Published in final edited form as:

Dent Mater. 2017 May ; 33(5): 564–574. doi:10.1016/j.dental.2017.03.013.

Probing the Neutralization Behavior of Zwitterionic Monomer-containing Dental Adhesive

Linyong Song¹, Qiang Ye^{1,*}, Xueping Ge¹, Anil Misra^{1,2}, Candan Tamerler^{1,3}, and Paulette Spencer^{1,3,*}

¹University of Kansas, Bioengineering Research Center, 1530 W. 15th Street, Lawrence, KS 66045-7609, USA

²University of Kansas, Department of Civil Engineering, 1530 W. 15th Street, Lawrence, KS 66045-7609, USA

³University of Kansas, Department of Mechanical Engineering, 1530 W. 15th Street, Lawrence, KS 66045-7609, USA

Abstract

Objective—To investigate the polymerization kinetics, neutralization behavior, and mechanical properties of amine-functionalized dental adhesive cured in the presence of zwitterionic monomer, methacryloyloxyethyl phosphorylcholine (MPC).

Methods—The control adhesive was a mixture based on HEMA/BisGMA/2-N-morpholinoethyl methacrylate (MEMA) (40/30/30, w/w/w). The control and experimental formulations containing MPC were characterized with regard to water miscibility of liquid resins, photopolymerization kinetics, water sorption and solubility, dynamic mechanical properties and leachables from the polymers (aged in ethanol). The neutralization behavior of the adhesives was determined by monitoring the pH of lactic acid (LA) solution.

Results—The water miscibility decreased with increasing MPC amount. The water sorption of experimental copolymer specimen was greater than the control. The addition of 8 wt% water led to improved photo-polymerization efficiency for experimental formulations at MPC of 2.5 and 5 wt %, and significant reduction in the cumulative amounts of leached HEMA, BisGMA, and MEMA, i.e. 90, 60 and 50% reduction, respectively. The neutralization rate of MPC-containing adhesive was faster than control. The optimal MPC concentration in the formulations was 5 wt%.

Conclusions—Incompatibility between MEMA and MPC led to a decrease in water miscibility of the liquid resins. Water (at 8 wt%) in the MPC-containing formulations (2.5–5 wt% MPC) led to higher DC, faster R_p^{max} and significant reduction in leached HEMA, BisGMA, and MEMA. The

Corresponding author: 1. Qiang (Charles) Ye, Ph.D., The University of Kansas, Bioengineering Research Center, School of Engineering, Learned Hall 5109, 1530 W. 15th Street, Lawrence, KS 66045-7609, Ph: 785-864-1746, FAX: 785-864-1742, yeq@ku.edu; 2. Paulette Spencer DDS, PhD, The University of Kansas, Dept. Mechanical Engineering, School of Engineering, 1530 W. 15th Street, Lawrence, KS 66045-7609, Ph: 785-864-8140, FAX: 785-864-1742, pspencer@ku.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

neutralization rate was enhanced with the addition of MPC in the amine-containing formulation. Promoting the neutralization capability of dentin adhesives could play an important role in reducing recurrent decay at the composite/tooth interface.

Graphical abstract

Keywords

Dental Adhesive; Neutralization Capacity; Zwitterionic; Tertiary Amine; Photo-polymerization Kinetics

1. Introduction

Resin-based composite is rapidly becoming the most popular dental restorative material and with the global emphasis on the elimination of dental amalgam, it is anticipated that the popularity of composite will continue to increase [1–3]. In spite of its popularity, clinical studies report a failure rate for composite restorations that is double to triple that of amalgam [4]. NIDCR points to an average lifespan of 6–7 years for dental composites [5] and patients at highest risk for decay are particularly susceptible to early failure [6, 7].

Since composite is too viscous to bond directly to the tooth, a low viscosity adhesive is used to form the bond between the tooth and composite. Acid-etching provides effective mechanical bonding between enamel and adhesive, but bonding to dentin has been fraught with problems. There are a variety of intrinsic and extrinsic factors that lead to acute and chronic degradation of the adhesive bond to dentin [8–13]. These factors include host-derived enzymes, e.g. matrix metalloproteinases (MMPs) that degrade exposed collagen at the adhesive/dentin (a/d) interface [13]; oral fluids, bacterial and salivary enzymes degrade the adhesive; [14] cariogenic and aciduric bacteria demineralize the tooth structure and the inability of the material to increase the local pH facilitates the outgrowth of more aciduric and cariogenic bacteria [15]. It is anticipated that the outgrowth of more aciduric and cariogenic bacteria would lead to increased acid production at the adhesive/dentin (a/d) interface. The acid could infiltrate the a/d interface—provoking demineralization of the tooth structure and potentially, degradation of the adhesive.

The primary reason that amalgam and composite restorations fail is recurrent caries, i.e. carious lesions on the margins of existing restorations, but the risk of recurrent caries is 3.5 times greater for composite [4]. Recurrent decay is most often localized at the gingival margin of Class II composite restorations and is linked to failure of the bond between the tooth and composite and increased levels of the cariogenic bacterium, *Streptococcus mutans*, at the perimeter of these materials [16–18]. At the vulnerable gingival margin, the adhesive is often the primary barrier between the prepared tooth and surrounding environment [2]. Degradation of the adhesive at the gingival margin leads to gaps at the composite/tooth interface—these gaps will be infiltrated by oral fluids, enzymes and bacteria. The infiltration of these noxious agents at the interface between the composite material and tooth structure will ultimately lead to failure of the restoration.

