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ABSTRACT

Polymeric dental adhesives require the formation of densely crosslinked network structures to best ensure mechanical strength and durability in clinical service. Monomeric precursors to these materials typically consist of mixtures of hydrophilic and hydrophobic components that potentially undergo phase separation in the presence of low concentrations of water, which is detrimental to material performance and has motivated significant investigation into formulations that reduce this effect. We have investigated an approach to network formation based on nanogels that are dispersed in inert solvent and directly polymerized into crosslinked polymers. Monomers of various hydrophilic or hydrophobic characteristics were copolymerized into particulate nanogels bearing internal and external polymerizable functionality. Nanogel dispersions were stable at high concentrations in acetone or, with some exceptions, in water and produced networks with a wide range of mechanical properties. Networks formed rapidly upon light activation and reached high conversion with extremely low volumetric shrinkage. Prepolymerizing monomers into reactive nanostructures significantly changes how hydrophobic materials respond to water compared with networks obtained from polymerizations involving free monomer. The modulus of fully hydrated networks formed solely from nanogels was shown to equal or exceed the modulus in the dry state for networks based on nanogels containing a hydrophobic dimethacrylate and hydrophilic monomethacrylate, a result that was not observed in a hydroxyethyl methacrylate (HEMA) homopolymer or in networks formed from nanogels copolymerized with HEMA. These results highlight the unique approach to network development from nanoscale precursors and properties that have direct implications in functional dental materials.

KEY WORDS: adhesive resin, hydrophilic, nanogel, phase separation, photopolymerization, polymer networks.

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Construction of Monomer-free, Highly Crosslinked, Water-compatible Polymers

INTRODUCTION

The versatility inherent in polymeric materials has led to their indispensable use in an array of dental materials, including impression and reline materials, denture base and artificial teeth, and endodontic obturation materials, as well as cements, adhesives, and esthetic composite restoratives. That versatility is in large part responsible for the ubiquitous nature of polymers, since their properties can be tailored over a wide range through choice of monomer that is further enhanced by simple copolymerization processing options. As an example, conventional dental adhesive resins are typically designed to accommodate the relatively hydrophobic character of the overlying restorative as well as adapting to the moist dentin substrate (Van Landuyt *et al.*, 2007; Marshall *et al.*, 2010; Park *et al.*, 2011; Pashley *et al.*, 2011). 2-Hydroxyethyl methacrylate (HEMA) or a small number of other neutral, water-compatible monomers are widely used in adhesive formulations to promote the hydrophilic, surface-wetting aspect (Guo *et al.*, 2007; Van Landuyt *et al.*, 2008; Zanchi *et al.*, 2011; Hiraishi *et al.*, 2014). Along with this, a more hydrophobic comonomer, such as BisGMA, is incorporated primarily to enhance polymer strength (Tay and Pashley, 2003). In addition, a compatibilizing solvent, such as ethanol or acetone, may be included to control resin viscosity or assist in resin infiltration and water dispersion (Hashimoto *et al.*, 2002; Yiu *et al.*, 2005). While BisGMA and HEMA are miscible in all proportions as monomers under ambient conditions, and they form single-phase copolymers, a significant concern arises when even small concentrations of water are introduced, since this promotes phase separation into water/HEMA-rich and BisGMA-rich domains (Spencer and Wang, 2002; Ye *et al.*, 2009) that seriously undermine the overall reactivity, strength, and practical performance potential of the adhesive (Holmes *et al.*, 2007).

We have demonstrated previously that ~10- to 20-nm reactive nanogels that are dispersed in and swollen by monomer can be used to significantly modify properties of the resultant hybrid polymer networks as applied to monomer reactivity (Liu *et al.*, 2012, 2014), shrinkage and stress in resin-based composites (Moraes *et al.*, 2011), and mechanical strength in model dental adhesives (Moraes *et al.*, 2012). Here, we focus on nanogels that are water-compatible or near-water-compatible for potential application in dental adhesives; however, for better appreciation of the relationship between nanogel structure and network properties conveyed by the nanogel component rather than nanogel entwined in a resin matrix, this work emphasizes nanogel-derived networks created in inert dispersion media. The nanogel series examined here represents a progressive disparity between the hydrophilic and hydrophobic character of the comonomer pairings where direct homogeneous copolymerization in water would not be possible because of spontaneous

monomeric phase separation or polymerization-induced phase separation. This approach of generating macroscopic polymer networks from nanogel precursors not only provides useful basic information regarding the design of nanogel-modified resin systems, but it also offers new water-compatible materials and a monomer-free route to dense networks with a range of properties that may provide alternative pathways to dental adhesives and other biomedical materials.

