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ABSTRACT

Current challenges in adhesive dentistry include over-hydrophilic bonding formulations, which facilitate water percolation through the hybrid layer and result in unreliable bonded interfaces. This study introduces nanogel-modified adhesives as a way to control the material's hydrophobic character without changing the basic monomer formulation (keeping water-chasing capacity and operatory techniques unaltered). Nanogel additives of varied hydrophobicity were synthesized in solution, rendering 10- to 100-nm-sized particles. A model BisGMA/HEMA solvated adhesive was prepared (control), to which reactive nanogels were added. The increase in adhesive viscosity did not impair solvent removal by air-thinning. The degree of conversion in the adhesive was similar between control and nanogel-modified materials, while the bulk dry and, particularly, the wet mechanical properties were significantly improved through nanogel-based network reinforcement and reduced water solubility. As preliminary validation of this approach, short-term micro-tensile bond strengths to acid-etched and primed dentin were significantly enhanced by nanogel inclusion in the adhesive resins.

KEY WORDS: adhesives, polymers, restorative dentistry, materials science(s), water, nanoparticles.

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Improved Dental Adhesive Formulations Based on Reactive Nanogel Additives

INTRODUCTION

Hydrophilic adhesive formulations have allowed for the improved bonding of direct esthetic restorations to water-rich dentin surfaces. Substrate hybridization depends on the ability of the adhesive resin to displace water to avoid collapse of the demineralized collagen network (Hashimoto *et al.*, 2009). Even being relatively hydrophilic, those formulations are still only marginally miscible with water, and water-induced phase separation prior to and during polymerization is common (Ye *et al.*, 2008; Sauro *et al.*, 2009). A strong correlation between adhesive layer heterogeneity and decreased mechanical properties has been established (Ye *et al.*, 2008), explained by locally compromised structural and mechanical properties (Spencer and Wang, 2002; Van Landuyt *et al.*, 2007). Ethanol wet-bonding techniques have been proposed (Sadek *et al.*, 2010a), with the rationale that the better solubility of the monomer in ethanol, as well as its capacity to displace water, would avoid phase separation and improve conversion within the adhesive layer (Sauro *et al.*, 2009). Even when well-polymerized, adhesives still behave as permeable membranes, and water percolation weakens the restoration bond over time, in a phenomenon described as “water-treeing” (Tay and Pashley, 2003). Long-term stability studies have shown that the combination of the ethanol wet-bonding technique and more hydrophobic monomers promotes more reliable adhesive interfaces (Sadek *et al.*, 2010a,b).

An alternate approach, considered here, involves the incorporation of functionalized prepolymers into adhesive formulations. One example of such prepolymers has been described recently (Morães *et al.*, 2011). Nanogels are 10- to 100-nm crosslinked globular particles synthesized through a versatile route allowing for adjustable hydrophobic character and level of methacrylate functionalization. Nanogels can be swollen by and dispersed in monomers such as BisGMA and HEMA (Morães *et al.*, 2011) and solvents (de Groot *et al.*, 2001), which, in dentin bonding, are anticipated to carry the nanoparticles into demineralized dentin. Since this approach allows for retention of the basic adhesive monomer formulation, the water-chasing capacity, which depends on both resin and solvent composition, is expected to be maintained. Upon solvent removal and polymerization shrinkage of the matrix resin, discrete nanogel particles partially or fully coalesce to potentially reinforce the adhesive resin network with some similarity to interpenetrating polymer networks (IPN) (Sahiner *et al.*, 2006) or as network precursors (Hamamoto *et al.*, 2010), with the aim to increase bond strength and limit water percolation as well as network swelling. The goal is to develop adhesive materials that allow for simple dentin-bonding procedures that provide long-term stability of bonded interfaces. This study assesses the effect of adding nanogels of varied hydrophobicity to a model solvated adhesive in terms of fundamental physical and

mechanical properties, along with a preliminary demonstration of dentin-bonding potential.