A dentin adhesive that is capable of neutralizing the acid in this local environment could mitigate the damage associated with oral fluid infiltration. It could play an important role in maintaining the integrity of the a/d bond and the seal at the composite/tooth interface. Reports of increased levels of the cariogenic bacterium, *Streptococcus mutans* [16–18] and the observation that the inability of the material to increase the local pH facilitates the outgrowth of more aciduric and cariogenic bacteria [15] highlight the need for materials that could buffer the pH at the microenvironment of the composite/tooth interface [19]. Thus, a dental adhesive that is capable of neutralizing the acid could play an important role in reducing recurrent decay at the vulnerable gingival margin.

The potential of amine-containing dental adhesives to provide buffering as well as the required physical and mechanical properties has been investigated recently [20, 21]. Due to the highly crosslinked network structures, the neutralization rate for the bulk polymer was relatively slow, i.e. 4 to 8 weeks were required to reach neutral pH [20]. While these results are promising, the slow rate of neutralization may offer limited benefit under *in vivo* conditions.

The biocompatible, hydrophilic zwitterionic monomer, e.g. 2-methacryloyloxyethyl phosphorylcholine (MPC), has been used to create protein-repellent surfaces [22–27]. To date, there has been limited investigation of MPC as a co-monomer for dental adhesive development [28–31]. The lack of attention may be related, in part, to the limitations of the zwitterionic functional groups—these groups can bind water molecules more strongly than other hydrophilic monomers via electrostatically induced hydration [30]. The addition of zwitterionic monomer led to adhesive materials with remarkably improved hydrophilicity [32] and strong protein-repellent capability. The bond strength between adhesive and dentin was not compromised with lower MPC concentration in the adhesive formulations [31].

In previous studies from our group [20, 21], tertiary amine-functionalized dental adhesive copolymers showed neutralization capabilities in lactic acid solution but, due to the highly crosslinked network structures, the neutralization rate was relatively slow. Understanding the relationship between neutralization kinetics and the crosslinked network is necessary for the development of dental adhesives that can offer buffering of the interfacial micro-environment while also providing the required physicochemical and mechanical properties. The objectives of this study were to investigate the neutralization behavior of dental adhesive

incorporating both amine monomer and zwitterionic monomer, MPC. The study tested the hypotheses that incorporating MPC into the amine-containing dental adhesive: (1) would improve the water compatibility; (2) would accelerate the rate of neutralization; and (3) would not significantly decrease the mechanical properties under wet conditions.

2 Materials and Methods

2.1 Materials

2,2-Bis[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]propane (BisGMA), 2-hydroxyethyl methacrylate (HEMA), 2-N-morpholinoethyl methacrylate (MEMA), and 2-methacryloyloxyethyl phosphorylcholine (MPC) were obtained from Sigma-Aldrich (St. Louis, MO) and used as received without further purification as monomers. Camphoroquinone (CQ), ethyl-4-(dimethylamino) benzoate (EDMAB), diphenyliodonium hexafluorophosphate (DPIHP), and L(+)-lactic acid (LA) were obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals were reagent grade and used without further purification.

2.2 Preparation of adhesive formulations

HEMA/BisGMA/MEMA (40/30/30, w/w/w) was used as the control (HBM). The formulations consisting of HEMA, BisGMA, MEMA, and MPC are noted as experimental and listed in Table 1. CQ (0.5 wt%), EDMAB (0.5 wt%), and DPIHP (0.5 wt%) were used as a three-component photoinitiator system, with respect to the total amount of monomers. Mixtures of monomers/photoinitiators are prepared in a brown glass vial under amber light as reported previously [33, 34].

2.3 Water miscibility and viscosity of adhesive formulations

The procedure for determining water miscibility of adhesive formulations has been reported [35]. In brief, about 0.5 g neat resin was weighed into a brown vial and water was added in increments of ~0.005 g until the mixture was visually observed to be turbid. The percentage of water in the mixture was noted (w_1). The mixture was then back-titrated using the neat resin until the turbidity disappeared and the percentage of water in the mixture was noted (w_2). The water miscibility (W_{wm} , %) of the liquid formulation was calculated as the average of w_1 and w_2 . Three specimens of each formulation were measured. The viscosities of the liquid resin formulated with or without water were carried out in a Brookfield DV-II +Pro viscometer (Brookfield Engineering Laboratories, Middleborough, MA) at varying shear rate from 112.5 to 165 s^{-1} . The sample volume was 0.5 mL and each formulation was measured three times.

2.4 Real-time conversion and maximum polymerization rate

The degree of conversion (DC) and polymerization kinetic behavior were determined by real-time FTIR as described previously [20]. The spectra were obtained by an infrared spectrometer (Spectrum 400 Fourier transform infrared spectrophotometer, Perkin-Elmer, Waltham, MA), equipped with an attenuated total reflectance (ATR) accessory (PIKE Technologies Gladi-ATR, Madison, WI) at a resolution of 4 cm^{-1} . A time-based spectrum collector (Spectrum TimeBase, Perkin-Elmer) was used for continuous and automatic

collection of spectra during polymerization. A minimum of three measurements ($n=3$) were carried out for each adhesive formulation. Methacrylic double bond conversion was monitored by the band ratio profile- 1637 cm^{-1} ($\text{C}=\text{C}$)/ 1608 cm^{-1} (phenyl). The average of the last 50 values of the time-based spectra is reported as the DC value. The maximum polymerization rate ($R_p^{\max}/[M]$) was determined using the maximum slope of the linear region of the DC vs. time plots [34, 36].

2.5 Specimens preparation

The rectangular beam specimens were prepared by injecting the prepared resin into a glass-tubing mold (Fiber Optic Center, Inc., part no.: ST8100, New Bedford, MA). Disc specimens were prepared by injecting the resin into a standard aluminum hermetic lid (Tzero®, P/N:901600.901) and covering them with a mylar film to prevent oxygen exposure. The specimens were light-cured for 40 s at $23\pm 2\text{ }^\circ\text{C}$ with an LED light curing unit (LED Curebox, 100 mW/cm^2 irradiance, Prototech, Portland, OR). The polymerized rectangular and disc specimens were stored in the dark at $23\pm 2\text{ }^\circ\text{C}$ for at least 48 h before testing. The resultant rectangular beam specimens of cross section $1\text{ mm} \times 1\text{ mm}$ and length 15 mm were used to determine water sorption and dynamic mechanical analysis. The disc specimens were used to study the neutralization behavior.

