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Thiol-functionalized Nanogels as Reactive Plasticizers for Crosslinked Polymer Networks

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Abstract

Significant efforts have been expended to mitigate plasticizer migration from crosslinked methacrylic and poly(vinyl chloride) polymer networks by synthesizing reactive plasticizers that can blend homogeneously within the networks to reduce polymer property change, acute toxicity and downstream environmental effects of plasticizer migration with limited and varying amount of success. We hypothesized that appropriate thiol-functionalized nanogels synthesized using the same monomers as the parent network to generate highly compact, crosslinked structures will form thermally stable, homogeneous networks and perform as optimal reactive plasticizers. Nanogels were synthesized via a thiol-Michael addition solution polymerization and incorporated at different mass ratios within a polyethylene glycol 400 urethane dimethacrylic monomer to form photo-crosslinked networks. While maintaining the inherent hydrolytic stability, thermal stability and biocompatibility of the parent matrix at ~ 99 % acrylic group conversion, the PEG400 urethane dimethacrylic -nanogel networks retained optical clarity with > 90 % visible light transmission at 20 wt % nanogel loading. The addition of the nanogels also enhanced the elongation of the parent matrix by up to 320 %, while a 37 °C reduction in glass transition temperature (T_g) and 50 % reduction in modulus was observed. A 52 % reduction in the shrinkage stress of the material was also noted. The results indicate that the application of thiol-functionalized nanogels as plasticizers to alter the bulk properties of the parent matrix while mitigating plasticizer migration by covalently crosslinking the nanogels within the polymer matrix provides a simple yet efficient technique to generate network-specific plasticizers with the ability to alter targeted properties within polymers.

Keywords

plasticizers; phthalate mimics; nanogels; thiols; crosslinked networks

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Introduction

A frequent challenge encountered in engineering synthetic polymers such as poly(vinyl chloride) (PVC) and polymethacrylates is the ability to design and manufacture a material that can fulfill the functional and mechanical requirements for a given end-application while keeping any plasticizers used within the network to alter its mechanical properties consistent, inherently efficient, and non-toxic [Chiellini et al., 2013; Mehta et al., 2015; Mohapatra and Nando 2014; Dube and Salehpour 2014.]. Traditionally, plasticizers are low molecular weight monomers incorporated within networks to improve the flexibility of the final network by obstructing secondary bonding between the polymer chains and altering the free volume within the polymer, thereby reducing the bulk glass transition temperature (T_g) [Carbonell-Verdu et al., 2016; Rahman and Brazel 2004]. Recently, the toxicity of commonly used plasticizers such as phthalates has raised environmental and health concerns due to their propensity to leach out into the immediate environment [Bui et al., 2016; Tuzum Demir and Ulutan 2013; Jobling et al., 1995]. Phthalates are found in high concentrations in biomaterials such as blood bags (30–50%), dialysis tubing (80%) dental adhesives and soft-denture linings (47%) and has been linked to a litany of health disorders including reproductive toxicity, genital disorders and toxicity during of fetal development [Parks et al., 2000; Jobling et al., 1995; Shea 2003; Heudorf et al., 2007]. Phthalate plasticizers are low molecular weight esters of phthalic acid such as dimethyl phthalate, di(n-butyl)phthalate, di(isodecyl) phthalate and di(2-ethylhexyl) phthalate (DHEP) [Mylchreest et al., 2000]. DEHP is used in biomaterials such as dental implants and intraocular lenses (IOL)s and has been shown to adversely impact cell viability even at very low concentrations [Mendell 2007]. As plasticizer migration also results in deteriorating mechanical properties of the parent material resulting in tackiness and embrittlement of the parent matrix, the ability of the material to function optimally is compromised [Tuzum Demir and Ulutan 2013].

