Polymerization Behavior of Hydrophilic-Rich Phase of Dentin Adhesive

Journal of Dental Research
2015, Vol. 94(3) 500–507
© International & American Associations
for Dental Research 2015
Reprints and permissions:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/0022034514565646
jdr.sagepub.com

F. Abedin^{1,2}, Q. Ye¹, R. Parthasarathy¹, A. Misra^{1,3},
and P. Spencer^{1,4}

Abstract

The 2-fold objectives of this study were 1) to understand whether model hydrophobic- and hydrophilic-rich phase mimics of dentin adhesive polymerize similarly and 2) to determine which factor, the dimethacrylate component, bisphenol A glycerolate dimethacrylate (BisGMA) or photoinitiator concentration, has greater influence on the polymerization of the hydrophilic-rich phase mimic. Current dentin adhesives are sensitive to moisture, as evidenced by nanoleakage in the hybrid layer and phase separation into hydrophobic- and hydrophilic-rich phases. Phase separation leads to limited availability of the cross-linkable dimethacrylate monomer and hydrophobic photoinitiators within the hydrophilic-rich phase. Model hydrophobic-rich phase was prepared as a single-phase solution by adding maximum wt% deuterium oxide (D_2O) to HEMA/BisGMA neat resins containing 45 wt% 2-hydroxyethyl methacrylate (HEMA). Mimics of the hydrophilic-rich phase were prepared similarly but using HEMA/BisGMA neat resins containing 95, 99, 99.5, and 100 wt% HEMA. The hydrophilic-rich mimics were prepared with standard or reduced photoinitiator content. The photoinitiator systems were camphorquinone (CQ)/ethyl 4-(dimethylamino)benzoate (EDMAB) with or without [3-(3, 4-dimethyl-9-oxo-9H-thioxanthen-2-yloxy)-2-hydroxypropyl]trimethylammonium chloride (QTX). The polymerization kinetics was monitored using a Fourier transform infrared spectrophotometer with a time-resolved collection mode. The hydrophobic-rich phase exhibited a significantly higher polymerization rate compared with the hydrophilic-rich phase. Postpolymerization resulting in the secondary rate maxima was observed for the hydrophilic-rich mimic. The hydrophilic-rich mimics with standard photoinitiator concentration but varying cross-linker (BisGMA) content showed postpolymerization and a substantial degree of conversion. In contrast, the corresponding formulations with reduced photoinitiator concentrations exhibited lower polymerization and inhibition/delay of postpolymerization within 2 h. Under conditions relevant to the wet, oral environment, photoinitiator content plays an important role in the polymerization of the hydrophilic-rich phase mimic. Since the hydrophilic-rich phase is primarily water and monomethacrylate monomer (e.g., HEMA as determined previously), substantial polymerization is important to limit the potential toxic response from HEMA leaching into the surrounding tissues.

Keywords: phase separation, polymerization kinetics, postpolymerization, photoinitiator, hydrophobic/hydrophilic components, water

Introduction

Infiltration of resin within interfibrillar space of demineralized dentin results in the formation of the hybrid layer, which consists of resin and collagen (Hashimoto et al. 2011; Pashley et al. 2011). The hybrid layer has been reported to be the weakest link in dentin/adhesive bond (Spencer et al. 2010). Demineralized dentin is 30% collagen and 70% water (Pashley et al. 2011). Retaining this moisture provides a more porous collagen network and more space for the adhesive infiltration. Under clinical conditions, it is difficult to control the amount of moisture present within the demineralized dentin matrix. In the presence of excess moisture, dentin adhesives can undergo phase separation into hydrophobic and hydrophilic-rich phases, leading to

limited infiltration of the cross-linker into the wet, demineralized dentin matrix (Spencer and Wang 2002; Toledano

¹Bioengineering Research Center, University of Kansas, Lawrence, KS, USA

²Bioengineering Graduate Program, University of Kansas, Lawrence, KS, USA

³Department of Civil Engineering, University of Kansas, Lawrence, KS, USA

⁴Department of Mechanical Engineering, University of Kansas, Lawrence, KS, USA

Corresponding Author:

Q. Ye, Bioengineering Research Center, University of Kansas, 1530 W. 15th St, Lawrence, KS 66045-7609, USA.
Email: yeq@ku.edu

et al. 2012). A ternary phase diagram of model dentin adhesive composed of hydrophobic bisphenol A glycerolate dimethacrylate (BisGMA), hydrophilic 2-hydroxyethyl methacrylate (HEMA), and water (Ye et al. 2011) has been used to determine quantitatively water miscibility, solubility, and phase partitioning behavior of dentin adhesive (Ye et al. 2013; Misra et al. 2014). The composition of the model hydrophilic-rich phase was determined quantitatively, and the results indicated limited cross-linkable dimethacrylate monomer and photoinitiators in the hydrophilic-rich phase (Ye et al. 2012). A separate but complementary investigation showed solvent- and polymerization-induced phase separation (SIPs and PIPs) in the hydrophilic-rich phase mimics (Abedin et al. 2014). The composition of the hydrophilic-rich phase is primarily water and HEMA. It is conceivable that, under clinical conditions, with limited polymerization of the hydrophilic-rich phase, there could be significant leaching of HEMA into the surrounding tissues. This is concerning since *in vitro* investigations report that HEMA can induce apoptosis, interfere with DNA synthesis and the production of reactive oxygen species (ROS), and also cause inhibition of mineral formation of dentin (Hanks et al. 1991; Spagnuolo et al. 2004; Chang et al. 2005; Paranjpe et al. 2005; Spagnuolo et al. 2006; Diamanti et al. 2013). In addition, low concentrations of HEMA can interfere significantly with the expression of type I collagen by gingival fibroblasts (Falconi et al. 2007).

The current study focuses on the etch-and-rinse adhesive system. This study gives insight into the polymerization behavior of the dentin adhesive when exposed to an excessive hydrated condition. The *in situ* polymerization kinetics of dilute dentin adhesive solutions was investigated; these solutions could be mimics for the hydrophilic-rich phase arising within the hybrid layer (Ye et al. 2011). The 2-fold objectives of the current investigation were 1) to understand the difference between the polymerization kinetics of hydrophobic and hydrophilic-rich phases and 2) to determine which factor, cross-linker or photoinitiator concentration, has a greater influence on polymerization of the hydrophilic-rich phase. The following null hypotheses were tested: 1) the degree of conversion and polymerization rate profile of the hydrophilic-rich phase are similar to the hydrophobic-rich phase, and 2) cross-linkable dimethacrylate and photoinitiator concentrations are equally important for substantial polymerization of the hydrophilic-rich phase.

Materials and Methods

Composition and Preparation of Hydrophobic- and Hydrophilic-Rich Phase Mimics

The monomers for the mimics consisted of HEMA (Acros Organics, Geel, Belgium) and BisGMA (Polysciences, Washington, PA, USA). The photoinitiators used in this

study were camphorquinone (CQ) as a hydrophobic photosensitizer and ethyl 4-(dimethylamino)benzoate (EDMAB) as a hydrophobic reducing agent (both from Aldrich, Milwaukee, WI, USA) (Ye et al. 2008). [3-(3, 4-Dimethyl-9-oxo-9H-thioxanthen-2-yloxy)-2-hydroxypropyl]trimethylammonium chloride (QTX, from Sigma-Aldrich, St. Louis, MO, USA) was selectively used as a hydrophilic photosensitizer based on our previous study (Ye et al. 2009). All of these materials were used as received, and each formulation was prepared in triplicate.

The composition for the formulations containing standard concentration of photoinitiator is given in Table 1, and the composition for the formulations containing a reduced amount of photoinitiators is given in Table 2. Hydrophobic-rich phase formulations were made from the neat resin, consisting of HEMA to BisGMA at a 45:55 wt% ratio, and approximately 10 wt% deuterium oxide (D_2O ; Cambridge Isotope Laboratories, Andover MA, USA) was added until the miscibility limit was reached (Ye et al. 2008). Model hydrophilic-rich phase mimics were made from HEMA/BisGMA neat resins containing 95, 99, 99.5, and 100 wt% HEMA, respectively (Ye et al. 2011). D_2O was added into these neat resins to reach the miscibility limit.

Past investigations indicate that 0.5 wt% photoinitiator concentration is adequate to initiate the polymerization of dentin adhesive (Ye et al. 2007a; Musanje et al. 2009; Schneider et al. 2009; Ye et al. 2009). To prepare the neat resins, 0.5 wt% of CQ and EDMAB each was added to the desired wt% of HEMA, and the mixture was agitated until the photoinitiators (CQ and EDMAB) dissolved. The desired wt% BisGMA was then added to the solution, and the sample was agitated overnight to ensure a homogeneous neat resin. To prepare samples at the miscibility limit, D_2O was added dropwise to the neat resin until a single drop turned the solution turbid. Then neat resin was added dropwise until the solution turned clear. The photoinitiator concentration was replenished based on the wt% of the added D_2O . Addition of the photoinitiators turned the solution turbid again, and then the neat resin was added dropwise until the solution turned clear.