2.6 Water sorption and solubility of copolymer

The experimental protocol for the water sorption analyses has been reported [33]. In brief, water sorption and solubility were measured using rectangular beam specimens ($1\text{ mm} \times 1\text{ mm} \times 15\text{ mm}$). Five specimens were prepared for each adhesive formulation. Samples were weighed (m_1) with a calibrated electronic balance (resolution of 0.01 mg, Mettler Toledo, XS 205 Dual range, Columbus, OH) and immersed in distilled water to prewash for 7 days at $37\text{ }^\circ\text{C}$. Following the 7-day prewash, the specimens were dried in a vacuum oven in the presence of freshly dried silica gel at $37\text{ }^\circ\text{C}$; the specimens were removed every 24 hours to determine the weight. This process was continued until a constant mass (m_2) was recorded for each beam specimen. After prewashing, the dried specimens were immersed in distilled water and at fixed time intervals, they were removed, blotted to remove excess water, weighed (m_3) and returned to the water until a constant weight was obtained. The values (%) for water sorption (W_{sp}) and solubility (W_{su}) were calculated by the following equations:

$$W_{sp} = \frac{m_3 - m_2}{m_2} \times 100\% \quad (1)$$

$$W_{su} = \frac{m_1 - m_2}{m_1} \times 100\% \quad (2)$$

2.7 pH measurements

The pH measurements were performed with a Titrator equipped with DGi 101-SG probe (Mettler Toledo T50, Switzerland) at $23\pm 2\text{ }^\circ\text{C}$. First, 5 mL LA solution was added in the

glass vessel under stirring and the pH values recorded during the first 5 minutes were noted as the initial pH of the LA solution. Then the disc specimen (~20 mg) was dropped into the LA solution and the pH data were collected continuously (LabX light titration, version 3.1.1.0) for 12 h. To investigate the neutralization behavior of copolymers, the non-prewashed (neat resin and water-containing) specimens and the prewashed water-containing samples (prewashed in ethanol over 14 days) were used.

2.8 Leachable study by High-Performance Liquid Chromatography (HPLC)

HPLC was used to determine the leachates from the specimens. Round disc samples (4 mm diameter $\times$ 1 mm thickness) were used for the leachable study [37]. The polymerized samples were stored in the dark at 23 ± 2 °C for at least 48 h before testing. The disc specimens were then submerged in 1 mL ethanol at 23 ± 2 °C, and the storage solutions were collected at various time intervals, i.e. 3, 6, 24, 48, 96, and 168h. Fresh ethanol was added to the disc samples after each collection. The leachates were analyzed using high performance liquid chromatography (HPLC) on a system (Shimadzu® LC-2010C HT) equipped with a 250 $\times$ 4.6 mm column packed with 5 μ m C-18 silica (Luna®, Phenomenex Inc., Torrance, CA). The mobile phase was gradient acetonitrile/water (50/50 to 70/30, v/v). The system was operated under the following conditions: 0.3 mL/min flow rate; detection at 208 nm; 20 μ L sampling loop; 40 °C.

The column was calibrated with known concentrations of HEMA, BisGMA, MEMA, and MPC, at concentrations of 5, 10, 20, 50, 100, 250, and 500 mg/L in ethanol. The calibration curves with the linear fittings of HEMA (5–500 mg/L, $R^2=0.9999$), BisGMA (5–100 mg/L, $R^2=0.9996$), MEMA (5–100 mg/L, $R^2=0.9997$), and MPC (5–250 mg/L, $R^2=0.9998$) were used to calculate the concentration of these species in the elution. The concentration was based on the intensity of the chromatographic peaks at the corresponding retention time. The HPLC analysis was performed using the extract of three samples for each formulation.

2.9 Dynamic mechanical analysis (DMA)

In this study, DMA tests were performed using a TA instruments Q800 DMA (TA Instruments, New Castle, USA) with a three-point bending clamp. The dynamic mechanical properties of methacrylate-based dental adhesives have been reported by our group [38–41]. The frequency that was used to measure the storage modulus is 1 Hz with an amplitude of 15 μ m and a preload of 0.01 N [38, 40]. In the dry condition, the storage modulus is measured from 10 to 150 °C with a ramping rate of 3 °C min⁻¹. In the wet condition, the test temperature was varied from 10 to 75 °C with a ramping rate of 1.5 °C min⁻¹ [37]. The glass transition temperature (T_g) is determined as the position of the maximum peak on the tan δ versus temperature plots. Rectangular beam specimens prepared as described previously are used for DMA measurements, and a minimum of three specimens of each material are measured.

2.10 Statistical analysis

The results were analyzed statistically using one-way analysis of variance (ANOVA), together with Tukey's test at $\alpha = 0.05$ (Origin Version 9.1, OriginLab Corp., Northampton, MA) to identify significant differences in the means.

3 Results

The water miscibility and viscosities of the control (HBM) and experimental adhesive versus MPC concentration are shown in Figure 1. With an increase in MPC concentration from 0 to 10 wt%, the W_{wm} decreased from 17.5 ± 0.1 to 12.2 ± 0.2 wt%, and the viscosity of the neat resin increased from 35.7 ± 0.6 to 91.3 ± 1.0 cp. Meanwhile, the viscosities decreased with the addition of 8 wt% water in the formulations and the higher the MPC concentration, the greater the decrease in viscosity.