To minimize plasticizer migration and maximize compatibility with the parent matrix, multiple strategies of differing complexity have been evaluated such as the use of plasticizers with lesser propensity to migrate (e.g. plasticizers with high molecular weight and increased branching within its structure), surface treatment of the plasticized polymer network via UV or gamma irradiation and choosing parent matrix/plasticizers combinations with better compatibility (i.e. similar solubility parameters and dielectric constants) [Messori et al., 2004; Barreto et al., 2012; Minna 2008; Stark et al., 2005, Lee et al., 2016]. The above approaches primarily rely on weak physical interactions such as hydrogen bonding and van der Waal's forces to suppress plasticizer migration and therefore have had limited success. Several of the more successful approaches to curb plasticizer migration have relied on covalently crosslinking plasticizers within polymer networks. Andreopoulos *et al.* studied the effect of PMMA copolymerization with diallyl phthalate (DAP) and demonstrated that the resulting network was a flexible material with lower T_g and improved impact resistance while successfully curtailing the migration of DAP by up to 20 parts per hundred [Andreopoulos 1985]. Building upon the ability of thiols to function as radical scavengers and stabilize thermally labile structural defects within the parent network, Navarro *et al.* have shown that the covalent binding of substituted aromatic thiols within PVC networks minimize plasticizer migration while enhancing the stability of the parent polymer network

by functioning as radical scavengers within the network [Navarro et al., 2008; Navarro et al., 2016]. Another advantage of implementing thiol-based solutions is that many of the principles that apply towards the design and use of crosslinking plasticizers hold true for both PVC and the growing methacrylic polymer market as thiol-functionalized plasticizers can be incorporated within methacrylic networks by facile thiol-vinyl reactions such as the thiol-Michael addition reaction and thiol-ene radical polymerization reaction. However, the odoriferous nature of lower molecular weight thiols prevent their implementation as plasticizers in their monomeric state while the multistep, synthetic protocols followed to generate the odorless, substituted aromatic thiol-functionalized molecules indicate challenges and opportunities that will have to be addressed [de Espinosa et al., 2014].

Building upon the advantages of thiol-based molecular networks to function as optimal plasticizers, we have taken a unique approach to demonstrate the efficiency of reactive nanogels as plasticizers by incorporating thiol-functionalized nanogels within acrylic networks and subsequently, photopolymerizing the monomer-nanogel mixture to generate plasticized networks. By utilizing a one-pot, step-growth thiol-Michael addition - solution polymerization reaction under ambient conditions, we synthesized thiol-functionalized nanostructured particles containing the monomer of the parent network within a highly cyclized, crosslinked structure that can then be dispersed and subsequently crosslinked to generate an optically clear, flexible network. As a first step towards demonstrating the versatility of this approach, a dimethacrylate monomer used to formulate dental adhesive materials, polyethylene glycol 400 urethane dimethacrylate (PEG400 UDMA; Exothane 9™), was utilized to generate a parent polymer matrix as well as the thiol-functionalized nanogels that were incorporated within the matrix. Our study demonstrates the ability of this approach to selectively alter the T_g of the parent polymer matrix while restricting the migration of plasticizers from the polymer network and maintaining the desirable bulk network properties of the network such as optical clarity, thermal stability, low shrinkage stress and biocompatibility.

Materials and Methods

Materials

Glycidyl methacrylate (GMA), triethylamine (TEA), triphenyl phosphine (TPP), pentaerythritol tetrakis(3-mercaptopropionate) >95 % (PETMP) were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO. and used without further purification. Polyethyleneglycol400 extended urethane dimethacrylate (E9, Mw; 1139.4 g/mol) and urethane dimethacrylate (UDMA) was donated by Esstech Inc., Essington, PA. Irgacure 819 (IR819) was donated by BASF. Minimum Essential Medium (MEM) and antibiotics (10000 international unit (IU)/mL penicillin and 10000 µg/mL streptomycin solution) were purchased from Gibco Life Technologies. Fetal bovine serum (FBS), was purchased from Sigma-Aldrich. Ultrapure water (18.2 MΩ-cm resistivity) was obtained from Milli-Q water purification system. All other reagents were of analytical grade and used as such without any further purification

Methods

Synthesis of thiol-functionalized Nanogels—E9, GMA, and UDMA at the molar ratio of 30:30:40 were added into a 500 mL round bottom flask with a magnetic stir bar. PETMP was added into the reaction mixture to maintain the ratio of functional groups of methacrylate:thiol, at 1:1.5. Four-fold excess of toluene relative to total weight of monomer was added and the solution was continuously stirred at 22 °C for 48 h in the presence of 4 wt % TPP and 2 wt % TEA. The resulting reaction mixture was added drop-wise to an eightfold excess of hexane. The precipitate was filtered and dispersed in dichloromethane (DCM). The precipitation and dissolution cycle was repeated three times to remove the trace quantities of catalyst and unreacted monomers. Residual solvent was removed under reduced pressure to obtain the purified nanogel.