Hydrophilic-rich mimics with reduced photoinitiator concentration were prepared similarly, but the photoinitiator was not refilled after the addition of the D_2O , causing the concentration of the photoinitiator to decrease (Table 2). More D_2O was added to the neat resins with increased hydrophilicity (Ye et al. 2011).

Figure 1 indicates the composition of all the formulations studied on the ternary phase diagram of HEMA, BisGMA, and D_2O . The formulations (HB45PB, HB95PB, HB95PB-QTX, HB99PB, HB99.5PB, and HB100PB) contained maximum single-phase D_2O content. Description of formulation name: HB stands for HEMA/BisGMA, and the number following HB is the weight percentage of HEMA in the neat resin from which the sample was made, and PB

Table 1. Composition of Each Component in the Formulations at Phase Boundary with Standard Photoinitiator Concentrations, Their Average Degree of Conversion, and Rate of Polymerization.

Formulation Name	HEMA in Neat Resin (wt%)	wt% of HEMA	wt% of BisGMA	wt% of D ₂ O at Miscibility	Degree of Conversion	Initial Polymerization Rate (s ⁻¹) × 10 ⁴	Post-polymerization Rate (s ⁻¹) × 10 ⁴	Appearance after Polymerization
HB45PB	45.0	40.26 ± 0.06	49.12 ± 0.04	10.62 ± 0.09	0.80 ± 0.07	297 ± 25	NA	Translucent
HB95PB	95.0	39.95 ± 0.50	2.09 ± 0.03	57.97 ± 0.52	0.94 ± 0.03 ^a	16.1 ± 5.4 ^b	3.4 ± 0.3	Turbid white
HB95PB-QTX	95.0	38.08 ± 0.08	2.00 ± 0.01	59.92 ± 0.09	0.83 ± 0.03	21.5 ± 0.5 ^b	2.0 ± 0.8	Turbid white
HB99PB	99.0	29.17 ± 1.37	0.29 ± 0.02	70.54 ± 1.39	0.95 ± 0.01 ^a	15.9 ± 3.3 ^b	2.1 ± 0.3	Turbid white
HB99.5PB	99.5	27.20 ± 1.50	0.14 ± 0.01	72.67 ± 1.50	0.97 ± 0.03 ^a	14.9 ± 2.9 ^b	2.4 ± 0.1	Turbid white
HB100PB	100	24.47 ± 0.11	NA	75.53 ± 0.11	0.78 ± 0.18	14.3 ± 3.3 ^b	1.4 ± 0.5	Translucent

Photoinitiators camphorquinone (CQ) and ethyl 4-(dimethylamino)benzoate (EDMAB) are both 0.5 wt% in the samples above, and in the case of sample HB95-QTX, CQ and [3-(3, 4-dimethyl-9-oxo-9H-thioxanthen-2-yloxy)-2-hydroxypropyl]trimethylammonium chloride (QTX) are each 0.25 wt%. BisGMA, bisphenol A glycerolate dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; NA, not applicable.

^aDegree of conversion of hydrophilic-rich phase is significantly different (one-way analysis of variance [ANOVA] at $\alpha = 0.05$) from control hydrophobic-rich phase (HB45PB).

^bInitial rate of polymerization of hydrophilic-rich phase is significantly different (one-way ANOVA at $\alpha = 0.05$) from control hydrophobic-rich phase (HB45PB).

Table 2. Composition of Each Component in the Formulations at Phase Boundary Containing Reduced Photoinitiator Concentrations, Their Average Degree of Conversion, and Rate of Polymerization.

Formulation Name	wt% of HEMA in Neat Resin	wt% of HEMA	wt% of BisGMA	wt% of D ₂ O at Miscibility	wt% of CQ	wt% of QTX	wt% of EDMAB	Degree of Conversion	Initial Polymerization Rate (s ⁻¹) × 10 ⁴	Post-polymerization Rate (s ⁻¹) × 10 ⁴	Appearance after Polymerization
HB95PB	95.0	39.15 ± 0.36	2.07 ± 0.03	58.34 ± 0.38	0.22 ± 0.01	NA	0.22 ± 0.01	0.24 ± 0.06	13.7 ± 3.3	NA	Transparent
HB95PB-QTX	95.0	37.98 ± 0.24	2.00 ± 0.01	59.61 ± 0.25	0.10 ± 0.01	0.10 ± 0.00	0.21 ± 0.01	0.57 ± 0.13	16.2 ± 1.0	1.7 ± 0.4	Turbid white
HB99PB	99.0	28.32 ± 0.45	0.29 ± 0.01	71.10 ± 0.46	0.14 ± 0.01	NA	0.15 ± 0.00	0.02 ± 0.01	11.3 ± 4.3	NA	Transparent
HB99.5PB	99.5	24.46 ± 0.30	0.13 ± 0.00	75.15 ± 0.30	0.13 ± 0.01	NA	0.13 ± 0.00	0.03 ± 0.01	13.2 ± 1.7	NA	Transparent

BisGMA, bisphenol A glycerolate dimethacrylate; CQ, camphorquinone; EDMAB, ethyl 4-(dimethylamino)benzoate; HEMA, 2-hydroxyethyl methacrylate; NA, not applicable; QTX, [3-(3, 4-dimethyl-9-oxo-9H-thioxanthen-2-yloxy)-2-hydroxypropyl]trimethylammonium chloride.