Real-time photopolymerization kinetics of the neat resin and water-containing formulations are shown in Fig. 2 and the data are summarized in Table 1. In the neat resin, with the increase in MPC concentration from 0 to 10 wt%, the DC decreased from 77.3% to 72.6%, the DC at the maximum polymerization rate decreased from about 34% to 30%, and the maximum polymerization rate increased from 0.12 to 0.17 s^{-1} . With the addition of 8 wt% water, the DC is significantly higher than that of the neat resin ($p < 0.05$), the DC at the maximum polymerization rate increased from about 35% to 39%. Meanwhile, the DC and maximum polymerization rate showed maximum values with 5 wt% MPC in the formulation.

With the increase of MPC concentration from 0, 2.5, 5, and 10 wt%, steeper sorption profiles can be observed in Figure 3. The time required to reach a balance is shortened from ~3 days to 1 day with the increase in MPC concentration. The W_{sp} values of the copolymers increased from $20.0 \pm 0.2\%$, $23.4 \pm 0.3\%$, $26.8 \pm 0.2\%$, to $34.1 \pm 0.1\%$. Meanwhile, the W_{su} values decreased slightly from 5.2 to 4.5 wt%.

Fig. 4 shows the pH values of LA solution containing the control or experimental disc specimens versus storage time. When the non-pretreated neat resin experimental specimens were soaked in 0.1 mM LA solution, the pH values increased from 4.1 to about 6.0 after 6 h. The non-pretreated neat resin control specimen achieved a pH value ~5.4 after 6h. When the non-pretreated water-containing specimens were soaked, the increase in the pH value was relatively slow as compared to the neat resin samples. Fig. 4C shows the pH value of 0.025 mM LA solution containing the pretreated specimen (pretreated in ethanol over 2 weeks). The pH value of LA solution containing the control (HBM-W) gradually decreased and reached a plateau (about 5.0) after 11 hours. Meanwhile, the pH value of LA solution containing experimental specimen dropped in the first 4–5 h from ~5.1 to ~4.9, then increased and reached about 5.5 after 12 h.

Fig. 5 shows the results of cumulative leachates from the neat resin and water-containing copolymers as a function of incubation time in ethanol at $23 \pm 2 \text{ }^{\circ}\text{C}$. In the neat resin specimens, with the increase of MPC concentration from 0 to 10 wt%, the cumulative release of HEMA, BisGMA, and MEMA are comparable; the cumulative release of MPC significantly increased to about $211 \pm 4 \text{ }\mu\text{g/mL}$ after 2 days. When 8 wt% water was added to the formulation, the cumulative release of HEMA, BisGMA, and MEMA were significantly reduced ($p < 0.05$) and the reduced percentage was 90, 65, and 60, respectively. Meanwhile, the amount of MPC in the elution from the water-containing sample is below the detection limit and was considered no leaching.

The dynamic mechanical properties of the water-containing control and experimental specimens in dry and wet conditions at various temperatures are shown in Fig. 6. In dry conditions, with the increase of MPC content from 2.5, 5, to 10 wt%, the storage moduli at 37 °C and the T_g values, obtained from the maximum peak of $\tan \delta$ profile, are greater than that of the control. In the wet conditions, the storage moduli of the experimental specimens at 37 °C and the T_g values are significantly lower than that of the control ($p < 0.05$).

4. Discussion

The results from our previous investigations indicate that the type of amine monomer and crosslink density of the adhesive formulation play vital roles in determining the neutralization capacity and kinetics [20, 21]. Additionally, in previous studies, 4 weeks or more time were required for the dental adhesive formulations to neutralize the lactic acid in solution. It was postulated that neutralization could be accelerated without compromising the mechanical properties by including a zwitterionic component, i.e. MPC, in the dental adhesive formulation. MPC is a methacrylate with phospholipid polar group in the side chain; it is more hydrophilic than HEMA. MPC has been used to develop a protein-repellent dental adhesive and the promising results were attributed to the extremely hydrophilic surface [31].

Due to the hydrophilicity of MPC, it was expected that the water miscibility (W_{wm}) of liquid resin would increase with the addition of MPC. However, in the present study, the W_{wm} of the experimental formulations showed an inverse relationship, i.e. decreased water miscibility with increasing MPC concentration. These results are attributed to the incompatibility between MPC and the amine monomer, MEMA (see Support Information of W_{wm} experimental with basic, acidic and neutral methacrylate co-monomers). From the water sorption experiment, the balanced W_{sp} exhibited a monotonic increasing trend with the increase of MPC concentration. This result indicated that the hydrophilicity of the monomer was the critical factor in the swelling of the copolymers. Compatibility of MPC and the amine monomer was the determining factor in the water miscibility of the liquid resin. Based on these results the first hypothesis, incorporating MPC into the amine-containing resin would improve the water miscibility, is rejected.

The photopolymerization kinetics play important roles in determining the network structure of the polymer. In the neat resins, the DC decreased and the maximum polymerization rate increased with an increase in MPC concentration. These results are attributed to the higher viscosity of the formulation in the presence of MPC (see Fig. 1B), which accelerated the auto-acceleration effect [42, 43]. The addition of water led to a decrease in viscosity (see Fig. 1B), and a significant improvement in the DC as compared to the neat resins (see Fig. 2E). Whether in neat resin or water-containing formulations, autoacceleration began at lower polymerization conversion and was steeper for the formulations containing higher MPC concentrations (Figure 2C and D). In neat resins, the conversion of the MPC-containing formulations at which the R_p^{max} occurred is slightly lower than that of the control. However, in water-containing formulations, an inverse relationship was observed. Furthermore, an unexpected phenomenon was observed in the polymerization kinetics of adhesives containing 2.5 and 5 wt% MPC. The R_p^{max} value of formulation with 2.5 or 5 wt% MPC was

significantly higher than that of the corresponding neat resin ($p < 0.05$). Usually, the R_p^{max} is proportional to the resin viscosity, since the termination in the free radical polymerization is diffusion-controlled and hence the rate constant of termination (k_t) is inversely related to the viscosity of resin [44]. With the addition of water, the increase in k_t is caused by the reduced viscosity, and lower R_p^{max} value should be the result. Meanwhile, the growing polymer chains containing MPC became more extended in polar solvent (zwitterions solvation effect) [45, 46]; the active radical center is exposed in favor of propagation and the termination reaction was retarded [46]. All in all, the competition between the reduced viscosity and long-lived macromolecular active sites led to polymerization rates that deviated from the usual relationship.