Preparation of E9NG covalently crosslinked polymer networks—The nanogel was added to the E9 monomer at mass fractions of 0, 5, 10, 20 and 30 with the resulting formulations subsequently photopolymerized with 0.1 wt % IR819 as the initiator. E9 with 0 wt % of nanogel was used as the control. To formulate and cast the E9NG networks with different nanogel mass fractions, the E9 monomer and the nanogel mixture were stirred well at 70 °C for 20 min and then cast between glass slides with 0.8 mm spacers. These laminated samples were then exposed to UV light (Omnicure, 365 nm at 350 mW/cm² for 10 min). Acrylate and thiol peaks were analyzed before and after UV exposure to quantify functional group conversion. The resultant films underwent an extraction procedure to remove any unreacted monomers in which the samples were swelled in acetonitrile at 40 °C for 3 days and subsequently dried under vacuum at 60 °C for another 3 days.

Characterization—The molecular weight of the nanogel was determined by a Viscotek-270 dual detector, VE3580 RI detector based gel permeation chromatography (GPC) with tetrahydrofuran (0.35 mL min⁻¹) used as the mobile phase at a column temperature of 35 °C. GPC calibration was based on a series of PMMA standards of known molecular weight and dispersity.

Viscosity measurements of E9 monomer with different nanogel mass fractions were performed using Brookfield Cap 2000 viscometer. A defined volume of each sample was tested at 25 °C under the following conditions: 100 RPM, spindle 06, hold time of 15 s and number of run 20.

Dynamic Mechanical Analyses was performed on DMA Q800 (TA Instruments) to measure the glass transition temperature (T_g) and rubbery moduli of E9 polymer films (16 mm*4 mm*0.8 mm) with different nanogel mass fractions. DMA multi strain mode was utilized by applying a sinusoidal stress of 1 Hz frequency with the temperature ramping from -30 to 100 °C at 3 °C/minute. The glass transition temperature, T_g was determined as the peak maximum of the tan delta curve. The modulus values in the rubbery state were measured at $T_g + 40$ °C whereas and the full width at half height (FWHH) of the transition was measured using the tan delta curve.

Thermogravimetric analyses (TGA) to test the thermal stability of the E9 polymer films with different nanogel mass fraction were carried out on a Pyris 7, Perkin Elmer

thermogravimetric analyzer. The temperature was ramped from 50 to 850 °C at a scan rate of 10 °C/min under inert atmosphere (Nitrogen purge at 20 ml/min).

To measure the equilibrium water content (EWC) of the E9NG polymer film, pre-weighed ~ 1 cm² E9NG films were incubated at 35 °C in 10 mL DI water for 5 days. The films were then removed from water, patted dry and the wet weight was measured using a microbalance. The samples were then dried under vacuum at 60 °C for 5 days. The EWC was calculated using the equation,

$$EWC = \frac{\text{Wet weight} - \text{Final Dry weight}}{\text{Wet weight}}$$

The surface hydrophilicity of the networks were studied using the water contact angles on the surface of the films. A NET GmbH 1394 Digital camera based contact angle measuring device (Rame-hart Instrument Co., software; Drop image advanced-Version 2-0-10) was used to carry out measurements at 22 °C. After dispersing the water drops on the polymeric surface, the contact angle was measured for both the right and left side of the water drop and the mean was determined. A minimum of five different measurements were taken for each set and averaged for this study.

The optical properties of the networks was studied by quantifying the refractive index of the E9NG polymer network, using an Abbe Refractometer (ATAGO NAR-1T solid)) along with % transmittance of light through the E9NG film at different nanogel loading using UV-visible spectroscopy (Evolution 201, Thermo Scientific). Films (0.8 mm thick) were attached onto the surface of a quartz cuvette and carefully introduced into the sample compartment of the spectrometer. For the wet RI measures, the samples were hydrated in 35 °C in 10 mL DI water for 5 days prior to measurement. Wavelengths in the range of 200–800 nm was passed through the film and % transmittance through each film was recorded.

Hydrolytic stability were studied for E9NG films at elevated temperature by measuring the weight difference of E9NG films before and after treatment. Samples were incubated with millipore water at 70 °C in the oven for 28 days. At different time periods (e.g., 7 days, 14 days, 21 days, 28 days) samples were taken out and dried at 70 °C under vacuum for 72 h and weighed. The dry weight of the sample were then compared with controls which consisted of E9NG films of different mass fractions.

