindrance of branched alkyl side chains.

Keywords

Photopolymerization; dentin adhesives; degree of conversion; enzymatic degradation; dynamic mechanical analysis

INTRODUCTION

Photopolymerization uses light to initiate and propagate a polymerization reaction to produce a linear or networked polymer structure. This technique has been widely used in drug delivery,¹ tissue engineering,² cell encapsulation,³ contact lenses,⁴ and dental restorative materials.^{4–7} Of particular interest here is the use of photopolymerizable methacrylate polymers as dentin adhesives, which serve to bind a composite resin to the underlying tooth structure in dental restorations. Photopolymerizable biomaterials such as methacrylates are especially advantageous as dentin adhesives due to flexibility in material

design and ease of handling. However, since methacrylate-based polymer networks have numerous ester groups that are subject to chemical and enzymatic hydrolysis in the oral cavity, degradation may limit the lifetime of the restoration. For example, while the typical lifetime of traditional mercury-containing dental amalgam restorations is 10–20 years, the lifetime for methacrylate-based restorations is about 8 years in anterior sites and as little as 2–4 years in posterior sites.^{8,9} Despite this limitation, light curable dental composite restorations have undergone a steady increase in use over the past decade due to their superior esthetics and the public's concern regarding the release of mercury from dental amalgam.

Water present in the mouth is a major interfering factor when bonding adhesives and/or composites to the tooth.¹⁰ The water content of the dentin surface varies as a function of depth,¹¹ the nature of the substrate (i.e. caries-affected or healthy dentin)¹² and the presence of residual rinse water. Under *in vivo* conditions, there is little control over the amount of water left on the tooth during dentin bonding. In the presence of water, methacrylate adhesives may undergo phase separation during photopolymerization, which leads to very limited infiltration of BisGMA, the critical dimethacrylate component.¹³ Adhesive phase separation inhibits the formation of an impervious, structurally integrated bond at the composite/tooth interface.^{14,15}

Water is also known to facilitate the chemical degradation of adhesives, and may be trapped within the matrix during photopolymerization or can enter the adhesive matrix by diffusion into the loosely cross-linked or hydrophilic HEMA-rich domains. A poorly polymerized adhesive does not attain its desired mechanical properties and is subject to ingress by oral fluids, and so may degrade rapidly in the moist oral environment.¹⁴ Under these conditions, the adhesive/dentin bond has limited structural integrity and durability.

In addition to the deleterious effects of water on dentin adhesives, human saliva contains enzymes which may participate in adhesive degradation.^{16,17} Since each methacrylate functional group contributes an ester bond to the polymerized network, methacrylate-based composites and adhesives are particularly susceptible to attack by salivary esterases. Esterase-catalyzed degradation of methacrylate-based dental materials has been documented in solution,^{18,19} in saliva samples,^{20,21} and *in vivo*.¹⁶

Traditionally, the mechanical properties of dental materials have been investigated using static tests. However these methods are not always well suited for measuring the complete material deformation and stiffness properties. This is particularly true for materials which show viscoelastic behaviour under load.²² It is now common in materials science to use dynamic methods to assess the mechanical properties of polymeric materials.²³ Dynamic tests such as dynamic mechanical analysis (DMA) are particularly well suited for viscoelastic materials, since they can determine both the elastic and viscous responses of the sample in one experiment. Since dental restorative materials are subjected to dynamic loading rather than static loading in the mouth, dynamic tests have become increasingly relevant.²⁴ Dynamic methods are often preferred²⁵ as they better mimic the cyclic masticatory loading to which these materials are subjected clinically. For this reason, dynamic mechanical measurements have been used in this study to analyze the dentin adhesives and provide information regarding their structural heterogeneity.

In our previous work, we reported the synthesis and characterization of a new monomer, trimethylolpropane mono allyl ether dimethacrylate (TMPEDMA) and evaluated the basic properties of dentin adhesives containing this new monomer to gain perspective on its potential use as a co-monomer in dentin adhesive.²⁶ The studies presented here further investigate the performance of dentin adhesives containing TMPEDMA, with particular

emphasis on the effects of water used during photopolymerization on the structure and properties of the crosslinked networks. The hypothesis of this investigation was that under simulated wet bonding conditions the experimental adhesive, which contains a newly synthesized methacrylate co-monomer, TMPEDMA, that provides high functionality with a branched side chain and low molecular weight between crosslinks, would have higher crosslink density and greater esterase resistance as compared to bisGMA/HEMA controls.