Figure 1. Phase diagram for ternary mixtures composed of D₂O, bisphenol A glycerolate dimethacrylate (BisGMA), and 2-hydroxyethyl methacrylate (HEMA). The dashed lines represent the phase boundary line. The filled squares and circles on the phase boundary line represent experimental formulations with standard and reduced photoinitiator concentrations, respectively.

represents phase boundary (at the miscibility point). Formulation HB95PB-QTX is made from neat resin containing both CQ and QTX as hydrophobic and hydrophilic photosensitizers. It is noted that 0.25 wt% of each was added instead of 0.5 wt% of CQ (Ye et al. 2009).

Photopolymerization and Kinetics Study

The mimics were light-cured for 40 s using a dental curing light (Spectrum 800; Dentsply, Milford, DE, USA) operated at 550 mW/cm². The photopolymerization kinetics was monitored in situ by a time-resolved spectrum collector using a PerkinElmer Spectrum 400 (PerkinElmer, Waltham, MA, USA) Fourier transform infrared spectrophotometer (FTIR) with a resolution of 4 cm⁻¹ in the ATR sampling mode (Ye et al. 2007a; Ye et al. 2007b). A fixed amount of liquid at 30 μ L was placed on the horizontal face of the internal reflectance crystal. A transparent plastic cover slip was placed on the sample, and its edges were sealed using tape to prevent D₂O evaporation during the collection of the data (Abedin et al. 2014). In this study, D₂O was used

instead of H_2O because D_2O does not exhibit overlapping spectral features with the aliphatic $\text{C}=\text{C}$ double bond that was monitored. The change in the ratio of band intensities measured at 1637 cm^{-1} ($\text{C}=\text{C}$) to that at 1716 cm^{-1} (carbonyl) was monitored. Measurements on 3 samples for each formulation were made. The degree of conversion (DC) was calculated using the following equation:

$$\text{DC} = \left(1 - \frac{\text{Absorbance}_{1637\text{ cm}^{-1}}^{\text{sample}} / \text{Absorbance}_{1716\text{ cm}^{-1}}^{\text{sample}}}{\text{Absorbance}_{1637\text{ cm}^{-1}}^{\text{monomer}} / \text{Absorbance}_{1716\text{ cm}^{-1}}^{\text{monomer}}} \right) \times 100\%.$$

The rate of polymerization was evaluated from the first derivative of the time against conversion curve.

Viscosity Measurement and Statistical Analysis

Viscosity of the liquid formulations was measured at various shear rates using a Brookfield DV-II+Pro (Brookfield, Middleborough, MA, USA) viscometer in a cone/plate setup. There were 3 samples per formulation. A volume of 0.5 mL of the formulation was placed in the viscometer, and the viscosity was measured at $25.0 \pm 0.2\text{ }^\circ\text{C}$. The differences in viscosity, degree of conversion, and polymerization rate between hydrophobic-rich and hydrophilic-rich samples were evaluated using one-way analysis of variance (ANOVA), together with Tukey's test at $\alpha = 0.05$ to identify significant differences.

Results

Polymerization Kinetics and Viscosity of Hydrophobic- and Hydrophilic-Rich Phase Mimics with Standard Photoinitiator Concentration

There was a statistically significant difference in the viscosities and polymerization kinetics of hydrophobic and hydrophilic-rich samples (Fig. 2). The viscosity of the hydrophobic-rich sample, HB45PB, was much higher ($P < 0.001$) than the hydrophilic-rich samples. Figure 2A, B shows that the hydrophobic-rich samples (HB45PB) polymerized much faster ($P < 0.001$) than the hydrophilic-rich samples and reached the DC plateau ($\sim 80\%$) within ~ 2 min. The hydrophilic-rich samples underwent postpolymerization, and they reached a substantial degree of conversion (at about 70 to 90 min). Table 1 shows that the initial rate of polymerization is significantly higher ($P < 0.001$) for the hydrophobic-rich phase compared with the hydrophilic-rich phase, and the former does not exhibit postpolymerization.