The biocompatibility of the E9NG network was evaluated by the direct contact test with monolayer of L929 mouse fibroblast cells according to ISO standards (ISO 10993-5, 1999). Briefly, L929 cells were sub-cultured from stock culture by trypsinization and seeded into 6 well tissue culture plates. Cells were fed with minimum essential medium (MEM) supplemented with fetal bovine serum and incubated at 37 °C in 5 % carbon dioxide atmosphere. Cells were incubated with E9NG films at different nanogel loadings at 37 °C for 48 h. Cell culture was examined microscopically for cellular response using a phase contrast inverted microscope (Leica, WLD MPS32, Germany). The morphology of the cells was assessed in comparison with a control (media only)

Results and Discussion

The ability of reactive, thiol-functionalized nanogels to function as efficient plasticizers within a commercial dimethacrylate monomer was evaluated by formulating and characterizing polymer networks generated with different nanogel mass fractions to form crosslinked networks. The nanogels were synthesized by reacting a PEG400 UDMA monomer, hereafter referred to as E9, a tetra functional thiol PETMP and methacrylate monomers (GMA and UDMA) via step-growth thiol-methacrylate Michael addition reaction. While GMA was chosen to lower the crosslinking density within the nanogel, the UDMA monomer was chosen as a crosslinker for its ability to impart toughness as well as flexibility within the network enabled via the secondary hydrogen bonding interactions. A simple, step-growth Michael addition polymerization was chosen to synthesize nanogels with low T_g and would thus, upon inclusion within the parent network, contribute towards lowering the overall bulk network T_g . By incorporating the E9 monomer as part of the nanogel backbone, the nanogels will readily swell and disperse evenly within the monomer with reduced propensity towards phase separation while preserving the ability to selectively alter the T_g and elongation of the network. The polymer networks were characterized for thermomechanical and mechanical properties (T_g , rubbery modulus, elongation, polymerization shrinkage stress, thermal stability) and optical properties (percentage light transmission and refractive index) along with their biocompatibility and hydrolytic stability. Additionally, the bulk and surface hydrophilicity of the networks and the propensity of the network to phase separate with time within an aqueous environment was also studied.

Initially a nanogel with a 1:1 stoichiometry of thiol to methacrylate functional groups was synthesized and observed to have a T_g of $-20\text{ }^{\circ}\text{C}$. The stoichiometric 1:1 thiol – methacrylate nanogel was utilized as a control to compare and evaluate an off-stoichiometric nanogel synthesized with a 1.5:1 thiol- methacrylate ratio, which was determined to have a T_g of $-31\text{ }^{\circ}\text{C}$. Detailed characterization of the nanogel has been included within the supplementary document. Predictably, the thiol-functionalized nanogels displayed a lower T_g than the control nanogel, as the presence of excess functional groups within the crosslinked nanogel networks have a plasticizing effect, thereby lowering the bulk nanogel T_g .

The gel permeation chromatography (GPC) results indicate that the thiol-functionalized nanogel had a molecular weight M_w of 6600 g/mol and hydrodynamic radius R_h of 1.42 nm. The thiol-functionalized nanogels were utilized for all remaining tests in this study. An advantage of this approach to synthesizing nanoscale plasticizers is the versatility afforded for tunable size scales and large surface areas that also allow multivalent conjugation and facile incorporation of different functional groups on its surface (e.g. OH, SH, COOH, vinyl) to react further with a variety of monomers and copolymer systems via different bonding mechanisms to form reactive plasticizers that can then be incorporated within linear and crosslinked polymers to form stable networks.

Different mass fractions of the thiol-functionalized nanogels - 5, 10, 20 and 30 %, were incorporated as reactive plasticizers within the E9 monomer and subsequently photopolymerized to form homogenous, crosslinked networks (hereafter referred to as

E9NG-5, E9NG-10, E9NG-20 and E9NG-30 networks respectively). The incorporation of the nanogels altered the free volume within the parent E9 network and in turn, the thermomechanical and mechanical properties of the E9 matrix were altered. As untethered plasticizer migration from the parent network can be triggered by extremes in external factors such as temperature, pressure and/or exposure to sunlight, covalently crosslinking the nanogels within the E9 matrix prevents the migration of the nanogels from the matrix. The functional group conversion of the thiols was observed to be at ~70 % (Supplementary Table 1) thereby indicating the presence of unreacted thiol groups within the network that can perform as radical scavengers at a later point in time.