MATERIALS AND METHODS

Materials

The dentin adhesives were prepared by the polymerization of mixtures of methacrylate monomers. The mixtures included two monomers commonly found in commercial dentin adhesives, 2-hydroxyethylmethacrylate (HEMA, Acros Organics, NJ) and 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]-propane (BisGMA, Polysciences, Warrington, PA), and a new trifunctional monomer, trimethylolpropane mono allyl ether dimethacrylate (TMPEDMA) (TABLE I).

BisGMA and HEMA were used as received without further purification. The new monomer (TMPEDMA) has been recently synthesized in our laboratories.²⁶ The control adhesive formulation was composed of BisGMA and HEMA with a mass ratio of 55/45 and formulated with 0 wt% (A0), 8 wt% (A8) and 16 wt% (A16) water to simulate wet bonding in the mouth. This control was compared to experimental adhesive formulations, HEMA/BisGMA/TMPEDMA=45/30/25 w/w ratio, which were also formulated with 0 wt% (A0T), 8 wt% (A8T) and 16 wt% (A16T) water. The synthesized dimethacrylate monomer, TMPEDMA, was used as a co-monomer in the experimental adhesive. Camphorquinone (CQ) and ethyl-4-(dimethylamino)benzoate (EDMAB) were obtained from Aldrich (Milwaukee, WI, USA) and used as photoinitiators without further purification. Shaking was required to yield well-mixed monomer/initiator mixtures. Porcine liver esterase (PLE, EC 3.1.1.1) was obtained from Sigma Chemical Co., St. Louis, USA. All other chemicals were reagent grade and used without further purification.

Specimen preparation, degree of conversion, and curing time

Two types of specimens were prepared. Pellet specimen discs, 4 mm diameter and 1 mm thick, were fabricated in an aluminum mold for enzymatic biodegradation studies. The resin solution was placed into the mold and covered with a transparent plastic film. Pressure was applied to extrude excess resin. Rectangular beam specimens (1×1×11 mm³) cured in a glass-tubing mold (Wilma Labglass, #LG-25001-100, Standard wall borosilicate tubing) were used for the determination of dynamic mechanical properties and degree of conversion (DC). The control and experimental adhesives were light-polymerized for 40 s at room temperature at a distance of 1 mm with a commercial visible-light-curing unit, (Spectrum® 800, Dentsply, Milford, DE, USA) at an intensity of 550 mW cm⁻², according to techniques published previously.^{6,7} The diameter of the cure tip was 9 mm and the intensity of the light-source was checked with a visible curing light meter (Cure Rite, DENSPLY Caulk, DE, USA) before the start of each experiment.

The DC of the methacrylate double bond was determined by using a LabRAM ARAMIS Raman spectrometer (LabRAM HORIBA Jobin Yvon, Edison, New Jersey) with a HeNe laser ($\lambda=633$ nm, a laser power of 17 mW) as an excitation source. The instrument conditions were: 200 μ m confocal hole, 150 μ m wide entrance slit, 600 gr/mm grating, and 10 × objective Olympus lens. Data processing was performed using LabSPEC 5 (HORIBA Jobin Yvon). The samples were mounted in a computer-controlled, high-precision x-y stage. To determine the DC of the samples, spectra of the unpolymerized resin mixtures and beam

samples (7 days dry storage in vacuum dry oven with dry agent at room temperature after photopolymerization) were acquired over a range of 700 – 1800 cm^{-1} . The change of the band height ratios of the aliphatic carbon-to-carbon double bond ($\text{C}=\text{C}$) peak at 1640 cm^{-1} and the aromatic $\text{C}=\text{C}$ at 1610 cm^{-1} (phenyl) in both the cured and uncured states was monitored and DC27:28 was calculated by using the following equation based on the decrease in the intensity band ratios before and after light curing.

$$DC(\%) = [1 - (R_{\text{cured}}/R_{\text{uncured}})] \times 100,$$

where R = band height at 1640 cm^{-1} /band height at 1610 cm^{-1} .

