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***In vitro* Performance of Nano-heterogeneous Dentin Adhesive**

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Abstract

Water is ubiquitous in the mouths of healthy individuals and routinely interferes with efforts to bond restorations to dental tissues. Our previous studies using tapping-mode atomic force microscopy (TMAFM) have shown that nanophase separation is a general feature of cross-linked polymethacrylates photocured in the presence of water. To explore the relationship between nanophase separation in dentin adhesives and their long-term mechanical properties, we evaluated model adhesives after 3 months of aqueous storage. The degree of contrast in the TMAFM phase image depended on the formulations used, ranging from ‘not observable’ to ‘very strong’. Correspondingly, the mechanical properties of these model adhesives varied from ‘minimal change’ to ‘significant depreciation’. The results support the hypothesis that a high degree of heterogeneity at the nano-scale is associated with poor mechanical durability in these model adhesives.

Keywords

dentin adhesive; durability; degree of nano-heterogeneity; phase contrast; aqueous storage

INTRODUCTION

Clinical studies have demonstrated that the strength, durability, and clinical lifetime of composite resin restorations do not match those of dental amalgam (Letzel, 1989; Collins *et al.*, 1998; Mjör *et al.*, 2002; Van Nieuwenhuysen *et al.*, 2003; DeRouen *et al.*, 2006). The breakdown of the composite/tooth bond has been linked to the failure of current adhesives to seal and adhere consistently to dentin (Meiers and Kresin, 1996; Hashimoto *et al.*, 2001; Murray *et al.*, 2002; Van Meerbeek *et al.*, 2005; Wang and Spencer, 2005; Spencer *et al.*, 2006).

Polymers used in dental restorative materials are subject to both hygroscopic and hydrolytic effects that may adversely affect their dimensional stability and mechanical properties (Ferracane, 2006). Although several investigators have suggested that these effects have a detrimental influence on the clinical lifetime of polymer restoratives, this relationship has not been established empirically. The general perception is that material failures account for a relatively small proportion of the total clinical failures, and thus, the effects of solvent uptake and polymer degradation may be of minimal consequence (Ferracane, 2006). Recent studies

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have suggested, however, that the short-term release of unreacted components and the long-term elution of degradation products from the polymer dental restorative into the oral cavity may contribute significantly to premature material failure (Ferracane, 2006; Joskow *et al.*, 2006).

Our previous work using tapping-mode atomic force microscopy (TMAFM)/Imaging showed that nano-scale phase separation is a general feature of model cross-linked polymethacrylates, even for visibly void-free polymerized resins (Ye *et al.*, 2007a, 2008). TMAFM and SEM were first used to examine the nano-heterogeneity of model adhesives in the presence of water and as a function of BisGMA/HEMA concentration (Ye *et al.*, 2008). Nanophase separation effects tended to disappear only at very low water concentrations and cross-linking densities. The aim of the present investigation was to study the relationship between nano-scale heterogeneity of phase-separated model dentin adhesives and the mechanical properties of these adhesives following aging. The hypothesis is that a high degree of heterogeneity at the nano-scale is associated with poor mechanical durability in these model adhesives.

MATERIALS & METHODS

Composition of the Model Adhesives Used in this Study

The model adhesive with/without 8.3 mass% water consisted of hydroxyethylmethacrylate (HEMA, Acros Organics, Morris Plains, NJ, USA) and 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]-propane (BisGMA, Polysciences Inc., Washington, PA, USA) with a mass ratio of 45/55 (HEMA/ BisGMA). This formulation was selected to simulate homogeneous adhesives. Based upon visual examination, these formulations present one solution phase prior to photopolymerization. In the presence of water, however, they show nano-heterogeneity in the copolymer network (Ye *et al.*, 2007a, 2008).

The following photoinitiators (all from Aldrich, Milwaukee, WI, USA) were selectively used in this study: camphorquinone (CQ) as a hydrophobic photosensitizer, 3-(3,4-dimethyl-9-oxo-9H-thioxanthen-2-yloxy)-2-hydroxypropyl trimethyl ammonium chloride (QTX) as a hydrophilic photosensitizer, ethyl-4-(dimethylamino)benzoate (EDMAB) as a hydrophobic co-initiator, 2-(dimethylamino) ethyl methacrylate (DMAEMA) as a hydrophilic co-initiator, and diphenyliodonium hexafluorophosphate (DPIHP) as the iodonium salt. The amounts of photosensitizer, co-initiator amine, and iodonium salt in the photoinitiator system were fixed at 0.5 mol%, 0.5 mol%, and 1.0 mass%, respectively, with respect to the total amount of monomer (Ye *et al.*, 2007b). All materials were used as received.

