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Modulating pH through Lysine Integrated Dental Adhesives

Linyong Song¹, Xueping Ge¹, Qiang Ye¹, Kyle Boone¹, Sheng-Xue Xie¹, Anil Misra^{1,3}, Candan Tamerler^{1,2}, and Paulette Spencer^{1,2}

¹Institute for Bioengineering Research, School of Engineering, University of Kansas, 1530 W. 15th Street, Lawrence, KS 66045-7609, USA

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

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

Abstract

Objectives.—The objective of this study was to explore the effect of lysine integration to dental adhesives with respect to the polymerization kinetics, neutralization capacities in the acidic microenvironment, dynamic mechanical properties, and thermal properties.

Materials and method.—Lysine was incorporated into liquid resin formulations at 2.5 and 5.0 wt % with additional water/ethanol co-solvents. The co-monomer system contained 2-hydroxyethyl methacrylate (HEMA) and Bisphenol A glycerolate dimethacrylate (BisGMA) with a mass ratio of 45/55. The kinetics of photopolymerization, neutralization capacities, lysine-leaching, dynamic mechanical properties and thermal properties of the control and experimental adhesives were analyzed.

Results.—The degree of conversion of the experimental adhesive was increased substantially at 2.5 wt% lysine as compared to the control. The experimental polymers provided acute neutralization of the acidic microenvironment. Approximately half of the lysine was released from the polymer network within one month. Under dry conditions and physiologic temperatures, the incorporation of lysine did not compromise the storage modulus. Comparison of the thermal properties suggests that the more compact structure of the control adhesive inhibits movement of the polymer chains resulting in increased T_g .

Significance.—Incorporating lysine in the adhesive formulations led to promising results regarding modulating pH, which may serve as one aspect of a multi-spectrum approach for enhancing the durability of composite restorations. The results provide insight and lay a foundation for incorporating amino acids or peptides into adhesive formulations for pH modulation or desired bioactivity at the interfacial margin between the composite and tooth.

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Keywords

Buffering; Dental adhesive; Lysine; Neutralization; Photo-polymerization; Hybrid Layer; Thermal degradation

1. Introduction

Over 170 million composite and amalgam restorations are placed annually in the United States and results from clinical studies suggest that more than half are replacements for failed restorations [1]. The primary reason for restoration failure is recurrent decay, i.e. decay on the margins of existing restorations, but the incidence of recurrent decay can be 3 to 3.5 times greater for composite restorations [2, 3]. A clear difference between amalgam and composite is the inherent ability of amalgam to seal marginal defects, i.e. corrosion products from amalgam can seal the tooth/material interface. Composites lack this inherent ability. The low viscosity adhesive that is used to bond the composite to the tooth is intended to seal the interface. However, the adhesive/dentin interface is susceptible to degradation over time by chemical and mechanical stresses [4–8]. Adhesive degradation leads to gaps at the interface between the composite and tooth. Bacteria and bacterial by-products, e.g., acids, enzymes, and solvents permeate the interfacial gaps, undermining the composite restoration and destroying tooth structure [3, 9].

Cariogenic plaque is the direct cause of decay at the composite/tooth margin and cariogenic plaques result when the low populations of aciduric bacteria increase following high-frequency exposure to sugars and fermentable carbohydrates [10]. The extended acidification of plaque ($\text{pH} < 5$) is a result of the metabolic activity of the microbiota [10]. The acid demineralizes the tooth and causes severe damage to the composite/tooth interface—this activity prompts further plaque attachment and deterioration of the restoration [11]. Composites accumulate more plaque than other restorative materials [3, 12].

Increased caries at the margins of composite restorations implies that the restoration itself may foster a local environment that promotes decay. The gingival margin of composite restorations is a particularly vulnerable site and at this margin, the adhesive and the adhesive seal to dentin is often the barrier between the prepared tooth and surrounding environment. As noted previously, adhesive degradation leads to gaps at the composite/tooth interface. Another potential weak link in the composite restoration is the inability of the material to increase the pH at the composite/tooth interface [13]. The depressed pH facilitates the outgrowth of more cariogenic and aciduric bacteria [13]. The lack of buffering capabilities can facilitate compositional shifts in plaque that are advantageous for cariogenic bacteria and detrimental for beneficial bacteria.