Curing time was evaluated by inserting a metal rod into the center of the adhesive resin immediately after placing the material into a two-end open glass tubing.²⁹ The curing time was taken as the period from which the light exposure was initiated to the moment at which the metal rod could not be moved by hand. The average was obtained from three readings.

Dynamic mechanical analysis (DMA) and mass loss

DMA is a thermo-mechanical analysis technique that may be used to characterize the rheological properties of a wide range of sample types as they are subjected to periodic loading under a range of temperatures. In DMA, a sinusoidal stress is applied and the resultant strain is measured. The properties measured under this oscillating loading are storage modulus, loss modulus, and $\tan \delta$ (ratio of loss to storage modulus). The storage modulus (E') represents the stiffness of a viscoelastic material and is proportional to the energy stored during a loading cycle. The loss modulus (E'') is related to the amount of energy lost due to viscous flow. The ratio of loss (E'') to storage modulus (E') is referred to as the mechanical damping, or $\tan \delta$. In this study, DMA tests were performed using a TA Instruments Q800 with a three-point bending clamp. The temperature range was from -20 to 200 $^{\circ}\text{C}$ with a ramping rate of 3 $^{\circ}\text{C}/\text{min}$ at a frequency of 1 Hz. No pre-heating cycle was applied, and the loss and storage moduli and $\tan \delta$ were recorded as a function of temperature. The $\tan \delta$ value goes through a maximum as the polymer undergoes the transition from the glassy to the rubbery state. The glass transition temperature (T_g) was determined as the position of the maximum on the $\tan \delta$ vs. temperature plot.

Rectangular beam specimens ($1 \times 1 \times 1 \text{ mm}^3$) were used for DMA measurement. To ensure complete postpolymerization, the polymerized samples were stored in the dark at room temperature for 2 days and then for 1 week in a vacuum oven in the presence of a drying agent. Mass loss (expressed as weight %) was calculated as the difference between the dry mass of the resin specimen before and after DMA test.

The results were analyzed statistically using analysis of variance (ANOVA), together with Tukey's test at $\alpha=0.05$ (Microcal Origin Version 6.0, Microcal Software Inc., Northampton, MA).

Enzyme solution preparation

The enzyme solution was prepared by dissolving porcine liver esterase (PLE, EC 3.1.1.1., Sigma E3019) at the required concentrations in 0.05 M phosphate-buffered saline (Dulbecco's phosphate-buffered saline). All solutions were sterile filtered using a 0.22 μm filter (MillexTM-GP 0.22 - μm filter unit; Millipore). PLE activity was assayed by test procedures provided by the manufacturer. The assay confirmed an activity of 27 units/mg of PLE powder as supplied. One unit of PLE catalyzed the hydrolysis of 1.0 mmol of ethyl butyrate to butyric acid and ethanol per minute at pH 8.0 at 25 $^{\circ}\text{C}$.

Enzymatic degradation and analysis of MAA

Adhesive discs were prepared as described above and exposed to PLE in triplicate sample groups to allow for statistical analysis. Five adhesive discs with an initial mass of ~100 mg and with a surface area of ~2.0 cm²/mL were placed in sterile vials and pre-washed in sterile 0.05M phosphate buffered saline (PBS) at pH 7.4 for three days to remove un-reacted monomer. Following the pre-wash, the adhesive discs were incubated in 1 mL buffer solution with/without 30 U/mL PLE at 37 °C for up to eight days with shaking. Enzyme activity was maintained at >90% using the substrates and procedures recommended by the manufacturer. The solution was removed daily and replaced with fresh enzyme solution. Solution samples removed each day were centrifuged and the supernatant collected and analyzed for methacrylic acid content (MAA) 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).¹⁹ An enzyme-free solution at pH 7 and 37 °C served as a negative control and a measure of the non-enzymatic hydrolysis of each material. A Phenomenex Luna 5 µm C₁₈ 4.6 × 250 mm (Phenomenex, Torrance, CA) column and security guard cartridge were used to isolate the products. The mobile phase was CH₃CN: 10 mM potassium phosphate buffer (80:20, v/v) at a flow rate of 1.0 mL/min. Under these conditions, the retention time of the HPLC peak for a standard solution of methacrylic acid (MAA) was 2.2 minutes. MAA concentrations were determined by comparing peak areas with a calibration curve prepared using MAA standards of 50, 100, 250, 500, and 1000 µM concentration.