Sample Preparation and Photopolymerization

The model adhesives were light-cured for 40 sec by means of a dental curing light (UltraLume® LED5, Ultradent, South Jordan, UT, USA) operated at 550 mW/cm². The photopolymerization of the model adhesives during irradiation was monitored *in situ* by means of a Perkin-Elmer (Waltham, MA, USA) Spectrum One Fourier transform infrared spectrophotometer (FTIR) with a resolution of 4 cm⁻¹ in the ATR sampling mode. A novel time-based spectrum collector (PerkinElmer™ Spectrum TimeBase) was also used to offer continuous and automatic collection of IR spectra of adhesives during polymerization (Ye *et al.*, 2007c). The change in the band ratio profile with 1637 cm⁻¹(C=C)/1608 cm⁻¹ (phenyl) was monitored, and degree of conversion (DC) was calculated based on the time-dependent decrease in the absorption intensity band ratios before and after light curing.

Mechanical Test

The model adhesives were injected into glass tubing (Vitrocom hollow square capillaries, 1.00-mm-square I.D., 0.200-mm wall thickness, borosilicate glass; Fiber Optic Center Inc., New

Bedford, MA, USA) by means of a micropipette (Centaur, LabSciences, Inc., Reno, NV, USA) and light-cured. After 24 hrs, the rectangular beam specimens ($1 \times 1 \times 11$ mm) were pushed out with the point of a small needle. The mechanical properties were determined by means of a SSTM-5000 mechanical tester (United Calibration Corporation, Huntington Beach, CA, USA) with a 150-lb load cell. The tensile properties were determined for all samples after dry storage at room temperature ($24 \pm 2^\circ\text{C}$), or after aqueous storage in distilled de-ionized water. Specimens were tightly and fully attached to the upper and lower grips by cyanoacrylate cement (Zapit, Dental Ventures of America, Corona, CA, USA) and were loaded at a cross-head speed of 0.5 mm/min. The elastic modulus (E , GPa) was measured as the slope of the linear portion of the stress-strain curve between 5% and 15% strain for all samples. Specimen toughness (T , MN/m^2) was calculated as the area under the stress-strain curves.

At least 24 specimens were prepared for each formulation. These specimens were randomly distributed into 3 groups, *i.e.*, one-day, one-month, and three-month storage. The specimens were carefully evaluated for defects under an optical microscope, and those with visible defects were discarded. Eight specimens were tested for each timepoint. For all experimental groups, the differences between modulus or toughness values were evaluated by one-way analysis of variance (ANOVA), together with Tukey's test at $\alpha = 0.05$ to identify significant differences in the means. We analyzed both toughness and modulus values at each timepoint by separate one-way ANOVA to determine if there was a statistical difference as a function of resin (cured in the presence or the absence of water) for each storage timepoint.

Tapping-mode Atomic Force Microscopy Study

The preparation of the pellet specimens used for AFM study has been described previously (Ye *et al.*, 2007a). In brief, the model resins were injected into circular aluminum molds (ID, 4.0 mm), sealed with a cleaned cover glass, and light-cured. After 24 hrs, the coverslips were carefully peeled off, and the pellet specimens (4.0 mm diameter $\times$ 1.0 mm thickness) were stored in a vacuum at ambient temperature for 1 wk before microscopy observations. The AFM images were obtained by means of a Nanoscope IIIa scanning probe microscope (Digital Instruments, Santa Barbara, CA, USA) operated in tapping mode under ambient conditions ($24 \pm 2^\circ\text{C}$, $40 \pm 5\%$ RH), according to the techniques published previously (Ye *et al.*, 2007a). In brief, a tapping-mode etched silicon probe (Prod No. TESPW, Veeco, Santa Barbara, CA, USA) with a resonant frequency of about 245–265 KHz was used. The probes were 130–140 and 3.5–4.5 μm in length and thickness, respectively. Images were recorded in the topographic (height) and phase mode simultaneously. The set-point amplitude (A_{sp}) used in feedback control was adjusted to 90% of the tip's free amplitude of oscillation (A_0), to minimize the interaction of the probe and sample. Images of each sample were recorded and analyzed with Nanoscope image processing software (5.30r2 version). In this study, the degree of heterogeneity of phase-contrast images was quantified with Nanoscope image processing software and calculated from section analysis of phase-contrast images of at least 4 specimens, as described previously (Ye *et al.*, 2007a).

RESULTS

The photoinitiators used in this study (Table) have been shown to be more efficient and sensitive for HEMA/BisGMA co-polymerization than conventional two-component counterparts (unpublished observations). We used these more efficient photoinitiator systems to ensure that the model adhesives underwent high monomer/polymer conversion after 40 sec of visible light exposure. Based on the comparisons shown in Fig. 1, the inclusion of the additional hydrophilic photoinitiators QTX and DMAEMA led to a degree of conversion comparable with that of the resins containing hydrophobic initiators for photopolymerization,

in both the presence and absence of water. However, the stored-in-water specimens prepared with the hydrophilic initiator show improved modulus and toughness values (Table).