Polymerization Kinetics Study of Hydrophilic-Rich Phase with Standard Photoinitiator Concentration

It was observed that standard hydrophilic formulations HB95PB, HB99PB, and HB99.5PB all exhibited 90% to 95%

Figure 2. Polymerization kinetics (A, B) and viscosities (C) of hydrophobic and hydrophilic-rich phases.

Figure 3. Polymerization kinetics study with different cross-linker and photo-initiator concentrations. (A) Degree of conversion of samples at miscibility containing standard concentration of photoinitiator. (B) Rate of polymerization of samples at miscibility containing standard concentration of photoinitiator. (C) Degree of conversion of samples at phase boundary containing reduced photoinitiator concentrations. (D) Rate of polymerization of sample HB95PB and HB95PB-QTX with reduced photoinitiator concentration compared with sample containing standard photoinitiator concentration.

degree of conversion, as shown in Figure 3A. At approximately 2 h, standard formulations HB95PB-QTX and HB100PB exhibited 83% and 78% average degree of conversion, respectively. All of the above samples, with the exception of HB100PB, turned opaque white after substantial polymerization, indicating PIPs and SIPs (Abedin et al. 2014). A lower postpolymerization rate maximum was observed for samples without BisGMA (HB100PB) compared with samples containing BisGMA, as shown in Figure 3B. Table 1 indicates that when the concentration of the cross-linker, BisGMA, is decreased, the degree of conversion is still substantial, indicating that BisGMA has limited influence on the degree of conversion of the model hydrophilic-rich phase.

Polymerization Kinetics of Hydrophilic-Rich Phase with Reduced Photoinitiator Concentration

Reduced photoinitiator concentration had an adverse impact on the degree of conversion of the hydrophilic-rich samples within 2 h, as shown in Figure 3C. Reduced photoinitiator

concentration also inhibited or delayed the postpolymerization within the 2 h, as shown in Figure 3D. The degree of conversion after 2 h was very low for samples with reduced photoinitiator concentration (Table 2), except for the formulation containing hydrophilic photosensitizer, QTX. The latter formulation exhibited delayed postpolymerization, within 2 h, which resulted in the higher degree of conversion. HB95PB-QTX (standard and reduced photoinitiator [PI]) exhibited a higher initial rate of polymerization compared with the other hydrophilic-rich formulations. Comparing the corresponding formulations for standard and reduced PI in Table 1 and Table 2, it can be seen that within the 2-h time frame, the decrease in photoinitiator concentration has a significant impact on the degree of conversion of the hydrophilic-rich phase mimics.

Discussion

With wet bonding techniques, the channels between the demineralized dentin collagen fibrils are filled with water, solvent, conditioner, and/or oral fluids. Since,

under clinical conditions, there is little control over the amount of water permeating the prepared dentin substrate, it is possible that as the adhesive interacts with the wet dentin surface, it will separate into hydrophobic- and hydrophilic-rich phases (Spencer and Wang 2002).

Commercial adhesives were not selected for the current study because of their unknown and complex composition. Previous results have shown that the model adhesive (HEMA/BisGMA 45/55) can mix with approximately 10 wt% water (Ye et al. 2008; Ye et al. 2012). The gel effect is responsible for the high rate of polymerization observed for the hydrophobic-rich phase (Norrish and Smith 1942). This is prominent in the case of the viscous system (i.e., HEMA/BisGMA 45/55) (Ye et al. 2007a; Ye et al. 2007b; Ye et al. 2008). For the hydrophobic-rich phase, formation of cross-linked polymer limits the movement of macro-radicals, and therefore the termination step becomes diffusion limited. The decrease in the termination rate results in an increase in the polymerization rate, and this is known as the auto-acceleration or gel effect (Zhang et al. 2008).

BisGMA is an important component of dental resin because of the chemical and mechanical properties it can impart to the material. The hydrophobic-rich phase is viscous due to the substantial concentration of BisGMA. The hydrophilic-rich phase mimic is not viscous and not rich in monomer concentrations like the hydrophobic-rich phase mimic. The composition of the hydrophilic-rich phase mimic is primarily D₂O and HEMA. Hence, despite having a similar photoinitiator composition, the possibility of free radicals to initiate a polymerization reaction within the hydrophilic-rich phase is low compared with the hydrophobic-rich phase. This could result in the low degree of conversion at the beginning and slow initial rate of reaction compared with the hydrophobic-rich phase. The reactive species within the hydrophilic-rich phase do not experience the restricted mobility that occurs in the hydrophobic-rich phase. Substantial postpolymerization is noted in the hydrophilic-rich phase with standard photoinitiator concentration. Therefore, we reject our first null hypothesis since the polymerization kinetics of the hydrophobic-rich phase is very different from the hydrophilic-rich phase.