MATERIALS & METHODS

All chemicals were obtained from Sigma-Aldrich Co. (Milwaukee, WI, USA) unless otherwise noted. Nanogel copolymers were synthesized at a 70:30 molar ratio of isobornyl methacrylate (IBMA) and either urethane dimethacrylate (UDMA – less hydrophobic) or ethoxylated bis-phenol-A-dimethacrylate (BisEMA – more hydrophobic), both from Esstech (Essington, PA, USA). 2-Mercaptoethanol (ME) was added (15 mol% relative to monomers) as a chain-transfer agent to avoid macrogelation, control molecular weight/nanogel particle size, and provide sites for post-polymerization refunctionalization. Materials were photopolymerized in solution (4-fold excess toluene relative to monomer) with bis(2,4,6-trimethylbenzoyl)-phenylphosphine oxide (Irgacure 819, Ciba, Basel, Switzerland) as photoinitiator and mercury arc lamp irradiation (Novacure, EXFO, Mississauga, ON, Canada; 320–500 nm, 2500 mW/cm² for 2 hrs). Methacrylate conversion was calculated from mid-IR spectra (Nicolet 6700, Thermo Scientific, Madison, WI, USA) obtained for the 1637 cm⁻¹ vinyl absorption before and after photoactivation. Nanogels I and II (prepared with UDMA and BisEMA, respectively) were purified by precipitation from hexanes (4-fold excess), filtration, and residual solvent removal under reduced pressure. Resulting nanogel powders were re-suspended in dichloromethane and reacted for 6 hrs at room temperature with a 10% molar excess of 2-isocynoethyl methacrylate relative to the original mercaptoethanol content, followed by re-precipitation.

Polymeric nanogels were characterized by triple-detector (refractive index, viscosity, light scattering) gel permeation chromatography (GPC; Viscotek, Houston, TX, USA) in tetrahydrofuran. The glass transition temperature (T_g) of nanogel powders (n = 2) was determined by dynamic mechanical analysis (DMA, Perkin Elmer 8000, Waltham, MA, USA) in thin metallic pockets subjected to single cantilever displacement (50 μm at 1 Hz) during a cooling temperature ramp (180 to -50°C at 2°C/min in air).

A model adhesive was prepared from bisphenol-A-diglycidyl dimethacrylate (BisGMA, Esstech) and 2-hydroxyethyl methacrylate (HEMA) (60/40 mass ratio). Camphorquinone and ethyl N,N-dimethylaminobenzoate were added at 0.2 and 0.6 wt%, respectively, as initiators. Ethanol was included in the adhesive resin at 40 or 12 wt% to simulate a fully solvated dental adhesive or the corresponding adhesive containing residual solvent following a clinically relevant air-thinning procedure (Yiu *et al.*, 2005). Methacrylate-functionalized nanogels (I or II) were added at 25 wt% with respect to the BisGMA/HEMA content of the resin.

We monitored the real-time polymerization kinetics of bulk adhesives (Nicolet 6700) by following the area of the methacrylate absorption band in near-IR (6165 cm⁻¹, n = 3) during a 5-minute irradiation with a mercury arc lamp (Acticure 4000, EXFO, 320–500 nm; 50 mW/cm²). Viscosity was evaluated with a cone-plate viscometer (CAP2000+; Brookfield, Middleboro, MA, USA) at 25°C (n = 3). To evaluate solvent evaporation

behavior, we weighed 1 drop of adhesive before and after spreading by a dental air syringe to simulate clinical air-thinning (applied for 10 sec at 5 cm from the adhesive, n = 3) during adhesive application. Passive solvent elimination was evaluated by thermogravimetric analysis (TGA, Pyris 1, Perkin Elmer, Shelton, CT, USA) operated isothermally at 40°C under nitrogen purge (20 mL/min) over 60 min (8-mg samples, n = 1).