RESULTS

Adhesive resin solution

The appearance of adhesive resin solutions formulated in the absence and presence of water is shown in Figure. 1. Resin solutions containing 0 wt% (A0 and A0T) and 8 wt% (A8 and A8T) water were transparent and homogeneous in appearance. In contrast, resin solutions containing 16 wt% water (A16 and A16T) were opaque, indicating macro-phase separation. All the resin solutions tested were yellow in color, due to the use of CQ as a photoinitiator (Figure 1).

Degree of conversion and curing time of adhesives

The presence of un-polymerized double bonds remaining in the adhesive during photopolymerization is shown by the peak height at 1640 cm⁻¹ in the Raman spectrum, which corresponds to the C=C stretching of methacrylate. The peak at 1610 cm⁻¹ assigned to aromatic C=C was used as an internal standard. The peak height ratios of the two bands at 1640 cm⁻¹ and 1610 cm⁻¹ were compared to determine the DC of the methacrylate C=C bonds. Table II shows the DC and curing time of the adhesive resins. Both control and experimental adhesives showed a final DC of 82–90 %. Samples polymerized in the presence of water (A8, A16, A8T, and A16T) showed a higher DC (85–90 %) than those polymerized without water, which may due to enhanced mobility of reactive species. The curing time indicates that samples cured significantly faster in the absence of water. The results demonstrate that the experimental adhesive achieves a degree of conversion comparable to the control with a similar curing time.

DMA results

Figure 2 shows the tangent δ curves as a function of temperature for the control and experimental adhesives cured in the absence and presence of water. The T_g values of control adhesives were in the range of 130 ~ 143 °C and decreased with increasing water content in the matrix. In contrast, the T_g values of experimental adhesives were higher (147 to 148 °C)

and the presence of water had minimal influence. As shown in Figure 2 and Table III, the height of $\tan \delta$ peak of adhesives containing 0% water was 0.65 for A0 and 0.46 for A0T, respectively, which is less than those of their corresponding adhesives containing 8 % (0.69 for A8 and 0.53 for A8T) and 16 % (0.67 for A16 and 0.53 for A16T) water. In addition, experimental adhesives showed lower $\tan \delta$ peak heights than those of the control. Based on the $\tan \delta$ curves, both the experimental and control adhesives show increased full-width-at-half-maximum values as the water content increases. As shown in Table II, mass loss values for both control and experimental adhesives ranged from 0.9 – 1.0 wt%.

Figure 3 shows the storage modulus(E') as a function of temperature for the control and experimental adhesives. The storage modulus values for both polymer networks at 25 °C are in the range of 4.4 ~ 2.6 GPa and decrease with increasing temperature, reaching the range of 0.014 ~ 0.054 GPa in the rubbery state. Storage modulus also decreased with increasing water content. The experimental polymer networks (A0T, A8T, A16T) showed higher storage modulus in the rubbery state than the controls (A0, A8, A16), regardless of the presence or absence of water. A0T exhibits the highest values for E' in the overall region of the curve.

Figure 4 shows the inverse ratio of the modulus in the rubbery region to temperature at which the modulus was measured (ζ) as a function of water content (%) in the polymer networks. The value of ζ is generally considered to be inversely correlated to the crosslink density of the network, with higher ζ values corresponding to lower crosslink density.³⁰ The lowest ζ values are observed for A0 and A0T photocured in the absence of water, suggesting higher crosslink density in these resins. As the water content in the resin mixture is increased, ζ increases. Interestingly, the control adhesives (A0, A8, A16) showed higher ζ values than those of the experimental adhesives (A0T, A8T, A16), indicating a greater crosslink density in the experimental adhesives. This difference is greatest at the highest water content (16%)