The fast water sorption of copolymer specimens is due to the rapid diffusion of water into the crosslinked network [47]. Therefore, the kinetics of water sorption for the adhesives is influenced significantly by the hydrophilicity of the copolymer and crosslink density of the network. With the addition of MPC, a faster rate of water uptake was observed. Based on the water sorption results, both the control and experimental were completely saturated within 3 days. A relatively slow neutralization rate of MEMA-containing samples was observed in our previous study [20, 21]. The results from the neutralization and water sorption experiments suggest that the diffusion coefficient of protons is relatively slow as compared to the diffusion coefficient for water molecules in highly crosslinked networks.

The non-prewashed samples were used to mimic the *in vivo* neutralization behavior of copolymer specimens. From Fig. 4A, fast neutralization rate was observed when the non-prewashed specimens were soaked in LA solution. Due to the incomplete conversion of C=C bonds, the un-polymerized MEMA could leach from the bulk specimen and neutralize the lactic acid. When the specimen was soaked into acidic solution, the neutralization process includes 1) the diffusion of water and protons from solution into the crosslinked network, 2) the leaching of unpolymerized monomers (mainly HEMA, MEMA, and MPC) and low molecular weight oligomers from the bulk polymer sample into the LA solution. With the increase of the MPC concentration, the faster water sorption rate and higher W_{sp} (Fig 3A) led to swelling and increased mobility within the crosslinked network. These changes prompted an increase in the rate at which protons diffused through the crosslinked network and the rate of MEMA leaching from the bulk specimen into the LA solution. Thus, the faster neutralization behavior was observed.

The neutralization rate of water-containing samples was slower than that of the neat resin and it took longer for the LA solution to reach neutral pH (Fig. 4B). The addition of water led to higher monomer to polymer conversion and a significant reduction in the amount of MEMA that was leached from the specimens (Table 2). Thus, the slower rate of neutralization in the water-containing samples is attributed to the higher crosslink density and significant reduction in the leachable MEMA.

In our previous investigation with prewashed HBM specimens (HEMA/BisGMA/MEMA=40/30/30, w/w/w), nearly 60 days were required for the LA solution (1 mM, initial pH 3.5) to reach neutral pH [20]. To evaluate the influence of MPC on proton diffusion into the bulk polymer and to reduce experimental time required for aging, in the current

investigation water-containing specimens were soaked in ethanol for 2 weeks and low concentration of LA solution (0.025 mM, initial pH 5.1) was used. Based on the HPLC results, MEMA was not leached from the polymers after soaking in ethanol for 7 days. From Fig. 4C, it can be observed that the pH value of the LA solution containing the control sample gradually decreased and reached a plateau after 11 h (pH 5.0). In comparison, the pH values of the LA solution containing the MPC-2.5-W or MPC-5-W specimens gradually decreased in the first 4–5 h, then increased and reached about 5.5 over 12 h. The increase of the pH value of the LA solution indicated that the proton concentration was decreasing and the polymer offered more buffering as compared to the control sample, which lacked MPC.

The enhanced buffering of the experimental formulation is likely attributed to the accelerated diffusion of protons into the polymer network by the bounded zwitterionic groups. Han *et al.* have investigated the water- and proton-diffusion in polymer electrolyte membrane, and their results indicated that the surface chemistry (sulfonic acid, and fluorocarbon) directly influenced proton transport [48]. Although the polymer material used in our experiment was different from Han's, the enhanced rate of neutralization associated with the experimental sample containing MPC indicated that proton diffusion was promoted. Based on these results, the second hypothesis, i.e. that the neutralization rate of MPC-containing adhesives could be promoted, was accepted.

Dynamic mechanical analysis (DMA) is suitable to characterize the viscoelastic behavior of polymers. DMA provides information on the relaxation of molecular motions, which are sensitive to the polymer network structure, composition, and environment [39, 49–51]. In this study, the DMA tests were carried out using both standard 3-point bending (for dry condition test) and 3-point bending submersion methods (for wet condition test). In dry conditions, these tests give the bulk mechanical properties of adhesive copolymer while the results acquired in wet conditions are more representative of the copolymer behavior in the oral environment. From Figure 6A and C, it can be observed that the storage moduli at 25 or 37 °C and T_g of MPC-containing samples were slightly higher than that of the control in dry condition, which could be mainly attributed to the zwitterionic group and the strong electrostatic interaction. The rubbery moduli of the experimental specimens were comparable (about 110 MPa), which was mainly attributed to the same concentration of crosslinker (BisGMA) in the formulations. Meanwhile, when the samples were soaking and saturated in water, the zwitterionic groups were ionized and the electrostatic interactions have been disrupted. Thus the storage modulus of MPC-containing formulations at 37 °C was lower than that of the control (Figure 6B). It has been reported that MPC concentrations up to 7.5 wt% did not compromise the bond strength under wet conditions [31]. However, in the present study, the mechanical properties of the MPC-containing adhesive in wet conditions were slightly diminished. Further study is required to comprehensively explain these differences. The third hypothesis, the mechanical properties of adhesives would not be significantly affected under wet conditions, was rejected.