Disc specimens (1 x 12 mm, n = 5) were produced between glass slides under the same photoactivation conditions used in the reaction kinetics experiments (5 min/side). Water sorption (WS) and solubility (SL) were calculated as:

$$WS = \frac{m_2 - m_3}{V} \quad SL = \frac{m_1 - m_3}{V}$$

where m₁ is the initial adhesive polymer mass (after complete removal of the 12 wt% ethanol present during photopolymerization) before water immersion, m₂ is the wet mass of the surface-blotted water-equilibrated polymer, and m₃ is the dry mass of the polymer after 7 days' storage in a desiccator. The water contact angle of the 12% ethanol-containing pre-immersion discs was measured by means of a goniometer (Ramé-Hart Instruments Co., Netcong, NJ, USA). One drop of distilled water was placed on the surface of the material, then a profile image was immediately recorded and the contact angle (n = 3) measured (DROPimage Advanced; Ramé-Hart).

Bar-shaped specimens (2 x 2 x 25 mm, n = 5) were fabricated between glass slides and photopolymerized as described for the water uptake experiments. Specimens were stored for 1 wk in dark containers (dry or in distilled water) at room temperature and then tested in three-point bending [MTS Mini Bionix 858, MTS, Eden Prairie, MN, USA; 20-mm span, crosshead speed = 1 mm/min (ASTM, 2011)] to obtain flexural modulus (FM) and strength (FS). Throughout this study, adhesives with the higher solvation level (40 wt% ethanol) were applied only to viscosity and solvent evaporation testing, while the lower solvation level (12 wt% ethanol) was used for all samples involving polymerization and bulk polymer property measurements.

A preliminary dentin-bonding study was conducted with a related acetone-solvated (15 wt%) BisGMA/HEMA (70:30 mass ratio) etch-and-rinse adhesive containing nanogels I and II at either 0, 17, or 23 wt% relative to the BisGMA/HEMA monomer content. Acetone was selected as solvent in the adhesives used for dentin bonding, according to a separate study that found a proportionally lower nanogel-induced viscosity rise in acetone rather than ethanol solutions, which allowed a lower solvent concentration to be used. With the materials used here, solvent-free nanogel-containing resins were deemed too viscous for practical use. For the bonding study, mid-coronal sectioned dentin of extracted human molars was prepared with Scotchbond etchant and multi-purpose primer (3M ESPE) following the manufacturer's recommendations. The applied adhesive layer was air-thinned, photocured for 10 sec (dental curing light), and covered with 2 2-mm increments of Z100 composite (3M ESPE) photocured for 60 sec each. The specimens (3 teeth/group, 1 x 1 x 8 bars, n = 20) were stored for 24 hrs in 37°C water prior to micro-tensile bond strength (μTBS) testing, as previously described (Lin *et al.*, 2010).

Table. Gel Permeation Chromatography Parameters*

Nanogel Sample	M_n (Da)	M_w (Da)	PDI	R_h (nm)	MH- α
IBMA/UDMA/ME (I)	784,000	3.46×10^6	4.42	19.2	0.333
IBMA/BisEMA/ME (II)	229,000	1.23×10^6	5.35	14.2	0.354

*Number and weight-averaged molecular weight (M_n and M_w , respectively), polydispersity index (PDI), hydrodynamic radius (R_h), and Mark-Houwink α exponent (MH- α). The data represent a single analysis.

Data from all measurements were tested with one-way ANOVA and Tukey's test ($\alpha = 5\%$).

RESULTS

GPC analysis (Table) demonstrated that both nanogels presented relatively broad distributions of high-molecular-weight polymer. The low Mark-Houwink exponent values indicated highly branched globular structure that yielded significantly reduced viscosity effects compared with similar-molecular-weight linear polymer (Graham *et al.*, 1996). Based on the distribution of solvent-swollen hydrodynamic radii determined by light-scattering, nanogel particle sizes ranged from approximately 10 to 80 nm. Dynamic mechanical analysis of nanogels I and II provided Tg values of 92 and 66°C, respectively.