MATERIALS & METHODS

Nanogel synthesis was modified from a previously described protocol (Moraes *et al.*, 2011). A 7:3 molar ratio of monomethacrylate [ethoxylated hydroxyethyl methacrylate (EHEMA10, EHEMA5) or HEMA; all materials (Appendix Fig. 1) were received from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise noted] to dimethacrylate [ethoxylated bisphenol A dimethacrylate (BisEMA, Esstech, Essington, PA, USA)] or tetraethylene glycol dimethacrylate (TTEGDMA), 15 mol% of mercaptoethanol (relative to total monomer content), and 1 wt% azobisisobutyronitrile (AIBN) were dissolved in a six-fold excess of methyl ethyl ketone. The solution was purged with nitrogen for 30 min and then stirred in a 75°C oil bath for 6 h. Following this reaction, 15 mol% of 2-isocyanatoethyl methacrylate (TCI America, Seekonk, MA, USA) was added with a trace amount (~10 mg) of dibutyltin dilaurate and stirred overnight at room temperature to introduce internal and surface-tethered polymerizable methacrylate groups. The product was precipitated into a 10-fold excess of hexanes with residual solvent removed under reduced pressure. The nanogels are referred to by their monomethacrylate and dimethacrylate components, *i.e.*, EHEMA10-BisEMA is the nanogel formed from the copolymerization of EHEMA10 and BisEMA.

Isolated nanogels were characterized by triple-detection gel permeation chromatography with THF as the mobile phase (Viscotek, Houston, TX, USA). Molecular weight, hydrodynamic radius, and intrinsic viscosity were simultaneously determined by refractive index, right-/low-angle light scattering, and differential viscometer detectors.

The general procedure for producing nanogel-based networks involved dispersal of the nanogels in acetone or water at 50 wt%, with 1.0 wt% (relative to nanogel mass) Irgacure 2959 (Ciba, Basel, Switzerland) as photoinitiator. These solutions were polymerized in silicon molds between glass slides under 365 nm or 320- to 390-nm ultraviolet (UV) irradiation at 10 mW/cm² for 10 min with a mercury arc lamp and quartz light guide (Acticure, EXFO, Quebec, PQ, Canada). For the testing described, all the nanogels were compared based on photopolymerization in acetone. Since only the EHEMA10-BisEMA, EHEMA10-TTEGDMA, and EHEMA5-TTEGDMA nanogels were fully soluble in water at the standardized 50 wt% loading level, these were polymerized in water in addition to acetone. Dynamic mechanical testing (Q800, TA, New Castle, DE, USA) was conducted on bar-shaped specimens (10 mm x 2 mm x 0.5 mm) to determine glass transition temperature (T_g). A cyclic strain of 0.025% at 1 Hz was applied to samples, and the temperature was increased at 3°C/min over an appropriate range to determine the maximum value of tan delta, which was taken as

the T_g . Disc-shaped specimens (5 mm x 0.5 mm) were prepared to determine the equilibrium water content of swollen nanogel-based networks. The polymerized networks were dried under vacuum at 60°C for 48 h (beyond which constant sample mass was observed). Discs were then immersed in 1 mL of deionized (DI) water and stored at room temperature. At 4, 10, and 14 days, each disc was removed from water, carefully blotted to remove surface water, weighed, and returned to fresh DI water. The swelling ratio was recorded as $(W_s - W_d)/W_d$, and the percentage of the network mass that was water was recorded as $(W_s - W_d)/W_s * 100$, where W_s = swollen mass and W_d = dry mass. Rheology of prepolymerized nanogel dispersions was tested on a parallel plate fixed-strain rheometer (Ares, TA). A strain sweep from 1% to 50% strain was applied at 10 or 100 rad/s on circular stainless steel parallel plates (diameter = 20 mm, gap = 0.2 mm). Viscosity was measured with the same rheometer configuration but with a steady shear sweep from 10–1,000/s.

