Acta Biomaterialia 10 (2014) 3038–3047

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Polymerization- and solvent-induced phase separation in hydrophilic-rich dentin adhesive mimic

Farhana Abedin [a,b], Qiang Ye [a,*], Holly J. Good [a], Ranganathan Parthasarathy [a], Paulette Spencer [a,c]

[a] Bioengineering Research Center, University of Kansas, 1530 W. 15th Street, Lawrence, KS 66045-7609, USA
[b] Bioengineering Graduate Program, University of Kansas, 1530 W. 15th Street, Lawrence, KS 66045-7609, USA
[c] Department of Mechanical Engineering, University of Kansas, 1530 W. 15th Street, Lawrence, KS 66045-7609, USA

ARTICLE INFO

Article history:
Received 25 October 2013
Received in revised form 12 February 2014
Accepted 3 March 2014
Available online 12 March 2014

Keywords:
Phase separation
Polymerization
Hydrophilic-rich phase
Dental adhesive

ABSTRACT

Current dental resin undergoes phase separation into hydrophobic-rich and hydrophilic-rich phases during infiltration of the over-wet demineralized collagen matrix. Such phase separation undermines the integrity and durability of the bond at the composite/tooth interface. This study marks the first time that the polymerization kinetics of model hydrophilic-rich phase of dental adhesive has been determined. Samples were prepared by adding varying water content to neat resins made from 95 and 99 wt.% hydroxyethylmethacrylate and 5 and 1 wt.% (2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl1]-propane prior to light curing. Viscosity of the formulations decreased with increased water content. The photopolymerization kinetics study was carried out with a time-resolved Fourier transform infrared spectrometer. All of the samples exhibited two-stage polymerization behavior which has not been reported previously for dental resin formulation. The lowest secondary rate maxima were observed for water contents of 10–30 wt.%. Differential scanning calorimetry (DSC) showed two glass transition temperatures for the hydrophilic-rich phase of dental adhesive. The DSC results indicate that the heterogeneity within the final polymer structure decreased with increasing water content. The results suggest a reaction mechanism involving both polymerization-induced phase separation and solvent-induced phase separation for the model hydrophilic-rich phase of dental resin.

1. Introduction

The median clinical lifetime of composite dental restoration is 3–8 years, compared to 5–15 years for mercury amalgam [1]. The primary factor in the premature failure of composite restorations is recurrent decay [2], and 80–90% of recurrent decay is located at the gingival margin [3]. At this margin, the adhesive and its seal to dentin provides the primary barrier between the tooth and the environment [4]. The failure of the adhesive allows *Streptococcus mutans* and other cariogenic bacteria to penetrate the composite/tooth interface; the bacteria colonize the subsurface tissues of the tooth initiating recurrent decay. Based on clinical investigations, the most significant factor in the long-term success of the composite restoration is the integrity of the adhesive bond layer [5–7].

The factors limiting the durability of the adhesive bond layer include incomplete polymerization, partial infiltration of the demineralized dentin matrix, phase separation and hydrolytic/enzymatic degradation of the adhesive as well as the underlying demineralized collagen [8–11]. Sub-optimal polymerization leads to loosely cross-linked domains that can be readily penetrated by water. Incomplete infiltration [12] leads to water retention within the collagen/adhesive interfacial zone. As the adhesive diffuses through the wet, demineralized dentin matrix, it undergoes phase separation into hydrophobic and hydrophilic-rich phases [11,13]. Water will diffuse into the loosely cross-linked or hydrophilic domains. Water-promoted degradation of the bonds within the adhesive [14–17] may undermine the dentin/adhesive bond integrity. The exposed demineralized collagen underneath the hybrid layer, arising due to incomplete impregnation of the dentin by the resin, also undergoes hydrolytic degradation [12,18]. Investigation by Pashley et al. [19] has also suggested possible degradation of exposed collagen by host-derived matrix metalloproteinases. Degradation of the exposed demineralized dentin underneath the hybrid layer could also promote access of saliva and oral bacteria, thereby compromising the dentin/adhesive bond integrity.

Previous investigation has shown the presence of limited cross-linkable dimethacrylate monomers (2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl1]-propane, BisGMA) and hydrophobic

---

* Corresponding author. Tel.: +1 785 864 1746; fax: +1 785 864 1742.
E-mail address: yeq@ku.edu (Q. Ye).

http://dx.doi.org/10.1016/j.actbio.2014.03.001
1742-7061/

initiator in the hydrophilic-rich phase of the dentin adhesive under wet conditions [20]. If the hydrophilic-rich phase that arises due to the phase separation fails to undergo substantial polymerization, the functional groups in the system become trapped as residual monomers and unreacted pendant radicals. The leaching of these unreacted monomers into the surrounding tissues has been associated with cytotoxicity. For example, the unreacted monomethacrylate component of the adhesive, 2-hydroxyethylmethacrylate (HEMA), can cause apoptosis, and interfere with the production of reactive oxygen species (ROS) and the expression of type I collagen by gingival fibroblasts [21–24].

Ye and colleagues developed a ternary phase diagram of model dentin adhesive composed of BisGMA, HEMA and water [25]. The ternary phase diagram provides valuable quantitative information regarding the miscibility, distribution ratio and phase partitioning of the three components. As noted in the phase diagram, the model hydrophilic-rich phase contains very little cross-linker and consists mostly of the monomethacrylate component, HEMA.

The objective of this study was to understand the photopolymerization kinetics of model hydrophilic-rich phases of dental adhesive as a function of water content. In vitro continuous monitoring of the polymerization kinetics of the model hydrophilic-rich phase in methacrylate-based dentin adhesive was investigated for the first time. We hypothesize that the lowest rate of polymerization will occur at a critical concentration of water.