Introducing neutralization capabilities by using amine-containing co-monomers in the adhesive formulation offers a promising approach for mitigating acid-provoked damage at composite/adhesive/tooth interfaces. In our previous work [14–17], amine-containing dental adhesives have been developed to buffer the acidic micro-environment and to enhance the lifetime of dental composite restorations. However, due to incomplete amine monomer conversion, there is a concern that amine-containing cytotoxic species could be leached.

Lysine, an essential amino acid, has been widely reported as an important component in biomaterials because of its biocompatibility [18–20]. Its lipophilic derivatives, lysine-based small molecules, showed commendable broad-spectrum antibacterial activity and biofilm-disrupting activity [21]. A recent study also showed that the residue of lysine may have antibacterial properties [22]. In addition, lysine can act as a weak base with a pKa value of 10.5 for the side amine group [23, 24]. Therefore, lysine may provide neutralization capacity for dental adhesives while also providing good biocompatibility (see Figure 1).

The two-fold objectives of this work were: 1) to study the effect of lysine integration in the adhesive formulation with respect to photo-polymerization and crosslinking density, and 2) to investigate the neutralization capacity and the leaching of lysine. To our knowledge, this investigation marks the first study on the neutralization capacity of a dental adhesive that contains an amino acid. The results may provide a path for developing adhesives that can provide buffering at the adhesive/dentin interface.

2. Materials and methods

2.1 Materials

Bisphenol A glycerolate dimethacrylate (BisGMA), 2-hydroxyethylmethacrylate (HEMA) and L-lysine were used as received (Sigma-Aldrich) without further purification. Camphorquinone (CQ), ethyl-4-(dimethylamino) benzoate (EDMAB), and diphenyliodonium hexafluorophosphate (DPIHP) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and used as a three-component photoinitiator system without further purification. TNBSA (2,4,6-trinitrobenzene sulfonic acid), 5% w/v in methanol, was received from Thermo Fisher Scientific.

2.2 Preparation of adhesive formulations

The preparation of the adhesive formulations has been reported [15–17]. There are two experimental adhesive formulations with 2.5 and 5.0 wt % of lysine, respectively. In order to dissolve lysine, D₂O and ethanol were used as the co-solvents. The lysine and co-solvents in experimental formulations were as follows: 2.5 wt % lysine, 2.5 wt % D₂O and 5.0 wt % ethanol (Lysine-1); 5.0 wt % lysine, 5.0 wt % D₂O and 10.0 wt % ethanol (Lysine-2). Two control formulations lacking lysine were prepared with the same amount of co-solvents, that is: 2.5 wt % D₂O + 5.0 wt % ethanol (C0–1) and 5.0 wt % D₂O + 10.0 wt % ethanol (C0–2). It should be noted that all the control and experimental formulations consisted of HEMA and BisGMA with a mass ratio of 45/55 [25–27], which is similar to widely used commercial dental adhesives. The composition of the three-component initiator system was CQ (0.5 wt %), EDMAB (0.5 wt %) and DPIHP (1.0 wt %) with respect to the total amount of monomers and solvents [28]. The resin mixtures were prepared in a brown glass vial. The solutions containing the monomers/photoinitiators/co-solvents/lysine were mixed for 24h at room temperature to promote complete dissolution and formation of a homogeneous solution. The details on weight percent (wt%) and molar concentration (mol/g) in the adhesive formulations are shown in Tables 1 and 2, respectively. Overall, we tested four groups, i.e. C0–1, C0–2, Lysine-1, and Lysine-2. These four groups were analyzed with respect to their polymerization kinetics, neutralization behavior, and mechanical properties.