Flexural modulus was obtained with a universal testing machine (Mini Bionix II, MTS, Eden Prairie, MN, USA) in three-point bending with a span of 10 mm and a cross-head speed of 1 mm/min. Testing was performed on bar specimens prepared from 2 mm x 2 mm x 15 mm elastomer molds sandwiched between glass slides and photopolymerized for 5 min *per side* by means of a mercury arc lamp (320–390 nm, 10 mW/cm²). Ten specimens were made for each material, of which 5 were stored dry (including the '60°C for 48 h' solvent removal step) and 5 were stored in distilled water at room temperature for 7 days prior to being tested.

Polymerization shrinkage was measured with a linometer as described previously (Moraes *et al.*, 2011). Specimens were irradiated for 10 min by means of a mercury arc lamp (320–390 nm, 10 mW/cm²).

Statistically significant differences between means were determined by analysis of variance (ANOVA) and Tukey's test ($\alpha = 0.05$). Error bars represent mean $\pm$ standard deviation for three replicates unless otherwise indicated. Gel permeation chromatography (GPC) data represent a single analysis.

RESULTS

Triple-detection GPC confirmed the formation of discrete, globular particles with swollen diameters ranging from 8 to 20 nm (Table). The average molecular weight was between 230 and 650 kDa for nanogels crosslinked with BisEMA and between 16 and 69 kDa for nanogels crosslinked with TTEGDMA. The larger BisEMA-crosslinked nanogels had a higher polydispersity than the smaller TTEGDMA-crosslinked nanogels, which is expected for high-molecular-weight polymers formed from chain-growth polymerizations and is consistent with previous results (Moraes *et al.*, 2011; Liu *et al.*, 2014). The Mark-Houwink alpha parameters are all below 0.5, which is indicative of highly branched polymeric structures. Optically transparent, covalently crosslinked networks were obtained from the photopolymerization of nanogels dispersed in acetone as well as the subset photocured in water. A loading level of 50 wt% was demonstrated to photopolymerize rapidly and to high conversion (Appendix Fig. 2), and was subsequently used for all materials tested. This demonstrates that the interparticle

Table. Number Average Molecular Weight (M_n), Hydrodynamic Radius (R_h), Mark-Houwink Alpha Parameter (MH- α), and Polydispersity Index (PDI) for All Nanogels

Nanogel	M_n (Da)	R_h (nm)	MH- α	PDI
EHEMA10-BisEMA	2.27×10^5	6.90	0.289	3.75
EHEMA5-BisEMA	6.42×10^5	8.78	0.324	2.60
HEMA-BisEMA	3.60×10^5	9.41	0.325	4.66
EHEMA10-TTEGDMA	5.13×10^4	5.10	0.485	1.48
EHEMA5-TTEGDMA	1.60×10^4	4.14	0.271	1.91
HEMA-TTEGDMA	6.88×10^4	5.28	0.331	2.16

Figure 1. Glass transition behavior for networks formed from nanogels polymerized in acetone (A) or in water (W). The peak of the tan delta curve is taken as the glass transition temperature (T_g).

polymerization is particularly rapid and efficient, which leaves no elutable materials. The 50 wt% loading produces an essentially confluent, interdigitated nanogel array (Liu *et al.*, 2012).

Dimethacrylate (DMA) analysis of dry samples revealed significant differences in T_g when either the monomethacrylate or dimethacrylate component was changed in the nanogel prepolymer (Fig. 1). Values of T_g ranged from 0 to 130°C for these 6 nanogel formulations. For networks formed in acetone or water, BisEMA-crosslinked nanogels produced networks with significantly higher T_g compared with those of TTEGDMA-crosslinked nanogels in all cases. Within nanogels crosslinked with BisEMA or TTEGDMA, changing the monomethacrylate component from EHEMA10 to EHEMA5 to HEMA produced increasing T_g in that order. The fully water-soluble nanogels were polymerized in both acetone and water for examination of the effects of solvent on final network properties. No significant change in T_g was observed as a function of solvent type, although differences in initial nanogel swelling behavior could be expected. The intensity of the tan delta peak was higher for all samples formed in water compared with those formed in acetone, which indicates higher chain mobility for the water-based networks.