2. Materials and methods

2.1. Model adhesive compositions and sample preparation

The model adhesive consists of a monomethacrylate component, HEMA (Acros Organics), a dimethacrylate component, BisGMA (Polysciences, Washington, PA, USA), camphorquinone (CQ) as a hydrophobic photosensitizer and ethyl 4-(dimethylamino)benzoate (EDMAB) as a hydrophobic reducing agent (both from Aldrich, Milwaukee, WI, USA) [26]. Model hydrophilic-rich phases were prepared from neat resins consisting of HEMA/BisGMA containing 95 and 99 wt.% of HEMA (HB95NR and HB99NR, respectively). To avoid spectral interference from water, deuterium oxide (D_2O; Cambridge Isotope Laboratories Inc., Andover, MA, USA) was added to the neat resins. The final composition of the D_2O was based on the total final weight of the mixture. The concentrations of CQ and EDMAB were kept constant at 0.5 wt.% each, based on the total final weight. The D_2O content was varied from 0 wt.% to maximum single-phase composition (phase boundary). The composition of the samples is given in Table 1. Three samples for each formulation were prepared. The neat resin samples were prepared by adding 0.5 wt.% CQ and EDMAB each into the desired weight percent of HEMA. The mixture was agitated until the CQ and EDMAB had dissolved completely. The required weight percent of BisGMA was then added, followed by agitation overnight to form a homogeneous mixture of neat resin. Samples with D_2O content less than maximum single-phase composition were prepared by adding the desired weight percent of D_2O to the neat resin. The formulations are shown in a ternary phase diagram (Fig. 1a). Then 0.5 wt.% of CQ and EDMAB each was added to the mixture based on the weight of D_2O. The final concentration of the photoinitiators CQ and EDMAB was 0.5 wt.% each, based on total weight of the mixture.

Table 1
Composition of each component in formulations made from neat resin containing 95 and 99 wt.% HEMA.

wt.% HEMA	wt.% BisGMA	wt.% D_2O
Formulations made from neat resin containing 95 wt.% HEMA		
94.95 ± 0.02	5.05 ± 0.02	N/A
84.95 ± 0.47	4.51 ± 0.04	10.54 ± 0.50
75.35 ± 0.60	4.00 ± 0.06	20.65 ± 0.65
65.92 ± 0.50	3.51 ± 0.07	30.57 ± 0.56
46.91 ± 0.22	2.50 ± 0.02	50.60 ± 0.23
39.95 ± 0.50	2.09 ± 0.03	57.97 ± 0.52
Formulations made from neat resin containing 99 wt.% HEMA		
98.99 ± 0.01	1.01 ± 0.01	N/A
78.55 ± 0.86	0.81 ± 0.01	20.64 ± 0.86
69.06 ± 0.13	0.71 ± 0.01	30.23 ± 0.13
49.00 ± 0.25	0.51 ± 0.01	50.49 ± 0.26
29.2 ± 1.4	0.29 ± 0.02	70.5 ± 1.4

[*] Photoinitiators CQ and EDMAB are both 0.5 wt.% in the samples above.

The miscibility limit for samples at maximum single-phase composition was determined by cloud point detection [11]. For these samples, D_2O was added dropwise to the neat resin until the mixture turned cloudy, then the neat resin was added dropwise until the cloudy mixture turned clear. This was done carefully, as a single drop could change the appearance of the mixture. CQ and EDMAB were added to the mixture based on the weight of D_2O so that the concentration of each remained constant to 0.5 wt.% of the total weight of the mixture. Since the photoinitiators were hydrophobic, their addition to the mixture turned the solution cloudy again, which was then back titrated with the neat resin until the mixture was clear. The final mixture was a single-phase solution containing the maximum D_2O (e.g. at the miscibility limit), as indicated by the triangular and circular points on the phase boundary line in Fig. 1a.

2.2. Photopolymerization kinetics study

The polymerization kinetics was monitored in situ for approximately 2 h using a Perkin-Elmer Spectrum 400 Fourier transform infrared spectrophotometer with a resolution of 4 cm^{-1} in the ATR sampling mode. This technique was used previously to monitor the photopolymerization kinetics [26–28]. The model hydrophilic-rich phases with varying D_2O content were cured for 40 s using dental curing light, Spectrum® 800, Dentsply, Milford, DE, USA at 550 mW cm^{-2}. A fixed volume of 30 μl of the sample was placed on the ATR crystal. A transparent coverslip was placed on the sample and the edges of the coverslip were sealed with tape to prevent evaporation of D_2O. The decrease in band intensity ratio for intensity at 1637 cm^{-1} to that at 1716 cm^{-1} (carbonyl) was monitored continuously during the polymerization. The polymerization kinetics of three samples for each formulation was studied. The degree of conversion (DC) was determined using the following equation which was based on the band intensity ratio before and after curing:

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

In order to reduce the number of variables that may impact the polymerization kinetics study, the evaporation of D_2O was prevented by sealing the sides of the coverslip with tape and the thickness of the sample on the ATR crystal was kept constant by drawing 30 μl of the formulation for each measurement. For the kinetic study, the light intensity was kept constant at 550 mW cm^{-2}. Fig. 2 shows a representative profile for D_2O and the band ratio of C=C bond intensity to C=O bond intensity. It can be seen that the D_2O profile remained almost constant throughout, whereas the band ratio decreased as the polymerization reaction continued. The slight decrease in D_2O profile at the beginning may be due to the slight difference in diffusion (D_2O is a small molecule compared with HEMA and BisGMA) from the center of the ATR crystal during the light curing. The rate of polymerization was determined by taking the first derivative of the time vs. conversion curve.