2.3 Real-time conversion and maximum polymerization rate

Real-time *in situ* monitoring of the photopolymerization of the adhesive formulations was performed using an infrared spectrometer (Spectrum 400 Fourier transform infrared spectrophotometer, Perkin-Elmer, Waltham, MA) at a resolution of 4 cm^{-1} [25–27]. One drop of adhesive solution was placed on the crystal top-plate of an attenuated total reflectance (ATR) accessory (Pike, GladiATR, Pike Technology, Madison, WI) and covered with a mylar film. A 40-s-exposure to the commercial visible-light-polymerization unit (Spectrum®, Dentsply, Milford, DE), at an intensity of 550 mW cm^{-2} , was initiated after 50 spectra had been recorded. Real-time IR spectra were recorded continuously for 600 s after light curing began. A time-resolved spectrum collector (Spectrum TimeBase, Perkin-Elmer) was used for continuous and automatic collection of spectra during polymerization. Three replicates were obtained for each adhesive formulation.

The change of the band ratio profile ($1637\text{ cm}^{-1}(\text{C}=\text{C})/1608\text{ cm}^{-1}(\text{phenyl})$) was monitored for calibrating the DC of the methacrylate group. DC was calculated using the following equation, which is based on the decrease in the absorption intensity band ratios before and after light curing. The average of the last 50 values of time-based data points is reported as the DC value at 10 minutes.

$$DC = \left(1 - \frac{\frac{\text{Absorbance}_{1637\text{ cm}^{-1}}^{\text{sample}}}{\text{Absorbance}_{1637\text{ cm}^{-1}}^{\text{monomer}}}}{\frac{\text{Absorbance}_{1608\text{ cm}^{-1}}^{\text{sample}}}{\text{Absorbance}_{1608\text{ cm}^{-1}}^{\text{monomer}}}}\right) \times 100\%$$

2.4 Preparation of polymer specimens

The preparation of the round-beam shaped polymer specimens for dynamic mechanical analysis and thermogravimetric analysis has been reported previously [17]. In brief, resin was first injected into glass-tubing molds with a diameter of 1 mm (Fiber Optic Center, Inc., part no.: ST8100, New Bedford, MA). Then, the resin was light-cured for 40 s with a LED light-curing unit (LED Curebox, 80 mW/cm^2 irradiance, Prototech, Portland, OR). Ten specimens were prepared for each formulation.

For neutralization and leaching study, disc-shaped samples ($\sim 4\text{ mm}$ diameter and $\sim 1.2\text{ mm}$ thick) were prepared by injecting the resin formulations into hermetic lids (Tzero®, P/N: 900794.901, TA instruments, New Castle, USA) and covering with a glass coverslip (Ted Pella, Inc., P/N: 26023). Five specimens were prepared for each formulation. The samples were light polymerized with a 40 s exposure to the commercial visible-light-polymerization unit (Spectrum®, Dentsply, Milford, DE) at an intensity of 550 mW cm^{-2} . The polymerized samples were stored in the dark at $23 \pm 2\text{ }^\circ\text{C}$ for two days to provide adequate time for post-curing polymerization. The samples were subsequently extracted from the molds.

2.5 Thermogravimetric analysis (TGA)

To investigate the thermal degradation behavior of the adhesives with or without lysine, the thermogravimetric experiments were performed on a PYRIS 1 analyzer (PerkinElmer, Akron, OH, USA) with a heating rate 20 °C/min under nitrogen (30 mL/min). The final temperature was 600°C and the sample mass used for the experiments was about 3–4 mg. The C0–2 (nonsoaked and soaked) and Lysine-2 (nonsoaked and soaked) samples were tested. The nonsoaked control beam samples (C0–2) and experimental samples (Lysine-2) were first dried in a vacuum oven at 37 °C to constant mass before testing. The soaked control (C0–2) and experimental (Lysine-2) samples were immersed in 0.1 M sodium bicarbonate solution at 23±2 °C for 7 days and then dried in a vacuum oven at 37 °C to constant mass. A minimum of three specimens of each formulation were measured.

2.6 Lysine leaching study

Disc samples were used for the leachable study [14]. The samples were extracted and the mass was recorded. The samples were stored in 1 mL 0.1 M sodium bicarbonate solution (pH=8.5) at 23±2 °C. The solution was replaced and reserved at fixed time intervals. The HPLC analysis was performed using the extract of 3 samples from each formulation.