Enzymatic biodegradation of adhesive resins

Figure 5 shows the net cumulative MAA release [$\text{MAA}_{(\text{in PLE})} - \text{MAA}_{(\text{in PB})}$] from control and experimental adhesive resins cured in the absence or the presence of 8 wt% or 16 wt% water (A0, A0T, A8, A8T, A16, and A16T) after incubation with PLE esterase for 8 days. No significant difference in MAA release was observed between control and experimental adhesives formulated in the absence of water (A0 vs. A0T) throughout the eight-day exposure period. In contrast, for adhesives formulated in the presence of 8 wt% and 16 wt% water, the net cumulative MAA release from the experimental adhesives (A8T=182 $\mu\text{g/mL}$ and A16T=205 $\mu\text{g/mL}$) was significantly less than from the control (A8=362 $\mu\text{g/mL}$ and A16=698 $\mu\text{g/mL}$) (Fig. 5, $P < 0.05$), indicating that the new experimental adhesive has greater esterase resistance than conventional adhesives when both are photopolymerized under conditions that simulate wet bonding in the mouth.

After 8-days of PLE incubation, increasing water content in the resin from 0% to 16% increased significantly the net cumulative MAA release for control adhesives. The experimental adhesives formulated with the new monomer showed a slight increase in MAA release (A0T=165 $\mu\text{g/mL}$, A8T=182 $\mu\text{g/mL}$ and A16T=205 $\mu\text{g/mL}$) with increasing water, but values were comparable regardless of whether the material was cured in the presence or absence of water.

DISCUSSION

The control adhesive resins used in this work consisted of a mixture of HEMA and Bis-GMA with a mass ratio of 45/55, a composition comparable to that of commercial dentin

adhesives.³¹ Commercial adhesives were not used, since differences in filler type and content, additives and processing conditions by the various manufacturers may influence results and adversely affect reproducibility. The controls were formulated with water to simulate wet bonding conditions in the mouth and to allow for possible phase separation of the adhesive during photopolymerization.

During acid etching, the mineral phase is extracted from a zone that measures between 1 and ~10 μm of the dentin surface.³² The composition of the exposed substrate differs radically from mineralized dentin. For example, mineralized dentin is 50% mineral, 30% collagen, and 20% water by volume,³³ whereas demineralized dentin is 30% collagen and 70% water. Water is a major interfering factor when bonding adhesive and/or composites to the tooth.¹⁰ This relationship was the basis for our investigations of the behavior of the adhesive in the presence of water. In this study, the water concentration values (0, 8 and 16 wt%) were selected on the basis of our previous work.^{35–37} Adhesive resins containing 0 wt% (A0 and A0T) and 8 wt% (A8 and A8T) water formed transparent solutions that were homogeneous in appearance, while those with 16 wt% water (A16 and A16T) were opaque and heterogeneous with visible macro-phase separation. According to our previously published phase diagram,¹³ when the water content is greater than 8 wt%, there is macro-phase separation in the adhesive resins. Here, it was confirmed that the resin was transparent in 8 wt% water, but visually turbid in 16 wt% water. The water concentration of 16 wt% used here is thus greater than that required for visible macro-phase separation in HEMA/BisGMA formulations with a mass ratio of 45/55.

Many studies have shown that free radical polymerization of multifunctional methacrylates does not result in the complete conversion of double bonds. This incomplete conversion is due in part to a reduction in mobility of the propagating free radicals and of the partially and fully polymerized methacrylate macromolecules as the reaction progresses. Most dental resins undergo between 40% and 85% conversion.³⁸ The extent of cure influences their bulk physical properties and may also contribute to their susceptibility to enzymatic degradation.^{9,10} In this study, we used Raman spectroscopy to determine the DC of rectangular beam specimens of photopolymerized methacrylates. Of the two available spectroscopic techniques (i.e., FTIR, Raman), infrared spectroscopy is the most commonly used, but during the past 10 year the use of FT-Raman spectroscopy has increased.^{27,39,40} A major advantage with FT-Raman spectroscopy is that the test rectangular beam specimen is not in contact with an attenuated total reflection (ATR) crystal as in the case with FTIR. In addition, the infrared intensity of the C=C stretching band is only medium-strong at best, while Raman gives a much more distinctive band which is high in intensity. The molecular vibrational frequencies observed by both methods are nearly the same, but the vibrational band intensities differ, because intensities in FT-IR are determined by changes in the dipole moments of the vibrations, whereas for Raman measurements, the relevant quantity is the change in the polarizability tensor.