It should be noted that although MPC-containing dental adhesive can accelerate the neutralization rate, the inherent hydrophilicity of MPC and the copolymers have higher water sorption. Further studies are needed to optimize the MPC-containing formulations and improve the durability under conditions that are relevant to the oral environment.

5. Conclusion

To understand the neutralization behavior of the MEMA-containing dental adhesive in highly crosslinked network and to mediate the neutralization rate, a zwitterionic monomer, MPC, was used as a co-monomer. The influence of MPC on the polymerization behavior, neutralization kinetics and mechanical properties were investigated. The water miscibility of liquid resin showed decreasing trend with increasing MPC content; this result was primarily attributed to the incompatibility of MPC with MEMA. The results indicate a higher degree of conversion and faster photo-polymerization rate can be achieved synchronously by formulating the MPC-containing resin with water. The cumulative amounts of leached BisGMA and MEMA showed a minimum in the 5wt% MPC-containing copolymer. With the incorporation of MPC at lower concentrations (2.5–5 wt%), the adhesive polymer showed a fast neutralization rate, without significantly compromising mechanical properties in dry conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This investigation was supported by Research Grant: R01 DE022054 and R01 DE025476 from the National Institute of Dental and Craniofacial Research, National Institutes of Health–United State, Bethesda, MD 20892

References

1. Ferracane JL. Resin composite-State of the art. *Dent Mater.* 2011; 27:29–38. [PubMed: 21093034]
2. Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, et al. Adhesive/Dentin Interface: The Weak Link in the Composite Restoration. *Ann Biomed Eng.* 2010; 38:1989–2003. [PubMed: 20195761]
3. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. *Dent Mater.* 2011; 27:1–16. [PubMed: 21112620]
4. Bernardo M, Luis H, Martin MD, Leroux BG, Rue T, Leitao J, et al. Survival and reasons for failure of amalgam versus composite posterior restorations placed in a randomized clinical trial. *J Am Dent Assoc.* 2007; 138:775–83. [PubMed: 17545266]
5. Ferracane JL, Hilton TJ. Polymerization stress - Is it clinically meaningful? *Dent Mater.* 2016; 32:1–10. [PubMed: 26220776]
6. DeRouen TA, Martin MD, Leroux BG, Townes BD, Woods JS, Leitao J, et al. Neurobehavioral effects of dental amalgam in children - A randomized clinical trial. *Jama-J Am Med Assoc.* 2006; 295:1784–92.
7. van de Sande FH, Opdam NJ, Rodolpho PAD, Correa MB, Demarco FF, Cenci MS. Patient Risk Factors' Influence on Survival of Posterior Composites. *J Dent Res.* 2013; 92:S78–S83.
8. Andersson-Wenckert IE, van Dijken JW, Kieri C. Durability of extensive Class II open-sandwich restorations with a resin-modified glass ionomer cement after 6 years. *Am J Dent.* 2004; 17:43–50. [PubMed: 15241909]
9. Brannstrom M. Communication between the oral cavity and the dental pulp associated with restorative treatment. *Oper Dent.* 1984; 9
10. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. Resin-tooth adhesive interfaces after long-term function. *Am J Dent.* 2001; 12:211–5.