We used the TMAFM/PhaseImaging technique to observe the model adhesive surface. Phase-contrast in the resin surface cured in the absence of water was barely discernible, regardless of the hydrophobic or hydrophilic photoinitiators that were used in the formulation (Table, Fig. 2a). A co-continuous structure was observed for the resin surface cured in the presence of water (Figs. 2b, 2c). The higher features of the topographic image show some corresponding features in the phase-contrast images, which appear brighter. The width of these worm-like features is ~15 nm based on the phase-contrast images. The roughness value (Ra) calculated from the section analysis is approximately two-fold greater for the hydrophobic photoinitiator system (Fig. 2b, Table) than for the hydrophilic photoinitiator system (Fig. 2c, Table), and is consistent with the appearance of the phase-contrast images.

The tensile properties of rectangular beams made with the different photoinitiator systems were determined after one-day, one-month and three-month aqueous storage. There was no significant change in modulus and toughness values during aging for specimens cured in the absence of water ($p > 0.05$) (Fig. 3). In contrast, there was a continuous decline of tensile properties for the specimens cured in the presence of water during 3 mos of storage ($p < 0.05$). The modulus and toughness values of resins containing hydrophilic photoinitiator did not decrease significantly ($p > 0.05$) during aging. However, model adhesives containing the hydrophobic photoinitiators and cured in the presence of water showed nearly a two-fold reduction in mechanical properties after three-month storage ($p < 0.05$).

DISCUSSION

A fundamental aspect of the development of new materials, particularly durable clinical materials, is the identification of phase separation and the conditions that promote phase separation. There have been reports of nanophase separation and nanostructural changes in polymer films detected through AFM measurements of surface morphology (Park *et al.*, 1995; Balsamo *et al.*, 2002; Reining *et al.*, 2002). However, there are few reports relating nanophase structure and nanophase separation to the physical and chemical durability of clinical materials (Soraru *et al.*, 2002), and no reports on this relationship for dentin adhesives.

The model dentin adhesives used in this work are a mixture of a modern hydrophobic component (BisGMA) and hydrophilic components (HEMA and water); the composition was based on that of conventional dentin adhesives (Pashley *et al.*, 1998; Spencer and Wang, 2002). Based on our previous finding of nanophase separation in dentin adhesives (Ye *et al.*, 2008), the water concentration (8.3 mass%) is below that required for visible macro-phase separation in HEMA/BisGMA formulations with a mass ratio of 45/55. We controlled this concentration to maintain visually homogeneous specimens prior to photopolymerization, and simulated the situation in which the homogeneous adhesives confront the threshold of water/monomers (liquid/liquid) phase separation. Thus, it was possible to prepare model adhesives with nano- as opposed to micron-sized phase separation, and to explore the relationship between nano-heterogeneity and durability. Commercial adhesives were not selected for investigation because of their unknown and complex composition. In addition, commercial adhesives routinely include a wide variety of additives which may interfere with the control, identification, and image analysis, especially at the nano-level.

Because water is present in the oral cavity, it is crucial to know the phase behavior of adhesives polymerized in the presence of water. In the absence of water, HEMA is a good solvent for BisGMA, so a relatively homogeneous solution can be formed, and specimens photopolymerized from this mixture would be expected to have a low degree of heterogeneity.

With increasing water concentration, the adhesive may exhibit a very different nanostructure. Water is a good solvent for HEMA, but a non-solvent for BisGMA. Given that water may also be a good solvent for relatively hydrophilic oligomers such as poly(HEMA)-rich segments, both the hydrophilic monomer and these oligomers may be nanophase-separated from poly(BisGMA)-rich segments in the presence of water. In the course of nanophase separation, water and poly(HEMA)-rich segments may associate with each other to form hydrophilic domains, whereas poly(BisGMA)-rich segments may form their own hydrophobic nanodomains. This nanophase-separated structure may be permanently locked into place by cross-linking copolymerization, which restricts translational diffusion and mixing of the chains. Water may also plasticize the hydrophilic domains and lower the degree of cross-linking. Thus, the degree of phase contrast between the two domains would increase with the water concentration in the hydrophilic domain, due to the softer material that is formed.