TNBSA is a rapid and sensitive reagent for the determination of free amino groups [29]. Primary amine group on lysine molecule, upon reaction with TNBSA, forms a highly chromogenic derivative, which can be measured at 335 nm with a plate reader. Since the working range of the TNBSA assay is quite narrow (2–20 ug/ml) for amino acid, the solutions for each sample were diluted to 10, 20, 30, 40 and 50 times to fit in the working range. The following procedure was used: 5% w/v TNBSA was diluted to 500-fold in 0.1M sodium bicarbonate buffer. Then, 0.25 mL TNBSA (0.01% w/v) was added to 0.5 mL leaching solution and incubated at 37 °C for 2 hours. 0.25 mL of 10% SDS and 0.125 mL of 1N HCl were added to each sample. Measurement of the absorbance at 335 nm was conducted by the plate reader (CYTATION 3 imaging reader, BioTek Instruments, Inc., Minooski, Vermont, USA) in bottom-read absorbance mode [15].

2.7 Neutralization experiments and pH measurements

Disc specimens were soaked in 1.0 mL volume of 0.1 mM lactic acid (pH=4.0). The pH measurement was carried out using a T50 titrator (METTLER TOLEDO) and Labx Titration software (Columbus, OH) was used to collect the data [14, 15].

2.8 Dynamic mechanical analysis (DMA)

As described previously [17], the viscoelastic properties of the dental adhesives were characterized using DMA Q800 (TA Instruments, New Castle, USA) with a 3-point bending clamp. The cylinder beam specimens (1mm× 15mm) were divided into two groups. The first group consisted of dry samples. It is noted that the solvent-containing specimens were stored in a vacuum oven at 37 °C to remove the solvents before DMA. These specimens were tested by a standard 3-point bending clamp. The test temperature was varied from 10 to 220 °C with a ramping rate of 3 °C/min, a frequency of 1 Hz, an amplitude of 15 µm, and a pre-load of 0.01 N.

The second group consisted of wet samples, which were stored in distilled water at 37 °C for 10 days. The wet samples were tested by 3-point bending using a submersion clamp. The test temperature varied from 10 to 80 °C with a ramping rate of 1.5 °C/min at a frequency of 1 Hz.

The properties measured under this oscillating loading were storage modulus (E') and $\tan \delta$. The ratio of the loss modulus (E'') to the storage modulus E' is referred to as the mechanical damping, or $\tan \delta$ (i.e., $\tan \delta = E''/E'$). Five specimens of each adhesive formulation were measured, and the results from the five specimens per each formulation were averaged.

2.9 Statistical analysis

The results (DC of C=C measured by FTIR, T_g and rubbery modulus obtained from DMA) were analyzed statistically using one-way analysis of variance (ANOVA), together with Tukey's test at $\alpha=0.05$ to identify the significant differences in the means. The statistical analysis was carried out using Microcal Origin (Version 8.0, Microcal Software Inc., Northampton, MA).

3 Results

Figure 2 shows the degree of conversion (DC) of the formulations. DC is 79.3% (C0-1) and 93.1% (C0-2), respectively for control with different content of solvent. The DC for the corresponding lysine containing experimental formulations: 91.6% (lysine-1) and 87.8% (lysine-2).

The neutralization results are shown in Figure 3. There is no neutralization capacity detected from the control polymers. The lysine-containing polymers show an obvious pH rise during the first several days. After one day soaking in LA solution, the pH value rose from 3.9 to 7.5 for the 5 wt% lysine formulation. The pH plateaued at 7.5 for the 5 wt% lysine during the 10 days observation. The pH value increased from 3.9 to 6.1 for the adhesive containing 2.5 wt % of lysine after three days soaking in LA solution. For the 2.5 wt% lysine, the pH plateaued at 6.5 during the 10 days observation.