The presence of the unreacted thiol groups can be attributed to the likely steric hindrance encountered by the high molecular weight, thiol-functionalized nanogels within the E9NG matrix. All polymerized samples were then subject to an extraction protocol to remove any unreacted monomers and/or untethered oligomers from within the networks. As the extractables quantified from the parent E9 matrix at 4.5 % are comparable to extractables obtained from the E9NG matrices (Supplementary Table 2), regardless of the nanogel content within the networks, the results from the extraction tests further indicate that the nanogels are covalently tethered within the matrix, thereby minimizing the possibility of leaching out as a function of time.

Thermomechanical analysis was used to characterize the plasticizing effect of the E9NG networks and is observed via the increasing drop in glass transition temperature (T_g) as a function of nanogel loading within the films (Fig. 1A). Interestingly, the absence of bimodal phases in the thermograms suggests that there is a weak contribution from the nanogel/matrix phase at low nanogel loading, which at 30 %, is still below confluent nanogel packing. The mono-modal tan delta peak at 30 % nanogel loading also indicates that the hybrid nanogel/matrix phase and the matrix phase have very similar T_g s and the absence of a consistent bulk matrix phase can be attributed to the progressive change in the network structure as a result of the thiol-based chain transfer events within the network. Increasing the nanogels within the films also enhanced the flexibility of the films at ambient temperature (22 °C) as shown in elongation test results in Fig. 1B with a 37 % increase in elongation seen in E9NG-5. Although a dramatic increase in elongation (i.e. as a result of the decrease in crosslinking density) is seen with higher loading levels of nanogels with a E9NG-20 exhibiting a 400 % increase in elongation, a concomitant decrease in the strength of the material is also observed with the E9NG-30 samples proving to be too weak to undergo the elongation tests.

From E9NG-5 to E9NG-30, the double bond concentration within the E9NG monomer formulations decreases (as the mass fraction of nanogels within the network increases) and this is observed as a reduction in the rubbery modulus (i.e. crosslinking density) of the photopolymerized films. However, the full width at half height (FWHH) of the tan delta curve also progressively reduced as a function of nanogel loading. As a narrow FWHH is indicative of the presence of a homogenous network such as those generated via a step-growth polymerization reaction (i.e. a thiol-ene reaction), the progressively narrow FWHH observed in Table 1 is a further indication of the thiol-functionalized nanogels being homogeneously incorporated within the E9 matrix [Ye et al., 2011].

The thermomechanical and mechanical data demonstrates the efficiency of this approach to utilize nanogels as plasticizers. The predictable relationship between the nanogel loading fractions and thermomechanical properties of the resulting networks can aid in formulating and designing polymer networks based on the specific demands of the end-application for the polymer-nanogel matrix.

In contrast to traditional plasticizer behavior where the inclusion of a small molecule typically reduces the viscosity of the resulting mixture, an increase in nanogel content within the E9 matrix results in an increase in viscosity of the formulations at 22 °C with a 70 % increase in viscosity recorded for E9NG-30 (Fig. 2). Both, the viscosity of the plasticizer-monomer formulations along with desirable end-properties at 22 °C (i.e. reduced T_g and increased elongation) are important considerations when choosing plasticizers as they dictate ease of processing and handling behavior of both the resin formulations and the films under ambient conditions. As the E9NG-20 formulation had a modest increase in viscosity of 27.5 % at 8700 cps while maintaining a T_g at 25 °C, all subsequent characterization tests were limited to nanogel loading levels of up to 20 %. Although a reduction in T_g and an increase in elongation was the primary goal in formulating the E9NG networks, the impact on the polymerization kinetics of the polymer matrix along with the shrinkage stresses generated within the bulk network is an important practical consideration in designing plasticizer systems. Formulated for dental applications, the E9 monomer is known to have efficient kinetics and generate low shrinkage stresses while attaining high functional group conversions during the polymerization process [Froes-Salgado et al., 2012]. As shown in the supplementary data (Supplementary Fig. 4A), the rapid kinetics of the parent E9 formulation is maintained while higher functional group conversion is observed in E9NG-5 to E9NG-20, as expected in low T_g formulations with less reactive group concentrations. The increased viscosity of the E9NG networks has little to no impact on the polymerization kinetics of the systems as the presence of the thiol-functionalized nanogels as chain transfer agents would result in shorter primary chain lengths that contribute to the delayed vitrification of the network which then results in high conversions. Similarly, the shrinkage stresses generated within the networks, which were also seen to reduce as a function of nanogel loading and reduction in crosslinking density (Supplementary Fig. 4B), can be attributed to both, the overall reduction of double bonds present within the NG-loaded network along with the increased thiol-methacrylate step-growth adducts generated within the network [Cramer et al., 2010; Kloxin et al., 2009].