11. Murray PE, Windsor LJ, Smyth TW, Hafez AA, Cox CF. Analysis of pulpal reactions to restorative procedures, materials, pulp capping, and future therapies. *Crit Rev Oral Biol Med*. 2002; 13:509–20. [PubMed: 12499243]
12. Van Meerbeek B, Van Landuyt K, De Munck J, Hashimoto M, Peumans M, Lambrechts P, et al. Technique-sensitivity of contemporary adhesives. *Dental Materials Journal*. 2005; 24:1–13. [PubMed: 15881200]
13. Carvalho RM, Manso AP, Geraldini S, Tay FR, Pashley DH. Durability of bonds and clinical success of adhesive restorations. *Dent Mater*. 2012; 28:72–86. [PubMed: 22192252]
14. Delaviz Y, Finer Y, Santerre JP. Biodegradation of resin composites and adhesives by oral bacteria and saliva: A rationale for new material designs that consider the clinical environment and treatment challenges. *Dent Mater*. 2014; 30:16–32. [PubMed: 24113132]
15. Nedeljkovic I, De Munck J, Slomka V, Van Meerbeek B, Teughels W, Van Landuyt KL. Lack of Buffering by Composites Promotes Shift to More Cariogenic Bacteria. *J Dent Res*. 2016; 95:875–81. [PubMed: 27146702]
16. Hansel C, Leyhausen G, Mai UE, Geurtsen W. Effects of various resin composite (co)monomers and extracts on two caries-associated micro-organisms in vitro. *J Dent Res*. 1998; 77:60–7. [PubMed: 9437400]
17. Leinfelder KF. Do restorations made of amalgam outlast those made of resin-based composite? *J Am Dent Assoc*. 2000; 131:1186–7. [PubMed: 10953536]
18. Santerre JP, Shajii L, Leung BW. Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. *Crit Rev Oral Biol Med*. 2001; 12:136–51. [PubMed: 11345524]
19. Kalesinskas P, Kacergius T, Ambrozaitis A, Peciulienė V, Ericson D. Reducing dental plaque formation and caries development. A review of current methods and implications for novel pharmaceuticals. *Stomatologija*. 2014; 16:44–52. [PubMed: 25209226]
20. Song LY, Ye Q, Ge XP, Spencer P. Compositional design and optimization of dentin adhesive with neutralization capability. *J Dent*. 2015; 43:1132–9. [PubMed: 26144189]
21. Ge XP, Ye Q, Song LY, Spencer P, Laurence JS. Effect of crosslinking density of polymers and chemical structure of amine-containing monomers on the neutralization capacity of dentin adhesives. *Dent Mater*. 2015; 31:1245–53. [PubMed: 26342639]
22. Ishihara K, Ueda T, Nakabayashi N. Preparation of Phospholipid Polymers and Their Properties as Polymer Hydrogel Membranes. *Polym J*. 1990; 22:355–60.
23. Wang ZT, Wang HX, Liu JD, Zhang YT. Preparation and antifouling property of polyethersulfone ultrafiltration hybrid membrane containing halloysite nanotubes grafted with MPC via RATRP method. *Desalination*. 2014; 344:313–20.
24. Wang BL, Jin TW, Han YM, Shen CH, Li Q, Lin QK, et al. Bio-inspired terpolymers containing dopamine, cations and MPC: a versatile platform to construct a recycle antibacterial and antifouling surface. *Journal of Materials Chemistry B*. 2015; 3:5501–10.
25. Kiritoshi Y, Ishihara K. Preparation of cross-linked biocompatible poly(2-methacryloyloxyethyl phosphorylcholine) gel and its strange swelling behavior in water/ethanol mixture. *J Biomater Sci-Polym Ed*. 2002; 13:213–24. [PubMed: 12022751]
26. Ma IY, Lobb EJ, Billingham NC, Armes SP, Lewis AL, Lloyd AW, et al. Synthesis of biocompatible polymers. 1. Homopolymerization of 2-methacryloyloxyethyl phosphorylcholine via ATRP in protic solvents: An optimization study. *Macromolecules*. 2002; 35:9306–14.
27. Yuan B, Chen Q, Ding WQ, Liu PS, Wu SS, Lin SC, et al. Copolymer Coatings Consisting of 2-Methacryloyloxyethyl Phosphorylcholine and 3-Methacryloxypropyl Trimethoxysilane via ATRP To Improve Cellulose Biocompatibility. *ACS Appl Mater Interfaces*. 2012; 4:4031–9. [PubMed: 22856677]
28. Zhang N, Zhang K, Melo MAS, Chen C, Fouad AF, Bai YX, et al. Novel protein-repellent and biofilm-repellent orthodontic cement containing 2-methacryloyloxyethyl phosphorylcholine. *J Biomed Mater Res Part B*. 2016; 104:949–59.
29. Zhang N, Melo MAS, Chen C, Liu J, Weir MD, Bai YX, et al. Development of a multifunctional adhesive system for prevention of root caries and secondary caries. *Dent Mater*. 2015; 31:1119–31. [PubMed: 26187532]

30. Zhang N, Weir MD, Romberg E, Bai YX, Xu HHK. Development of novel dental adhesive with double benefits of protein-repellent and antibacterial capabilities. *Dent Mater.* 2015; 31:845–54. [PubMed: 25990262]
31. Zhang N, Melo MAS, Bai YX, Xu HHK. Novel protein-repellent dental adhesive containing 2-methacryloyloxyethyl phosphorylcholine. *J Dent.* 2014; 42:1284–91. [PubMed: 25234652]
32. Chen SF, Li LY, Zhao C, Zheng J. Surface hydration: Principles and applications toward low-fouling/nonfouling biomaterials. *Polymer.* 2010; 51:5283–93.
33. Guo X, Wang Y, Spencer P, Ye Q, Yao X. Effects of water content and initiator composition on photopolymerization of a model BisGMA/HEMA resin. *Dent Mater.* 2008; 24:824–31. [PubMed: 18045679]
34. Ye Q, Park J, Topp E, Spencer P. Effect of photoinitiators on the in vitro performance of a dentin adhesive exposed to simulated oral environment. *Dent Mater.* 2009; 25:452–8. [PubMed: 19027937]
35. Song LY, Ye Q, Ge XP, Misra A, Laurence JS, Berrie CL, et al. Synthesis and evaluation of novel dental monomer with branched carboxyl acid group. *J Biomed Mater Res Part B.* 2014; 102:1473–84.
36. Abedin F, Ye Q, Song LY, Ge XP, Camarda K, Spencer P. Effect of Partition of Photo-Initiator Components and Addition of Iodonium Salt on the Photopolymerization of Phase-Separated Dental Adhesive. *JOM.* 2016; 68:1090–9. [PubMed: 27158215]
37. Song LY, Ye Q, Ge XP, Misra A, Spencer P. Mimicking nature: Self-strengthening properties in a dental adhesive. *Acta Biomater.* 2016; 35:138–52. [PubMed: 26883773]
38. Song LY, Ye Q, Ge XP, Misra A, Spencer P. Tris(trimethylsilyl)silane as a co-initiator for dental adhesive: Photo-polymerization kinetics and dynamic mechanical property. *Dent Mater.* 2016; 32:102–13. [PubMed: 26616688]
39. Singh V, Misra A, Parthasarathy R, Ye Q, Park J, Spencer P. Mechanical properties of methacrylate-based model dentin adhesives: Effect of loading rate and moisture exposure. *J Biomed Mater Res Part B.* 2013; 101:1437–43.
40. Park JG, Ye Q, Topp EM, Lee CH, Kostoryz EL, Misra A, et al. Dynamic Mechanical Analysis and Esterase Degradation of Dentin Adhesives Containing a Branched Methacrylate. *J Biomed Mater Res Part B.* 2009; 91B:61–70.
41. Misra A, Spencer P, Marangos O, Wang Y, Katz JL. Micromechanical analysis of dentin/adhesive interface by the finite element method. *J Biomed Mater Res Part B.* 2004; 70B:56–65.
42. Oshaughnessy B, Yu J. Autoacceleration in Free-Radical Polymerization. *Phys Rev Lett.* 1994; 73:1723–6. [PubMed: 10056867]
43. Dvornic PR, Jacovic MS. The Viscosity Effect on Auto-Acceleration of the Rate of Free-Radical Polymerization. *Polym Eng Sci.* 1981; 21:792–6.
44. Odian, G. Principles of Polymerization. Fourth. John Wiley & Sons, Inc; Hoboken, New Jersey: 2004.
45. Sato T, Miyoshi T, Seno M. Kinetic study on the radical polymerization of 2-methacryloyloxyethyl phosphorylcholine. *J Polym Sci Pol Chem.* 2000; 38:509–15.
46. Wang HW, Miyamoto A, Hirano T, Seno M, Sato T. Radical polymerization of 2-methacryloyloxyethyl phosphorylcholine in water: kinetics and salt effects. *Eur Polym J.* 2004; 40:2287–90.
47. Parthasarathy R, Misra A, Park J, Ye Q, Spencer P. Diffusion coefficients of water and leachables in methacrylate-based crosslinked polymers using absorption experiments. *J Mater Sci-Mater Med.* 2012; 23:1157–72. [PubMed: 22430592]
48. Song J, Han OH, Han S. Nanometer-Scale Water-and Proton-Diffusion Heterogeneities across Water Channels in Polymer Electrolyte Membranes. *Angew Chem Int Edit.* 2015; 54:3615–20.
49. Fadda HM, Khanna M, Santos JC, Osman D, Gaisford S, Basit AW. The use of dynamic mechanical analysis (DMA) to evaluate plasticization of acrylic polymer films under simulated gastrointestinal conditions. *Eur J Pharm Biopharm.* 2010; 76:493–7. [PubMed: 20800682]
50. Misra A, Spencer P, Marangos O, Wang Y, Katz JL. Parametric study of the effect of phase anisotropy on the micromechanical behaviour of dentin-adhesive interfaces. *J Roy Soc Interface.* 2005; 2:145–57. [PubMed: 16849175]