Figure 4 shows the results of the thermogravimetric analysis for lysine, and the nonsoaked and soaked C0-2 and Lysine-2 polymers. The major decomposition of lysine occurs at 217 and 240 during heating. The nonsoaked specimens (C0-2 and Lysine-2) show a slight weight loss from 100 to 250 °C. The nonsoaked Lysine-2 sample has a maximum temperature of weight loss at 220 °C. Meanwhile, the soaked samples (C0-2 and Lysine-2) do not show any weight loss from 100 to 250 °C. When the temperature is over 250 °C, for the C0-2 (whether the nonsoaked or soaked samples), the degradation includes two steps, one is from 250 to 430 °C, and another is from 430 to 500 °C. Based on the DTG curves, for the C0-2 specimens two maximum temperatures of weight loss are found around 380 and 464 °C. To the nonsoaked Lysine-2 polymer, only one degradation step is observed from 250 to 500 °C and the DTG curve shows one maximum temperature at 447 °C. However, the soaked Lysine-2 sample exhibits three degradation steps and the maximum temperatures were 384, 444, and 535 °C, respectively.

Lysine-leaching results are shown in Figure 5. In the first 24 h, the accumulative concentration of lysine from the Lysine-1 and Lysine-2 polymer could reach 160 and 213 $\mu\text{g/mL}$, respectively. After 9 days, the leach rates decreased gradually and after 30 days, the cumulative concentration of lysine in solution for Lysine-1 polymer (2.5 wt % lysine) and Lysine-2 (5 wt % lysine) polymers are 300 and 425 $\mu\text{g/mL}$, respectively.

The results of DMA under dry conditions are shown in Figure 6 and Table 3. At the rubbery region, the storage modulus for lysine containing formulations was significantly lower than control formulations ($p<0.05$). The rubbery modulus of Lysine-1 polymer at 180 °C is 46.6 MPa, which is significantly lower than C0-1 (180 °C: 52.8 MPa) with the same solvent content ($p<0.05$). Meanwhile, the modulus at rubbery region of the Lysine-2 polymer is 27.5 MPa, which is significantly lower than C0-2 with the same amount of solvent ($p<0.05$). The glass transition temperature (T_g) was also decreased with the presence of lysine. T_g of Lysine-1 polymer is 116.6 °C, while the T_g of the C0-1 is 123.6 °C. With increase in lysine concentration to 5 wt%, the T_g of Lysine-2 polymer decreases to 101.7 °C, which is significantly lower than that of the C0-2 (109.0 °C) ($p<0.05$).

Figure 7 and Table 4 show the DMA results in wet condition. The storage modulus of lysine containing formulations was also lower than control formulations with the same amount of solvent. The T_g of Lysine-2 adhesive (48.8 °C) was significantly lower ($p<0.05$) than the corresponding C0-2 (49.7 °C). While, the T_g of Lysine-1 (50.2 °C), is not significantly different from the corresponding control's (C0-1, 50.2 °C) ($p>0.05$).

Discussion

The adhesive that bonds the composite to the tooth is intended to seal the interface, but the adhesive degrades, which can breach the composite/tooth margin. Bacteria and bacterial byproducts such as acids and enzymes infiltrate these marginal gaps and the composite's inability to increase the interfacial pH facilitates cariogenic and aciduric bacterial outgrowth. Together, these characteristics encourage recurrent decay, pulpal damage, and composite failure. Recurrent decay at the composite-tooth interface has consistently been the primary reason for the failure of composite restorations and *S. mutans* is a primary pathogenic bacterium responsible for recurrent decay. *S. mutans* adsorbs to the tooth surface and changes the local environment, which further supports subsequent colonization on the tooth surface by other bacterial species (forming a biofilm). In addition, *S. mutans* increases the acidity of the environment by producing lactic acid, which both damages the tooth and hydrolyzes adhesives and composites [13, 31].

Introducing lysine as a biocompatible amino acid offers a promising approach for providing buffering at the adhesive/dentin interface. The ability to buffer this micro-environment offers promise for mitigating the damage caused by lactic acid. The buffered micro-environment will potentially retard the chemical hydrolysis and thus, the stability of the adhesive layer will be enhanced. The neutralized micro-environment will reduce the potential for compositional shifts in plaque to more cariogenic and aciduric bacteria [13]. Recently, arginine-based dental adhesive system has been developed and exhibited antibacterial effects [32]. The capability of increasing the pH of local oral biofilms through the arginine-release

could retard the outgrowth of the acid-producing cariogenic bacteria and reduce the risk for secondary caries [33–35]. Based on the evidence accumulated from the literature [13, 32–35], there is a clear potential that the buffering capabilities provided by the lysine-containing adhesives could protect both the polymer adhesive and tooth structure.