The kinetics of photopolymerization suggests that there are minimal differences in overall photo-reactivity and average degree of polymerization for formulations made with hydrophobic or hydrophilic photoinitiators. However, differences in the mechanical property measurements of hydrated specimens suggest that there are differences in polymer structure, despite the similar degree of conversion. As an example, for specimens cured in the presence of water, the roughness of the specimens with a hydrophobic initiator system is two-fold greater than that of the same resin with a hydrophilic initiator. These differences are related to the structure of the model adhesives and quite probably to the degree of heterogeneity at the nano-scale in these model adhesives. Previous work has shown that the surface feature/structure in these model adhesives is representative of bulk characteristics (Ye *et al.*, 2007a, 2008).

Limited reports in the literature indicate that different types of materials may show different relationships between the degree of nano-heterogeneity and mechanical durability. One study on silicon oxycarbide (SiOC) glasses claimed that the chemical durability in alkaline and hydrofluoric acid solutions decreased as the amount of phase separation increased (Soraru *et al.*, 2002). Another study on magnetic nanocomposite films, synthesized by the copolymerization of urethane acrylate non-ionomer and acrylic acid in different solvents, reported a relatively highly nanophase-separated structure, resulting in improved mechanical properties and an increased glass-transition temperature (Kim *et al.*, 2004). In contrast, the results presented here show that a high degree of heterogeneity at the nano-scale is associated with poor mechanical durability in model dentin adhesives. Materials with a high degree of nano-heterogeneity are expected to have both “strong” and “weak” domains, with the latter having greater susceptibility to chemical and biological stress. These weak domains may undermine the mechanical integrity of the resin as a whole by providing a route for water ingress into the resin. On prolonged exposure of the restoration to oral fluids, water may initially enter the matrix by diffusion into these weak domains. Over years of exposure to salivary fluids, the weak domains may become sufficiently degraded and/or hydrophilic to permit access by esterases, which greatly accelerate ester bond hydrolysis and the degradation of the resin.

The results presented here suggest that nanophase-separation effects may be of general importance in the performance of dental resins and deserve consideration when durable dentin adhesives are being developed.

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Figure 1. Effect of hydrophilic component in photoinitiators on degree of conversion (**left**) and mechanical properties (**right**) of model resin cured in the presence/absence of water. Traces represent a single determination for each type of sample; average values of final degree of conversion and mechanical properties ($n = 4$ or $8 \pm SD$) are reported in the Table.

Figure 2.

TMAFM images of model adhesive cured in the absence of water **(a)** and model nanophase-separated specimens cured in the presence of water and with different photoinitiators: **(b)** CQ/EDMAB/DPIHP and **(c)** QTX&CQ/DMAEMA/DPIHP. Topographic images **(left)** are shown with corresponding phase images **(right)**. Section analysis of phase contrast for **(b)** and **(c)** is shown on the far right, and the average values of phase contrast ($n = 4$) are reported in the Table. The adhesive **(a)** was cured in the presence of hydrophobic photoinitiators. Images for adhesives cured in the presence of hydrophilic photoinitiators were similar (not shown here). The magnification of these images is indicated by the scan dimension, which is 1 μm. The Z range of topographic images and phase images is 10 nm and 10°, respectively.

Figure 3.

Effect of 3 months' aqueous storage on the (a) modulus of elasticity and (b) toughness of model adhesives made with hydrophobic or hydrophilic photoinitiators and cured in the absence or presence of water; $n = 8 \pm SD$ at each timepoint. *Significantly different from the mechanical property of neat resin at the same timepoint, $\alpha = 0.05$. #Significantly different from the mechanical property of the same resin at $t = 1d$, $\alpha = 0.05$.

Table
Degree of Conversion Values and Mechanical Properties of Model Nanophase-separated Specimens

Initiator Composition	Degree of Conversion (%), N = 4	Modulus of Elasticity (GPa), N = 8	Toughness (MN/m ²), N = 8	Phase Contrast (Ra, °), N = 4
Cured in the absence of water				
* CQ/EDMAB/ DPIHP	73.7 ± 0.9	2.194 ± 0.103	2.42 ± 0.44	N/A
** QTX&CQ/DMAEMA/ DPIHP	71.5 ± 1.5	2.215 ± 0.132	2.77 ± 0.55	N/A
Cured in the presence of water				
* CQ/EDMAB/DPIHP	92.5 ± 1.3	1.519 ± 0.113	5.47 ± 0.23	8.5 ± 0.6
** QTX&CQ/DMAEMA/ DPIHP	90.6 ± 2.2	1.757 ± 0.155	9.84 ± 0.54	4.3 ± 0.9

* Hydrophobic photoinitiator system: 0.5 mol% CQ; 0.5 mol% EDMAB; 1.0 wt% DPIHP.

** Hydrophilic photoinitiator system: 0.25 mol% QTX; 0.25 mol% CQ; 0.5 mol% DMAEMA; 1.0 wt% DPIHP.