Previous investigations have also reported a bimodal rate of polymerization for the methacrylate system (Horie et al. 1975; Anseth et al. 1994; Yu et al. 2001). Horie et al. (1975) suggested that there could be an accumulation of pendant double bonds during polymerization due to their reactivity being lower than the free monomers. During later stage of the polymerization, these pendant double bonds could form higher cross-linked regions called microgels. The trapped pendant double bonds trigger a secondary gel effect within the microgels, allowing the reaction to continue through the postpolymerization and resulting in the secondary rate maxima (Horie et al. 1975).

For the hydrophilic-rich phase, we suggested a possible polymerization mechanism involving PIPs and SIPs (Abedin et al. 2014). Based on the proposed mechanism, microgels form following auto-acceleration, which are localized regions of a higher degree of conversion and/or cross-linking density. During polymerization, the microgels precipitate out due to PIPs and SIPs. Reactive species are able to diffuse into the microgels and become trapped, resulting in a secondary gel effect. At a later stage, the D₂O could separate as droplets when the miscibility limit of the D₂O in the polymer matrix is exceeded (Abedin et al. 2014). Although the hydrophobic-rich phase may also undergo PIPs and SIPs, it is possible that the phase separation is taking place at the same time as the auto-acceleration. Diffusion of reactive species is very likely restricted for the hydrophobic-rich phase—restricting the diffusion of the reactive species prevents a substantial postpolymerization stage.

Variables that may affect the polymerization kinetics study are evaporation, sample thickness, light intensity, and dissolved oxygen. Evaporation of D₂O was prevented by sealing the sides of the cover slip, and by drawing the same

volume of the sample, the thickness of the sample was kept constant. Constant light intensity was ensured by maintaining the energy and time of exposure at 550 mW/cm² and 40 s, respectively. To eliminate the interference of the viscosity change arising from the evaporation of D₂O with the reaction mechanism, the D₂O profile was monitored in situ during the polymerization (Abedin et al. 2014). Since the hydrophilic-rich phase contained a large amount of D₂O, these samples would possess dissolved oxygen, which could affect the polymerization behavior. Samples without BisGMA (HB100PB) were very dilute, and the viscosities were very low. As a result, it is likely that there could be rapid diffusion of oxygen within these samples during the polymerization. Since these samples contained the highest D₂O composition, it is reasonable that the substantial amount of dissolved oxygen interfered with the polymerization. These factors may have contributed to the large standard deviation in the degree of conversion for this formulation.

The hydrophilic-rich mimics with standard photoinitiator content but without BisGMA show the lowest postpolymerization. For this formulation, the microgels that form during initial polymerization may just be linear poly(HEMA), and this can be soluble in the cosolvent system (e.g., HEMA and D₂O) at an early stage. It is likely that the formation of microgels is limited and the precipitation of microgels is much lower, trapping fewer reactive species. This could lead to lower postpolymerization. Even if PIPs and SIPs are taking place for this composition (HB100PB), large-scale phase separation in excess of the micrometer scale may be difficult. All of the hydrophilic-rich phases containing cross-linker have similar viscosity and exhibit similar polymerization behavior. For the samples containing reduced photoinitiator concentration, the average degree of conversion is lower compared with the corresponding formulations with standard photoinitiator concentration. Therefore, we reject our second null hypothesis since photoinitiator concentration plays a vital role in the degree of conversion of the hydrophilic-rich phase.

The overall availability of free radicals is decreased for formulations with reduced photoinitiator concentration. Gao and Nie (2006) demonstrated that with increasing concentration of photoinitiators, the number of radicals produced per unit volume increased. Samples with reduced photoinitiator led to limited availability of sufficient free radicals to result in postpolymerization within the 2 h. Although the initial polymerization rates are similar for the formulations HB95PB, HB99PB, and HB99.5PB with reduced photoinitiator (PI), the degree of conversion for HB95PB and HB95-QTX (reduced PI) is higher than the other 2 formulations. It is possible that for HB95PB (reduced PI), the generation of effective free radicals is higher compared with HB99PB (reduced PI) and HB99.5PB (reduced PI) since the former contains a higher concentration of PI. Moreover, the

monomer concentration for HB95PB (reduced PI) is higher than the other 2. QTX is more water soluble than CQ (Ye et al. 2009). It is possible that for the formulations containing QTX and CQ, the free radicals produced are able to diffuse more freely since they are more compatible with the current hydrophilic-rich formulations that are being studied. This could result in higher initial rate of polymerization and also the delayed postpolymerization for the reduced PI formulation. This shows that efficiency and compatibility of photoinitiators for the system are also important factors that should be considered.