The degree of conversion (DC) plays an important role in determining the quality of the adhesive/dentin interface [36, 37]. It has been widely reported that the DC of C=C bond in dental adhesive formulations is rarely complete due to rapid vitrification, which causes the limited mobility of the propagating free radical species within the crosslinked network [38–40]. The introduction of low concentrations of solvents, e.g. water, ethanol, and so forth usually leads to increased DC due to the delay in vitrification [25, 41]. In this study, the DC of the control formulations increased significantly with an increase in solvent concentration from 7.5 to 15 wt%. The experimental formulation with 2.5 wt% lysine exhibited even higher DC than the control. This effect could be related to lysine's two primary amines. Amine compounds have been observed as co-initiators in the photo-curing process and improve the monomer's conversion [14, 17]. With further increase in lysine concentration to 5 wt%, the DC is slightly lower than that of the control. The lower conversion was mainly caused by the calculation method, which used the band ratio between C=C (1637 cm^{-1}) and phenyl group (1608 cm^{-1}). With 5 wt% lysine, the broad peak of primary amine (N-H) bending and the C=O stretching of lysine around $1650\text{--}1580\text{ cm}^{-1}$ overlap with the peaks of C=C and phenyl group, which leads to the significant rise of the baseline and results in a higher peak intensity of C=C bond after light irradiation. Therefore, the calculated DC of 5 wt% lysine-containing formulation becomes lower.

Usually, with the increase in solvent concentration, the maximum polymerization rate decreases [25]. For the control formulations, the maximum polymerization rate decreased significantly ($p < 0.05$) with the increase of solvent concentration from 7.5 wt% to 15 wt%. In comparison, the maximum polymerization rates of the lysine-containing formulations are comparable (see SI Figure 1) and higher than that of the control formulations. This result is mainly attributed to the higher free radical concentration during light irradiation, and supported the coinitiator effect of amine group [14].

The neutralization behavior of the control and experimental samples are presented in Figure 3. With the control formulations, the pH values of the LA solution are similar, i.e. about 4.0 after 10 days. When the lysine-containing specimens were soaked in LA solution, the pH values showed a rapid increase, e.g. within 2 days the pH plateaued at 7.5 for the 5 wt% lysine-containing samples. The main reason is attributed to the leaching of lysine from the polymer. The neutralization rate is dependent upon the lysine concentration in the polymer.

TGA was used to investigate the thermal degradation behavior (the results are shown in Figure 4) and with this technique, the detection limit for lysine in lysine-containing polymer is lower than 1 wt% (see SI Figure 2). Based on the neutralization test, Lysine-2 sample showed a very promising result in neutralizing the lactic acid, and therefore, it was selected for the TGA test. For the nonsoaked samples, a mass loss began at $150\text{ }^{\circ}\text{C}$, which was probably attributed to the presence of low molecular weight fractions, such as unpolymerized monomers. This result was supported by the DC results, which indicated that

the conversion of C=C bond was about 90% and unpolymerized monomers remained in the crosslinked network. For the soaked samples, no mass loss was observed below 250 °C, which indicated that the polymers are stable and do not degrade by heating below 250 °C. Since all samples (nonsoaked or soaked) were first dried to constant weight before TGA testing, the difference in mass loss for the control and experimental samples is not attributable to solvent loss. The nonsoaked Lysine-2 polymer showed a maximum temperature of degradation at 220 °C, which was attributed to the degradation of lysine via dehydration or deamination reaction [42]. Meanwhile, only one maximum temperature of degradation at 447 °C was detected with further heating. Comparing with the C0-2 or soaked Lysine-2 polymer, the maximum temperature of degradation at around 370–380 °C may be attributed to the loosely crosslink regions (polyHEMA-rich) [43]. On the other hand, the soaked Lysine-2 sample decomposed slower than soaked control and showed some thermal stabilization as the degradation proceeds, especially when temperature is below 450 °C. These results indicated that the thermal stability of lysine-containing samples was improved. The enhanced thermal stability may be due to an improvement in the crosslink density of the loosely crosslink regions as a result of the co-initiator effect of lysine [14].