In this study, the DCs obtained by Raman spectroscopy are in the range of 82 ~ 90 %, which is relatively high. Although the DC for the experimental adhesive (A0T) photocured in the absence of water was slightly lower than that of the corresponding control, experimental adhesives cured in the presence of water exhibited similar or higher DCs than those of controls. DCs for both types of samples increased in the presence of water, which may be due to enhanced mobility of reactive species as a result of lower viscosity with the dilution in water.

Because DMA gives information on the relaxation of molecular motions which are sensitive to the structures and variations in the stiffness of materials, it may be used to provide information on the properties of polymer networks. DMA can be used to determine storage

modulus, glass transition temperature and assess structural heterogeneity. It is particularly suitable for determining glass transitions because the change in modulus is much more pronounced in DMA than, for example, the c_p change in a DSC measurement. Because storage after initial light activation and polymerization significantly affects the dynamic physical properties,⁴¹ the DMA specimens used in this study were stored at room temperature for 2 days in the dark and then dried for 1 week prior to evaluation. The results of mass loss for the adhesives may be associated with impurities, e.g. additives, that were present in the commercial monomers. No difference in mass loss between adhesives cured in the absence of water and adhesives cured in the presence of water indicates that there is little residual water in the polymer matrix after all specimens were dried in a vacuum oven. Further studies should be performed to examine water sorption and solubility and how these characteristics relate to the dynamic mechanical properties of these adhesives following immersion in water.

The widths of the $\tan \delta$ curves [Figure 2(a,b)] indicate that the glass transition occurs over a wide range of temperature rather than at a specific temperature. Further, the temperature range is more extended in the case of adhesives cured in the presence of water. This broad glass transition can be attributed to the fact that the polymerization of multifunctional monomers produces networks with highly heterogeneous environments, containing both highly crosslinked regions and less densely crosslinked regions.⁴² Such an inhomogeneous distribution of environments results in a very broad distribution of mobilities or relaxation times.⁴³⁻⁴⁴ Because the glass transition occurs over a wide temperature range, it is difficult to give a specific temperature as the glass transition temperature of the polymer; here, the peak of the $\tan \delta$ curve is reported as the T_g of the network (see Fig. 2). The main peaks correspond to polymer main chain relaxation. The shoulder at lower temperature for A0 (Fig. 2a) corresponds to relaxation of chain segments for different crosslinked regions; this response may be influenced by the restriction of mobility of the propagating radicals in this solution of relatively high viscosity (195 cP for A0; 36 cP for A0T).²⁶

As the water content increases from 0 to 16 wt%, the T_g of control adhesives decreases from 143 to 130 °C. This decrease in T_g may be the result of a less crosslinked network and/or the larger free volume of the relatively loosely crosslinked control samples where the water was initially located. In contrast, the water content had negligible influence on the T_g for the experimental adhesive. This difference may be related to a higher degree of cross-linking contributed by the TMPEDMA with low molecular weight and multifunctionality and a reduction in the number of hydroxyl groups in the network.

The intensity of the $\tan \delta$ peak at the T_g reflects the extent of mobility of the polymer chain segments at this temperature.⁴⁵ Higher values of $\tan \delta$ indicate higher energy loss and more viscous behavior, while lower $\tan \delta$ values indicate increasingly elastic behavior (more energy is stored in the material).⁴⁶ All the materials showed higher $\tan \delta$ values when cured in the presence of water than when cured in the absence of water and experimental adhesives exhibited lower $\tan \delta$ peak heights than those of the control. The lower $\tan \delta$ peak heights in experimental adhesives may be explained by the fact that the addition of the TMPEDMA, a monomer with a multifunctional group and a smaller molecular weight between crosslinks, is thought to produce a higher crosslink density and correspondingly to reduce the intensity of the $\tan \delta$ peak in experimental adhesives.