51. Mark, HF. Encyclopedia of Polymer Science and Technology. 4th. Wiley; 2014.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlight

- Dental adhesive containing zwitterionic monomer shows tuning neutralization rate.
- Resin exhibits faster photo-polymerization rate and higher conversion simultaneously.
- Polymer shows improved neutralization rate without compromised mechanical properties.

Figure 1.
Water miscibility (A) and viscosity (B) of MPC-containing formulations.

Figure 2.

Real-time conversion of neat resin (A) and water-containing resin (B), polymerization rates vs. conversion of neat resin (C) and water-containing resin (D), degree of conversion (E) and maximum polymerization rate (F) vs. MPC concentration.

Figure 3. Water sorption (A) and solubility (B) of the control and MPC-containing copolymers (control: HEMA/BisGMA/MEMA=40/30/30, w/w/w).

Figure 4.

Real-time pH value of LA solution containing the control and experimental disc specimen versus time at 23 ± 2 °C. (A) Non-prewashed neat resin samples soaked in 0.1mM LA, (B) non-prewashed water-containing samples soaked in 0.1mM LA, and (C) prewashed sample soaked in 0.025 mM LA solution.

Figure 5. Cumulative release from the neat resin and water-containing copolymers as a function of incubation time in ethanol at 23 ± 2 °C: (A) HEMA, (B) BisGMA, (C) MEMA, (D) MPC.

Figure 6. Representative storage modulus (A and B) and tan δ (C and D) vs. temperature profiles of the water-containing control and experimental adhesives in dry (A and C) and wet (B and D) conditions.

Table 1

Formulations of the neat resin and water-containing adhesives, and evaluation of their photo-polymerization behaviors

Run	HEMA (%)	BisGMA (%)	MEMA (%)	MPC (%)	D ₂ O (%)	DC of C=C (%)	$\frac{R_p^{max}}{[M]} \times 100(1/S)$
Neat resin	40	30	30	–	–	77.3 (0.1)	12.07 (0.64)
	37.5	30	30	2.5	–	75.9 [*] (0.2)	13.39 (0.88)
	35	30	30	5	–	74.3 [*] (0.2)	14.32 [*] (0.01)
	30	30	30	10	–	72.6 [*] (0.1)	16.93 [*] (0.42)
Water-containing	36.8	27.6	27.6	–	8	80.0 (0.6)	11.34 (0.08)
	34.5	27.6	27.6	2.3	8	81.1 (0.6)	16.70 [#] (0.36)
	32.2	27.6	27.6	4.6	8	83.5 [#] (0.3)	17.40 [#] (0.69)
	27.6	27.6	27.6	9.2	8	79.9 (0.9)	15.14 [#] (0.81)

* Significantly ($p < 0.05$) different from the HBM formulation.# Significantly ($p < 0.05$) different from the HBM-W formulation. The value in the () is the standard deviation.

Table 2

The mass ratio of leachates from the adhesive copolymers

Release amount* (mg/g copolymer)	HEMA	BisGMA	MEMA	MPC
HBM	27.1	11.6	47.0	–
MPC-2.5	24.9	11.4	45.5	2.4
MPC-5	23.9	12.0	45.5	5.2
MPC-10	21.1	13.4	46.2	10.6
HBM-W	4.4(84%)	4.5(61%)	20.1(57%)	–
MPC-2.5-W	3.1(88%)	3.9(66%)	20.3(55%)	X
MPC-5-W	1.8(92%)	3.5(71%)	19.4(57%)	X
MPC-10-W	1.2(94%)	5.4(60%)	24.1(48%)	X

* : the release amount (mg/g copolymer) was calculated based on the cumulative concentration obtained from HPLC results. The value in the () is the decreasing percentage of leachate from the water-containing specimens than that from neat resin sample. -: no leaching. X: the concentration in the eluate is lower than that of the detection limit and the release amount cannot be calculated.