Passive solvent evaporation probed by the thermogravimetric technique under nitrogen purge showed approximately four-times-faster ethanol evaporation for the control adhesive compared with either nanogel-containing formulation (Fig. 1A). However, solvent evaporation by active air-thinning produced an equivalent weight loss for the control and the nanogel II formulation, while nanogel I had marginally lower evaporation efficiency (Fig. 1B). With 40% ethanol, viscosity was higher for the nanogel-containing materials compared with the controls (Fig. 1C), more so for the nanogel I composition. Upon solvent evaporation (simulated by the difference from 40 to 12 wt% ethanol content), the relative increase in viscosity was similar in magnitude (around 7- to 10-fold) for nanogel-modified and control materials.

Final conversion for the nanogel I adhesive was statistically equivalent to that of the control (Fig. 2A), while nanogel II slightly reduced the limiting resin conversion. The nanogels promoted more active early-stage polymerization, and the inclusion of nanogel I increased the overall reaction rate maximum (Fig. 2B). The degree of conversion at which the maximum rate of polymerization occurred was 5 to 6% lower for nanogel-modified materials.

Water contact angles associated with the nanogel I and II adhesives ($64.5 \pm 4.0^\circ$ and $64.9 \pm 2.2^\circ$, respectively) were significantly greater than those of the control ($57.2 \pm 3.8^\circ$). While UDMA provided a similar degree of hydrophilicity compared with BisGMA, both BisEMA and particularly IBMA were significantly more hydrophobic than the other monomers used here. We previously determined (unpublished observations) the equilibrium water uptake of UDMA and BisEMA homopolymers to be 3.3 and 0.6 wt%, respectively. The more hydrophilic nanogel I yielded water sorption equivalent to that of the control, while nanogel II provided reduced sorption (Fig. 3A). Both prepolymer additives significantly lowered solubility, with nanogel I being more effective because of the higher overall conversion achieved.

Without reliance on any enhancement in conversion, both reactive nanogel additives significantly increased dry flexural modulus and strength relative to the control (Figs. 3B, 3C). With nanogel-containing adhesive resins, flexural strength and modulus were effectively preserved after 7 days' immersion in distilled water. This differs dramatically from the approximately 35 and 55% reductions in these properties obtained with the water-equilibrated control compared with the dry state. In the 24-hour μ TBS testing with acetone-solvated BisGMA/HEMA adhesives, all the experimental adhesives, except those with the lower content of nanogel II, provided statistically significant improvements relative to the control (Fig. 3D).

DISCUSSION

The goal here was to modify the polymer network structure of a model adhesive to favorably alter its interaction with water and improve wet mechanical properties. The size and globular structure of individual nanogel particles (Szaloki *et al.*, 2008; Morães *et al.*, 2011) are such that their dispersion into dentinal tubules and interfibrillar collagen spaces is practical. Crosslinked particles not only impair water percolation and swelling but also provide mechanical reinforcement (Sahiner *et al.*, 2006; Szaloki *et al.*, 2008). Residual solvent present in adhesives at the point of polymerization influences resin reactivity (Ye *et al.*, 2007; Cadenaro *et al.*, 2009), polymeric network structure (Ye *et al.*, 2007), adhesive layer thickness (Hashimoto *et al.*, 2009), and interfacial morphology. The active solvent evaporation results mean that the nanogel incorporation at the loading levels used here did not practically impair clinically relevant solvent removal. The passive solvent evaporation testing suggests that nanogel addition may restrict oxygen diffusion rates at the material surface, which may reduce oxygen inhibition during adhesive layer polymerization. The increase in bulk monomer viscosity with nanogel addition has been shown to be fairly limited up to loading levels of 20 to 30 wt% (Morães *et al.*, 2011), above which particle-particle interactions dominate (percolation threshold; Rubinstein and Colby, 2008). The effective concentrations of nanogels in the ethanol-solvated materials (*ca.* 22 or 18 wt% for the 12 and 40 wt% solvent concentrations, respectively) approximate that threshold, thus providing a balance between potential network reinforcement and a viscosity that allows for controlled adhesive layer thickness.