The samples are mimics of the hydrophilic-rich phase, and the polymerization kinetics was investigated under conditions that limit D₂O evaporation and oxygen diffusion. These conditions do not represent the clinical setting. The variation of the dissolved oxygen under in vitro and in vivo conditions may be a significant factor influencing the photopolymerization behavior of these samples. High degree of conversion of the hydrophilic-rich phase at the miscibility limit may still yield a degradable polymer or a polymer with very poor mechanical integrity due to the low cross-linking density (Misra et al. 2014). Degradation of poorly polymerized hydrophilic-rich phases would result in leaching of HEMA and low-molecular-weight degradants into the surrounding tissues. Leaching of HEMA is of clinical concern for reasons discussed previously (Hanks et al. 1991; Spagnuolo et al. 2004; Chang et al. 2005; Paranjpe et al. 2005; Spagnuolo et al. 2006; Falconi et al. 2007). To address the clinical issues associated with the leaching of HEMA and low-molecular-weight degradants, the results from this study indicate that sufficient photoinitiator content is required to achieve substantial polymerization of the hydrophilic-rich phase. It is conceivable that incorporation of hydrophilic photoinitiators in resin will allow photoinitiators to diffuse more freely within the hydrophilic-rich phases in higher concentrations. Current dentin adhesives should be optimized to include an adequate concentration of efficient hydrophilic photoinitiators, but it must also be considered that photoinitiators in high concentration could elicit cytotoxic effect (Wessels et al. 2014). Ongoing research is exploring novel, efficient hydrophilic photoinitiators that will be compatible with resin/water in the oral cavity and capable of imparting optimal polymerization to the hydrophilic-rich phase.

Author Contributions

F. Abedin, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; Q. Ye, P. Spencer, contributed to conception, design, data analysis, and interpretation, drafted and critically revised the manuscript; R. Parthasarathy, contributed to conception and data interpretation, critically revised the manuscript; A. Misra, contributed to conception and data interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Acknowledgments

This investigation was supported by research grants R01DE14392 (PS) and R01 DE022054 (PS, JSL) from the National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

References

- Abedin F, Ye Q, Good HJ, Parthasarathy R, Spencer P. 2014. Polymerization- and solvent-induced phase separation in hydrophilic-rich dentin adhesive mimic. *Acta Biomater.* 10(7):3038–3047.
- Anseth KS, Wang CM, Bowman CN. 1994. Reaction behaviour and kinetic constants for photopolymerizations of multi(meth)acrylate monomers. *Polymer.* 35(15):3243–3250.
- Chang HH, Guo MK, Kasten FH, Chang MC, Huang GF, Wang YL, Wang RS, Jeng JH. 2005. Stimulation of glutathione depletion, ROS production and cell cycle arrest of dental pulp cells and gingival epithelial cells by HEMA. *Biomaterials.* 26(7):745–753.
- Diamanti E, Mathieu S, Jeanneau C, Kitraki E, Panopoulos P, Spyrou G, About I. 2013. Endoplasmic reticulum stress and mineralization inhibition mechanism by the resinous monomer HEMA. *Int Endod J.* 46(2):160–168.
- Falconi M, Teti G, Zago M, Pelotti S, Breschi L, Mazzotti G. 2007. Effects of HEMA on type I collagen protein in human gingival fibroblasts. *Cell Biol Toxicol.* 23(5):313–322.
- Gao X, Nie J. 2006. Low-temperature photopolymerization and post-cure characteristics of acrylates. *Polymer Int.* 56(6):707–710.
- Hanks CT, Strawn SE, Wataha JC, Craig RG. 1991. Cytotoxic effects of resin components on cultured mammalian fibroblasts. *J Dent Res.* 70(11):1450–1455.
- Hashimoto M, Nagano F, Endo K, Ohno H. 2011. A review: biodegradation of resin-dentin bonds. *Japan Dent Sci Rev.* 47(1):5–12.
- Horie K, Otagawa A, Muraoka M, Mita I. 1975. Calorimetric investigation of polymerization reactions, V: crosslinked copolymerization of methyl methacrylate with ethylene dimethacrylate. *J Polymer Sci Polym Chem Ed.* 13(2):445–454.
- Misra A, Parthasarathy R, Ye Q, Singh V, Spencer P. 2014. Swelling equilibrium of dentin adhesive polymers formed on the water-adhesive phase boundary: experiments and micro-mechanical model. *Acta Biomater.* 10(1):330–342.
- Musanje L, Ferracane JL, Sakaguchi RL. 2009. Determination of the optimal photoinitiator concentration in dental composites based on essential material properties. *Dent Mater.* 25(8):994–1000.
- Norrish R, Smith R. 1942. Catalyzed polymerization of methyl methacrylate in the liquid phase. *Nature.* 150(3803):336–337.
- Paranjpe A, Bordador LCF, Wang MY, Hume WR, Jewett A. 2005. Resin monomer 2-hydroxyethyl methacrylate (HEMA) is a potent inducer of apoptotic cell death in human and mouse cells. *J Dent Res.* 84(2):172–177.
- Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, Tezvergil-Mutluay A. 2011. State of the art etch-and-rinse adhesives. *Dent Mater.* 27(1):1–16.
- Schneider LF, Cavalcante LM, Consani S, Ferracane JL. 2009. Effect of co-initiator ratio on the polymer properties of