The potential impact of lysine on the crosslinked structure is noted in the TGA results at 535–600 °C. At 600 °C the mass loss for the control (both nonsoaked and soaked) and nonsoaked lysine specimens is about 90%. For these specimens at 600 °C there is about 10% remaining in the backbone of the polymer. In comparison with the soaked lysine specimens there is total mass loss at 600 °C. These results suggest that lysine impacts the crosslinked structure and due to the hydrophilic nature of lysine the impact on the crosslinked structure appears potentiated during soaking.

Lysine, as one of the essential amino acids, is a biocompatible weak base and has a positive effect on the absorption of calcium and the formation of collagen with other nutrients [44]. By using lysine instead of the amine monomers that were used in our previous work to provide neutralization capacity [16, 17], the risk of cytotoxicity could be reduced. Meanwhile, the neutralization experiments have shown that the lysine-containing adhesives provide promising neutralization capacity in the short-term. Moreover, the leaching study showed that the accumulative concentrations were 160 and 213 µg/mL for 2.5 and 5 wt% lysine-containing polymer, respectively in the first 24 h. The leach rates decreased gradually with soaking time. The calculated release percentage indicated about 50 wt% of the lysine was leached within one month (calculation method sees SI). The TGA results indicated that lysine was not retained in the polymer after soaking and these results contradicted the HPLC analysis. The primary reason for these contradictory results was the lack of stability of lysine under conditions of the leaching study [45].

The storage modulus and T_g of the lysine-containing formulations decreased slightly as compared to the control in dry conditions. Lysine lacks free radical polymerizable functional group and could not be copolymerized into the polymer network—the monomer molar concentration (HEMA and BisGMA) was slightly diluted (shown in Table 2), therefore, decreasing the crosslinking density.

Lysine-containing adhesives are expected to provide an acute neutralization to the acidic micro-environment at the tooth/composite interface. This effect offers promise as one component of a multi-faceted approach to reduce the negative impact of bacteria at the margins of composite restorations. Future work will focus on reducing the leaching of lysine and providing delayed neutralization ability by exploring approaches to incorporate amino acids into dental adhesives.

Conclusion

Lysine-containing dental adhesives have been evaluated in this work. The presence of lysine can increase the degree of conversion of methacrylate and the thermal stability. The lysine-containing adhesives show promise in terms of acute buffering of the acidic micro-environment. These capabilities offer potential for reducing the outgrowth of aciduric bacteria and mitigating the damage associated with lactic acid. These results lay the foundation for the future development of bio-macromolecule-containing dental adhesives, which will offer pH modulation at the interfacial margin between the composite and tooth.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Dental adhesive capable of neutralization was achieved by incorporating lysine.
- Lysine promoted the degree of conversion of methacrylate and thermal stability of polymer
- Lysine did not compromise the mechanical properties of polymer in dry and wet conditions.

Figure 1.
L-lysine structures

Figure 2.

Real-time conversion of the adhesive resins. The adhesives were light-cured for 40 sec using a commercial visible-light-curing unit (Spectrum® 800, Dentsply, Milford, DE. Intensity is 550 mW/cm⁻²). Real-time IR spectra were continuously recorded for 600 s after light activation began. N= 3 ± SD. *Significantly ($p < 0.05$) different from the control formulation (C0-1).

Figure 3.
pH values of lactic acid (0.1 mM) solution containing control and experimental disc specimen versus time at 23 ± 2 °C.

Figure 4. Thermogravimetric analysis curves (TGA and DTG) for the lysine, control and experimental polymers in nitrogen.

Figure 5.
Cumulative lysine release from the adhesives as a function of incubation time (in 30 days) in buffer solution (NaHCO_3 , 0.1 M). $N=3 \pm \text{SD}$.