Three-point bending tests were conducted to determine the flexural modulus of dry and water-swollen materials (Figs. 2A, 2B). For samples formed in acetone and tested in the dry state, no difference was observed between nanogels formed from EHEMA10 or EHEMA5 for both crosslinkers. When HEMA was used as the monomethacrylate, the modulus increased over several orders of magnitude in both cases. Nanogels containing BisEMA exhibited a higher modulus than nanogels containing TTEGDMA for the same monomethacrylate component.

Samples formed in water followed the same trend, in which EHEMA10-BisEMA had a higher modulus than either EHEMA10-TTEGDMA or EHEMA5-TTEGDMA, while the 2 TTEGDMA-crosslinked nanogels had similar moduli. Samples formed in acetone and swollen in water had either no change or an increase in modulus compared with the dry state in the majority of the samples. Only HEMA-TTEGDMA decreased in modulus from the dry to the water-swollen state. Samples formed in water and swollen in water either main-

tained the same modulus or decreased, with the exception of EHEMA10-BisEMA, which increased in modulus compared with the dry state. For comparison of monomer-free nanogel-based networks with conventional crosslinking monomer polymerization, HEMA-BisEMA nanogels were copolymerized with glycerol dimethacrylate (GDMA), which forms densely crosslinked networks, or HEMA, which generates a lightly crosslinked matrix. Adding this nanogel to reactive monomers either matched the modulus of the corresponding homopolymer network, in the case of GDMA, or greatly increased the modulus, in the case of HEMA (Fig. 2C). The dry and wet moduli of nanogel-modified HEMA compared with HEMA-BisEMA polymerized in acetone or neat HEMA demonstrated a decrease in wet modulus when HEMA was included but no significant change in modulus for the nanogel-based network (Fig. 2D).

Samples for swelling testing were dried under vacuum at 60°C for 48 h, after which the characteristic near-infrared water peak at 5,200 cm^{-1} (Lin and Stansbury, 2004) was indistinguishable from the baseline. Dry samples were allowed to equilibrate in water for 4 days, and the water content was monitored over a 14-day period. No significant change in sample mass was observed during this time (Fig. 3). Networks formed in acetone from nanogels containing BisEMA (a more hydrophobic component) had a lower swelling ratio and water content than nanogels containing TTEGDMA (more hydrophilic) for all formulations (Figs. 3A, 3B). Water content for nanogels containing EHEMA10 and EHEMA5 was either very similar or identical, while HEMA-BisEMA and HEMA-TTEGDMA had dramatically lower water content. Networks formed in water all had statistically similar water content (Figs. 3C, 3D), which was

invariant over the 14-day period, indicating that elastic limits imposed during macroscopic network formation supersede the hydrophilic character of the nanogel constituent monomers.

Polymerization-induced volumetric shrinkage was determined for EHEMA5-TTEGDMA nanogels dispersed in hydrogenated glycerol dimethacrylate (an inert matrix) (Lovell *et al.*, 1999), or GDMA (a reactive matrix) as well as neat GDMA as a control (Fig. 4). Nanogels polymerized in a non-reactive matrix exhibited low levels of shrinkage (1.0%) compared with those in the GDMA control (8.1%). The addition of nanogel to the reactive monomer decreased the volumetric shrinkage significantly to 6.3%. The final conversion of both inert and reactive GDMA-containing nanogels was increased compared with that of neat GDMA, indicating that low shrinkage was achieved even at high conversion of reactive species.