- experimental resin composites formulated with camphorquinone and phenyl-propanedione. *Dent Mater.* 25(3):369–375.
- Spagnuolo G, D'Anto V, Cosentino C, Schmalz G, Schweikl H, Rengo S. 2006. Effect of N-acetyl-L-cysteine on ROS production and cell death caused by HEMA in human primary gingival fibroblasts. *Biomaterials.* 27(9):1803–1809.
- Spagnuolo G, Mauro C, Leonardi A, Santillo M, Paternò R, Schweikl H, Avvedimento EV, Rengo S. 2004. NF- κ B protection against apoptosis induced by HEMA. *J Dent Res.* 83(11):837–842.
- Spencer P, Wang Y. 2002. Adhesive phase separation at the dentin interface under wet bonding conditions. *J Biomed Mater Res.* 62(3):447–456.
- Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, Wang Y, Bohaty BS, Singh V, Sene F, et al. 2010. Adhesive/dentin interface: the weak link in the composite restoration. *Ann Biomed Eng.* 38(6):1989–2003.
- Toledano M, Yamauti M, Osorio E, Monticelli F, Osorio R. 2012. Characterization of micro- and nanophase separation of dentin bonding agents by stereoscopy and atomic force microscopy. *Microsc Microanal.* 18(2):279–288.
- Wessels M, Leyhausen G, Volk J, Geurtsen W. 2014. Oxidative stress is responsible for genotoxicity of camphorquinone in primary human gingival fibroblasts. *Clin Oral Invest.* 18(6):1705–1710.
- Ye Q, Park J, Laurence J, Parthasarathy R, Misra A, Spencer P. 2011. Ternary phase diagram of model dentin adhesive exposed to over-wet environments. *J Dent Res.* 90(12):1434–1438.
- Ye Q, Park J, Parthasarathy R, Pamatmat F, Misra A, Laurence JS, Marangos O, Spencer P. 2012. Quantitative analysis of aqueous phase composition of model dentin adhesives experiencing phase separation. *J Biomed Mater Res B Appl Biomater.* 100(4):1086–1092.
- Ye Q, Park J, Topp E, Spencer P. 2009. Effect of photoinitiators on the in vitro performance of a dentin adhesive exposed to simulated oral environment. *Dent Mater.* 25(4):452–458.
- Ye Q, Park J, Topp E, Wang Y, Misra A, Spencer P. 2008. In vitro performance of nano-heterogeneous dentin adhesive. *J Dent Res.* 87(9):829–833.
- Ye Q, Parthasarathy R, Abedin F, Laurence JS, Misra A, Spencer P. 2013. Multivariate analysis of attenuated total reflection Fourier transform infrared (ATR FT-IR) spectroscopic data to confirm phase partitioning in methacrylate-based dentin adhesive. *Appl Spectrosc.* 67(12):1473–1478.
- Ye Q, Spencer P, Wang Y, Misra A. 2007a. Relationship of solvent to the photopolymerization process, properties, and structure in model dentin adhesives. *J Biomed Mater Res A.* 80(2):342–350.
- Ye Q, Wang Y, Williams K, Spencer P. 2007b. Characterization of photopolymerization of dentin adhesives as a function of light source and irradiance. *J Biomed Mater Res B Appl Biomater.* 80(2):440–446.
- Yu Q, Nauman S, Santerre JP, Zhu S. 2001. UV photopolymerization behavior of dimethacrylate oligomers with camphorquinone/amine initiator system. *J Appl Polym Sci.* 82(5):1107–1117.
- Zhang Y, Kranbuehl DE, Sautereau H, Seytre G, Dupuy J. 2008. Study of UV cure kinetics resulting from a changing concentration of mobile and trapped radicals. *Macromolecules.* 41(3):708–715.