The photopolymerization kinetic studies demonstrate few if any effects of nanogels on final conversion. Because of its lower Tg, nanogel II might have been expected to allow for higher conversion, which was not the case. This discrepancy could indicate preferential partitioning of BisGMA into the BisEMA-based nanogel particles to create higher viscosity and reduced

Figure 1. Weight loss during passive TGA-based solvent evaporation (A) and following active air-thinning (B) for the control and nanogel-modified materials. Weight loss experiments were conducted with materials at 40 wt% ethanol solvation. Viscosity of adhesive compositions (C) as either fully solvated adhesives (40 wt% ethanol) or the analogous materials with partial residual ethanol content (12 wt% ethanol).

Figure 2. Degree of conversion (A) and rate of polymerization (B) as a function of time for control and nanogel-modified materials solvated with 12 wt% ethanol. Materials were photopolymerized with a mercury arc lamp (320-500 nm; 50 mW/cm²).

reactivity in/around these particles. Inclusion of fairly hydrophobic prepolymeric particles into a relatively hydrophilic formulation might potentially cause de-mixing; however, only homogeneous (transparent) nanogel-modified polymers were observed both before and after exposure to water, which indicates a lack of any significant nanogel aggregation. It appears that the higher initial viscosity environment encountered by monomer located in and around nanogel particles results in earlier autoacceleration, with the UDMA-containing nanogel additive providing an enhanced maximum reaction rate. Nanogel additives may counter the negative effects of solvent dilution on autoacceleration without significant compromise to limiting

conversion, which is potentially important in solvated adhesive systems.

Water-contact angle probes structural features within a few angstroms of a surface, and therefore, the higher values presented by the experimental materials demonstrate that nanogel particles are present at the adhesive surface. The more hydrophobic nanogel II limited overall water uptake in the adhesive, while both nanogels reduced water solubility in approximately the same proportion as their concentration, which strongly suggests that neither of the high-molecular-weight nanogels is leachable. For polymer reinforcement, the need for covalent attachment between nanogel additives and the polymer matrix has been demonstrated (Morães *et al.*, 2011). The nanogels used here displayed Tg values higher than those available in bulk polymer networks photocured under ambient conditions. Greater initial strength is desirable for higher early bond strengths when the adhesive layer is challenged clinically by stress development during composite placement. Under dry conditions, both experimental adhesives produced higher flexural modulus and strength than the control, with nanogel II providing the more pronounced effect. Of particular interest for dental adhesive materials, and in stark contrast to the control, the excellent

bulk mechanical properties were not reduced in the experimental materials upon water equilibration. These positive results are continued with an initial demonstration of the practical dentin-bonding potential of nanogel-based adhesives where 24-hour μ TBS results showed significant improvements with respect to the nanogel-free control. It is notable that the introduction of reactive nanogel additives in dental adhesives does not require modification of conventional bonding techniques. The negative short- and long-term effects of water on adhesive materials represent a major drawback of current commercial formulations. Based on the present findings and the results of an extended dentin-bonding study (Yang *et al.*, unpublished observations), we

Figure 3. Water sorption/solubility (A) and dry/wet flexural strength (B) and modulus (C) mechanical properties for control and nanogel-modified materials (solvated with 12 wt% ethanol). Materials were photopolymerized with a mercury arc lamp (320-500 nm; 50 mW/cm²). Micro-tensile dentin bond strength (D) measured after 24 hrs of storage in 37°C water (asterisk indicates statistically significant difference relative to the control).

speculate that the structure and stability of the adhesive bond will benefit from this nanotechnology approach to materials design.

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