All nanogel dispersions in either acetone or water were transparent, moderately viscous, and fluid-like (Appendix Fig. 3) with the exception of EHEMA10-BisEMA, which formed a paste-like gel in water but remained fluid-like in acetone. A strain sweep of EHEMA10-BisEMA in acetone at a high shear rate (100 rad/s) resulted in the loss modulus, G'' , dominating over the storage modulus G' , indicating that energy supplied to the system was predominantly dissipated by viscous interactions (Appendix Fig. 4). The same nanogel in water showed crossover of G' and G'' at 10 rad/s and dominance of G' at 100 rad/s, indicating that energy was being stored through viscoelastic interactions and physical gelation was occurring. This behavior was not observed for the other water-soluble nanogels, EHEMA10-TTEGDMA and EHEMA5-TTEGDMA.

DISCUSSION

The formation of densely crosslinked networks without relying on conventional small monomers is possible from the solution polymerization of overlapping reactive nanogel prepolymers in inert carriers that have little or no appreciable toxicity (Dahl, 2007). While the compatibility of nanogels with water as a dispersant is important to avoid phase separation, the use of acetone or ethanol as a carrier allows the nanogel loading level at the point of polymerization to be varied significantly based on solvent evaporation (Moraes *et al.*, 2012). This, along with the

Figure 2. Flexural modulus of dry or water-equilibrated networks as indicated. **(A)** All nanogels polymerized in acetone. **(B)** Water-dispersible nanogels polymerized in water. **(C)** HEMA-BisEMA nanogels polymerized in glycerol dimethacrylate (GDMA), hydroxyethyl methacrylate (HEMA) or acetone, and neat GDMA or HEMA. **(D)** HEMA-BisEMA nanogels polymerized in HEMA or acetone, and neat HEMA. Nanogel loading is 50 wt% in all cases.

Figure 3. Swelling ratio and equilibrium water content for networks formed from nanogels polymerized in acetone **(A, B)** or water **(C, D)** for EHEMA10-BisEMA (●), EHEMA5-BisEMA (■), HEMA-BisEMA (▲), EHEMA10-TTEGDMA (○), EHEMA5-TTEGDMA (□), or HEMA-TTEGDMA (△).

ready ability to independently alter the internal nanogel branching density (Liu *et al.*, 2012) and the reactive group concentration, gives extensive control over the final polymeric structure and crosslink density that can be achieved. The described method for synthesizing nanogels easily accommodates a wide range of comonomer formulations, which allows for significant tunability of both the isolated nanogel and the networks formed from polymerized nanogels. Covalently combining monomers into a macromolecular prepolymer assembly prevents phase separation of the constituent monomers, which is advantageous when hydrophobic materials are applied to hydrated surfaces. This feature is critical for obtaining sufficient mechanical

Figure 4. Volumetric shrinkage and final conversion of networks formed from the homopolymerization of glycerol dimethacrylate (GDMA), 50 wt% EHEMA5-TTEGDMA in hydrogenated GDMA, or 50 wt% EHEMA5-TTEGDMA in GDMA. All materials were photopolymerized for 10 min at 10 mW/cm² at 320 to 390 nm.

reinforcement and adhesive strength for application toward dental adhesives and restoratives.

Our study was designed to investigate nanogel formulations with systematically varying overall hydrophilic or hydrophobic characteristics and internal disparity, as well as the impact of starting nanogel chemistry on final network mechanical properties. Acetone, being of intermediate polarity, readily disperses all nanogels without settling or phase separation prior to or during polymerization. Water effectively solvates only a subset of these materials but can easily stabilize nanogels containing water-insoluble BisEMA and a monomethacrylate with sufficient hydrophilic character, which, in this case, is EHEMA10. In addition, HEMA-TTEGDMA and EHEMA5-BisEMA were soluble in a 50:50 water:acetone solution, while HEMA-BisEMA was soluble in a 25:75 water:acetone solution, indicating that these nanogels can interface with a significant quantity of water before the onset of precipitation. This approach to forming crosslinked networks from nanogels can therefore lead to water-compatible materials with chemical and physical characteristics that are not possible through direct monomer polymerization. A related imaging study with confocal laser scanning microscopy with a fluorescently labeled nanogel demonstrated that the nanogel dimensions (even with a relatively hydrophobic nanogel) allowed for efficient interpenetration throughout the hybrid layer in demineralized dentin (Appendix Fig. 5).