The modulus behavior is well captured within the whole temperature range (−20 ~ 200 °C) (Fig. 3), with no indication of a postcured reaction during the temperature scans. The storage modulus indicates a change from a glassy state to the rubbery state over the temperature range. This change is clearly observed for all the samples tested. At very low temperature, both control and experimental adhesives show gradual decreases in storage moduli with

increasing temperature. Near the glass transition, storage moduli decrease drastically. As heating continues, all the samples reach the rubbery plateau, in which storage modulus is insensitive to further increases in temperature. This rubbery modulus value has been related to the crosslink density of the material.⁴⁷ The ratio of rubbery modulus to the absolute temperature at which that modulus was measured, ζ , is inversely related to the crosslinking density of polymer network and directly proportional to the molecular weight between crosslinks.^{47,48} By this measure, the new adhesives formulated using TMPEDMA showed higher crosslink density than those of the control (Fig. 4). This behavior may be explained by a greater degree of cross-linking contributed by the addition of the TMPEDMA with a reactive vinyl side chain and low molecular weight between reactive groups, as described above. Both the control and experimental adhesives showed reductions in crosslink density when cured in the presence of water, suggesting that water interferes with the formation of crosslinks.

The properties of the polymers formed by free-radical polymerization are strongly influenced by the selection of the monomers.^{49,50} Yourtee *et al.* have demonstrated that dimethacrylates containing branched methacrylate linkages show greater resistance to chemical and enzymatic degradation.¹⁹ In the present work, the high functionality and branched side chain of TMPEDMA were expected to contribute to increased esterase resistance of resins containing this material, especially in the presence of water. In the enzymatic biodegradation study, MAA was monitored as a hydrolytic biodegradation product. Since MAA may also be produced from hydrolysis of unreacted monomers, the adhesive discs were prewashed for 3 days to eliminate most of the unreacted monomers prior to enzyme exposure.³⁷ Unreacted methacrylate double bonds may also be present in pendant groups on the photopolymerized network. Most of the unreacted carbon-carbon double bonds are on molecules which have reacted at one end and are thus bound to the polymer network and are not free to elute. Inoue⁵¹ has suggested that only 10% of the unreacted monomer in dental composites is elutable. Similarly, Tanaka *et al.*⁵² have reported that only 2–7% of the unreacted monomer in dental composite is elutable. Generally, elution from cured resins is rapid for most species, essentially reaching completion within 1–3 days.¹⁰

Salivary esterases (SE), including cholesterol esterase (CE), pseudocholinesterase (PCE), porcine liver esterase (PLE) and acetylcholinesterase (ACE) can break down the ester linkages in methacrylate-based monomers and polymers of dental resins.^{19,53} However, not all esterases have demonstrated the same specificity for monomer components. For example, kinetic studies have demonstrated that PCE preferentially hydrolyzes TEGDMA over BisGMA, while CE's activity with respect to BisGMA is much greater than that of PCE.⁵³ The use of PLE in the esterase resistance studies does not duplicate the substrate specificity of the individual salivary esterases. However, its broad substrate specificity provides a general test of esterase resistance and may mimic the cumulative effects of mixed salivary esterases better than any single enzyme from that pool.

The improvement in esterase resistance afforded by adhesives containing the synthesized dimethacrylate monomer is greater when this material is photopolymerized in the presence of water, suggesting better performance in the moist environment of the mouth. The factors affecting the enzymatic degradation of methacrylate resins include the DC, crosslink density, monomer structure and morphology of polymer network. The improved esterase resistance of the new adhesive could be explained in terms of the densely crosslinked network structure and/or the steric hindrance afforded by the branched alkyl side chain of new monomer. The ester bond of BisGMA is relatively unhindered and therefore susceptible to hydrolytic degradation, while the branched alkyl side chain of the new monomer may protect the ester groups from attack by water or hydrolytic enzymes. The improved esterase

resistance of the experimental adhesive cured in the presence of water is consistent with its DMA results showing higher crosslink density and supports previous reports on the degradation of dental materials with highly crosslinked networks and branched groups.^{10,19}

CONCLUSIONS

In this study, enzymatic degradation and dynamic mechanical properties have been studied for new dentin adhesives containing a multifunctional methacrylate with a branched side chain (TMPEDMA) and compared with a BisGMA/HEMA control. When polymerized in the presence of water, experimental adhesives containing TMPEDMA showed higher crosslink density and improved esterase resistance relative to BisGMA/HEMA controls, without compromising DC and mechanical properties. Thus, TMPEDMA, when included as a component of methacrylate dentin adhesives, may offer enhanced durability in the moist environment of the mouth. Future studies will include evaluation of the rate of hydrolysis of TMPEDMA monomer and polyTMPEDMA by PLE to obtain direct evidence of its resistance to esterase activity using BisGMA as a control.