Figure 6. Representative storage modulus (A) and $\tan \delta$ (B) vs. temperature curves of the control and experimental specimens in dry conditions.

Figure 7. Representative storage modulus (A) and tan δ (B) vs. temperature curves of the control and experimental specimens in wet conditions.

Table 1 -

Weight percent (wt%) of the monomers, initiators and solvents in the two controls and two experimental adhesives

Formulations	HEMA wt%	BisGMA wt%	Lysine wt%	D ₂ O wt%	Ethanol wt%	CQ wt%	EDMAB wt%	DPIHP wt%
CO-1	40.7	49.8	0.0	2.5	5.0	0.5	0.5	1.0
CO-2	37.4	45.7	0.0	5.0	10.0	0.5	0.5	1.0
Lysine-1	39.6	48.4	2.5	2.5	5.0	0.5	0.5	1.0
Lysine-2	35.1	42.9	5.0	5.0	10.0	0.5	0.5	1.0

The mass ratio of HEMA/BisGMA is 45/55 for all the formulations.

Table 2 -

Molar Concentration (mol g^{-1}) of the components in the two controls and two experimental adhesives

Formulations	HEMA $\text{mol/g} \times 10^{-3}$	BisGMA $\text{mol/g} \times 10^{-4}$	Lysine $\text{mol/g} \times 10^{-4}$	D ₂ O $\text{mol/g} \times 10^{-3}$	Ethanol $\text{mol/g} \times 10^{-3}$	CQ $\text{mol/g} \times 10^{-5}$	EDMAB $\text{mol/g} \times 10^{-5}$	DPIHP $\text{mol/g} \times 10^{-5}$
CO-1	3.1	9.7	0.0	1.2	1.1	3.0	2.6	2.3
CO-2	2.9	8.9	0.0	2.5	2.2	3.0	2.6	2.3
Lysine-1	3.0	9.4	1.7	1.2	1.1	3.0	2.6	2.3
Lysine-2	2.7	8.4	3.4	2.5	2.2	3.0	2.6	2.3

Table 3 -

DMA data for two controls and two experimental adhesives tested under dry conditions

Sample	Storage modulus at 25 °C (MPa)	Storage modulus at 37 °C (MPa)	Storage modulus at 180 °C (MPa)	T _g (°C)*
CO-1	3741±102	3422±99	52.8±1.8	123.6±0.6
Lysine-1	3517±271	3189±256	46.6 ^a ±1.6	116.6 ^a ± 1.8
CO-2	2882±44	2499±42	39.2±1.7	109.0± 1.1
Lysine-2	2650±479	2246±423	27.5 ^b ±1.4	101.7 ^b ±2.4

Values are mean (± standard deviation) for n = 5 in each group.

^aSignificantly ($p<0.05$) different from the control (C0-1)

^bSignificantly ($p<0.05$) different from the control (C0-2)

* The glass transition temperatures (T_g) values of the polymer networks were taken to be the maximum of the tan δ versus temperature curve determined using a dynamic mechanical analyzer.

Table 4 -

DMA data for two controls and two experimental adhesives tested under wet conditions

Sample	Storage modulus at 25°C (MPa)	Storage modulus at 37°C (MPa)	Storage modulus at 70°C (MPa)	T _g (°C)*
CO-1	1932±52	1639±43	509.3±15.8	50.2± 0.2
Lysine-1	1741 ^a ±28	1474 ^a ±29	488.3±8.6	50.2±0.1
CO-2	1484±17	1255±13	448.3±2.4	49.7±0.1
Lysine-2	1380±26	1159±22	436.3±8.4	48.8 ^b ± 0.2

Values are mean (± standard deviation) for n = 5 in each group.

^aSignificantly ($p<0.05$) different from the control (C0-1)

^bSignificantly ($p<0.05$) different from the control (C0-2)

* The glass transition temperatures (T_g) values of the polymer networks were taken to be the minimum of the Deriv. storage modulus versus temperature curve determined using a dynamic mechanical analyzer.