A wide range of network T_g and flexural moduli is achievable through the solution polymerization of nanogels into crosslinked networks. Nanogels containing EHEMA10 or EHEMA5 and either BisEMA or TTEGDMA exhibited moderate differences in T_g and formed rubbery networks within the same order of magnitude of flexural modulus, while HEMA-BisEMA and HEMA-TTEGDMA formed very glassy systems with T_g and modulus values that are comparable with those of commercially available dental resins. The water content of the more rubbery networks

approached values typical for hydrogels, while the very low water content for HEMA-BisEMA is reflective of the increased hydrophobicity of the material as well as the greatly increased cross-linking density. We evaluated the polymerization-induced volumetric shrinkage of EHEMA5-TTEGDMA as a representative material *via* linometry, since evaluating and mitigating shrinkage stress in polymeric dental materials is an extensive area of investigation. Evaporation effects complicated the analysis of volumetric shrinkage for nanogel polymerizations in water or acetone, so hydrogenated glycerol dimethacrylate was used to demonstrate the very low levels of shrinkage that occur when nanogels constitute the reactive species for polymerizations in inert solvent. Adding nanogels to either an inert or reactive matrix lowers the volumetric shrinkage compared with that of the network formed from the unfilled monomer, which is consistent with previous results that applied nanogels as resin-phase fillers for the reduction of shrinkage and stress (Liu *et al.*, 2012).

Flexural modulus was chosen for the evaluation of network mechanics under conditions that more closely represent the stresses on dental restoratives compared with those of tension or compression alone. Examination of the mechanical properties of both the dry and water-swollen networks confirmed that incorporating hydrophobic components such as BisEMA consistently increased both the dry and the wet modulus compared with nanogels formed from purely ethylene-glycol-based monomers (Figs. 2A, 2B). In addition, a significant reduction in chain mobility occurred when HEMA was used as the monomethacrylate component and resulted in a large increase in dry modulus. Unlike TTEGDMA-crosslinked nanogels, the wet modulus for BisEMA-crosslinked nanogels was equal to or greater than the dry modulus in all formulations. Analysis of rheology data indicated that water drives supramolecular gelation in EHEMA10-BisEMA, likely due to self-association and subsequent pi-pi stacking of the hydrophobic centers of BisEMA. The viscosity of the nanogel solutions was comparable with that of conventional dental resins, indicating that these materials are easily workable from a clinical perspective. Higher viscosity was observed for the glassy nanogels (HEMA-TTEGDMA and HEMA-BisEMA) and for EHEMA10-BisEMA, which exhibited interesting physical gelation in water and retained a high viscosity in acetone due to intramolecular interactions that are unique in this set of materials. TTEGDMA is fully soluble in water only below ~1 wt%, and polymerizing TTEGDMA-crosslinked nanogels in acetone may permit a higher degree of physical interpenetration than in water, leading to an increased modulus in wet conditions. However, it is clear (Fig. 2B) that only the BisEMA reinforcing interactions persist in swollen materials formed from water-based polymerizations. The significantly lower water content of all BisEMA-containing materials formed in acetone was further indicative of this effect, and though the water content was identical for all materials formed from water-based polymerizations, the differences in modulus highlight the impact of secondary interactions on the mechanics of nanogel-based materials. We further examined the properties of GDMA and HEMA networks formed with or without HEMA-BisEMA nanogels to demonstrate that densely crosslinked networks can form from nanogels polymerized in inert solvent or

with reactive monomer, and that even at the 50 wt% nanogel loading level in an inert solvent, the resulting polymers have properties that are similar to or exceed those of networks formed from undiluted (neat) small monomers. Additionally, the inclusion of HEMA decreased the modulus of water-equilibrated networks, while monomer-free nanogel-based networks were remarkable for their general ability to either match or increase the modulus compared with the dry state. This approach to synthesizing densely crosslinked networks from macromolecular prepolymer constructs with nanoscale control over material chemistry is a promising avenue for the development of functional materials. This study offers methods for designing nanogel-based networks that may lead toward improvements in dental adhesives and other high-strength materials whose function is traditionally impaired in aqueous environments.

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