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Figure 1.

The appearance of adhesive resin solutions containing 0, 8, and 16 wt% water prior to photopolymerization. The appearance of adhesives with 0% (A0 and A0T) and 8% (A8 and A8T) water was transparent while those with 16% water (A16 and A16T) were opaque.

Figure 2.

The $\tan \delta$ versus temperature curves for (a) control and (b) experimental adhesives with 0%, 8% and 16% water contents. The polymerized samples were stored in the vacuum oven under drying agent at room temperature for 1 week after curing. DMA (TA Instruments, Q800) with a three-point bending clamp was conducted over a temperature range of $-20 - 200$ °C with a ramping rate of 3 °C/min at a frequency of 1 Hz.

Figure 3.

Comparison of the storage modulus versus temperature curves for experimental adhesives (A0T, A8T, A16T) with those for control adhesives (A0, A8, A16). The polymerized samples were stored in the vacuum oven under drying agent at room temperature for 1 week after curing prior to evaluation. DMA (TA Instruments, Q800) with a three-point bending clamp was conducted over a temperature range of $-20 - 200$ °C with a ramping rate of 3 °C/min at a frequency of 1 Hz.

Figure 4.

The inverse ratio (ζ) of the modulus in the rubbery region to temperature at which the modulus was measured plotted as a function of water content(%) in adhesives. ζ is inversely related to the crosslinking density of the copolymer. Values are mean (error bar : standard deviation) for n=5 in each group.

Figure 5.

Net cumulative MAA release from control and experimental adhesives [A0 vs. A0T (A)26; A8 vs. A8T (B)26; A16 vs. A16T (C)]. $N = 3 \pm \text{S.D.}$

TABLE I

The Chemical Structure and Molecular Weight of Monomers

Monomer	Molecular Weight (g mol ⁻¹)	Chemical Structure
HEMA	130.1
Bis-GMA	512.6
TMPEDMA	310.5

TABLE II

DC, Curing Time and Mass Loss of Control and Experimental Adhesives

Sample	DC (%) ^a	Curing time (sec) ^b	Mass loss (%) ^c
A0	84.9 ± 0.6	9	1.1 ± 0.1
A8	85.2 ± 0.4	19	1.1 ± 0.1
A16	89.7 ± 0.2	18	0.9 ± 0.1
A0T	82.0 ± 0.6	10	0.9 ± 0.1
A8T	89.9 ± 0.3	18	1.0 ± 0.1
A16T	89.3 ± 0.9	18	1.0 ± 0.1

^aValues are mean (± standard deviation) for n=5 in each group

^bCuring time was taken as the period from which the light exposure was initiated to the moment at which the metal rod inserted into the resin mixture could not be moved by hand. The average was obtained of three readings.

^cMass loss (expressed as weight%) was calculated as the difference between the dry mass of resin specimen before and after DMA test.

TABLE III

DMA Data for Control and Experimental Adhesives

Sample	Tg (°C)	Height of tan δ peak	Full-width-at-half-maximum values of tan δ peak (°C)
A0	143.4 (0.8) ^a	0.647	35.94
A8	138.1 (1.5)	0.694	44.84
A16	129.5 (1.0)	0.671	48.02
A0T	147.7 (0.7)	0.461	43.92
A8T	146.9 (0.8)	0.526	53.69
A16T	147.6 (0.8)	0.532	54.40

^aEntries are mean values of five specimens with standard deviations in parentheses. Symbols: A0, A8, and A16, control formulations polymerized at 0%, 8%, and 16% water; A0T, A8T, and A16T, experimental formulations polymerized at 0%, 8%, and 16% water. The glass transition temperatures (Tg) values of the polymer networks were taken to be the maximum of the tan δ versus temperature curve determined using a dynamic mechanical analyzer.