As small molecule plasticizers are designed to impact a targeted network property such as its glass transition temperature, they can inadvertently alter other desirable bulk properties such as the polymer's inherent hydrophilicity or hydrophobicity. To characterize the impact of nanogel loading on the bulk hydrophilicity of the E9NG networks, the equilibrium water content (EWC) of the network was measured. The EWC of a network is a measure of the propensity of the bulk polymer to swell in water upon immersion as a function of time and subsequently exclude water from the network upon drying. As shown in the Fig. 3A, the average EWC values indicate a trend that points towards reduction in the hydrophilicity of the bulk networks as a function of the nanogel content. The marginal increase in hydrophobicity of the network (17 % decrease observed between E9NG-20 and parent E9 matrix) despite a decrease in the crosslinking density of the networks can be attributed to the

increased presence of the relatively hydrophobic UDMA and GMA within the nanogels within the bulk of the network.

However, the overall reduction in crosslinking density within the E9NG networks dominated the surface hydrophilicity of the films when the surface properties of the networks were quantified using water contact angle tests. Although a reduction in the contact angle was observed from E9 to E9NG-20, at low loading levels the surface characteristics of E9 remains unchanged (Fig. 3B). Primarily depending on the end-application for which the networks are formulated, the impact of nanogel mass fractions to alter the degree of hydrophilicity of the network may or may not be a desirable outcome. However, the nanogels can be trivially modified to accommodate a change in hydrophilicity by varying the monomers selected to synthesize the nanogels. Preserving the bulk optical properties of a film while altering its mechanical properties via reactive additives can be challenging. Previous studies have shown that increasing the plasticizer content within networks can result in an overall reduction in light transmittance through the bulk of the material. Herrera *et al.* demonstrated that in the presence of 20 wt % glycerol triacetate plasticizer, the light transmittance of plasticized polylactic acid films decreased from 84 % to 33 % [Herrera *et al.*, 2015]. A change in the optical properties such as refractive index (RI) and translucency of the plasticizer-parent matrix is also indicative of the presence of phase separation and lack of homogeneity within networks. The RI of the E9 and E9NG networks was 1.506 (dry state, 22 °C) at the wavelength of 589 nm and this remains unchanged at different nanogel mass fraction, indicating the compositional similarity between the matrix and nanogel polymers and the absence of heterogeneous phase formation within the E9NG networks. The RI was also measured in the wet state in which the films were immersed in water at 35 °C for 5 days prior to measurement at 22 °C. Although predictably, the wet RI measurements were lower in comparison to the dry RI measurements (1.489 vs 1.506), there were no significant changes in RI between the different E9NG formulations in their wet and dry states (Fig. 4B). The stability of the networks generated from two or more monomers to withstand phase separation when exposed to polar solvents as a function of time and temperature was also studied by implementing a modified glistening tests. Glistening tests are typically utilized within optical devices as an accelerated test to study phase separation within networks [Thomes and Callaghan 2013]. As evident from the images (Supplementary Fig. 5) the absence of microvacuoles within the bulk of the parent network E9 and E9NG-20 indicates the absence of phase separation. This result is potentially significant as the absence of microvacuoles within the network opens up the implementation of this approach in developing devices that are subject to prolonged exposure to aqueous environments such as intraocular lenses in which optical clarity as a function of time is a prerequisite for any aspiring polymer formulation.

Often, the addition of plasticizers have the potential to adversely impact the thermal stability of the networks [Shah and Shertukde 2003]. Even though the ultimate application of the polymer may not involve exposure to high temperatures, the thermal stability of the material is an important parameter for the storage and handling considerations of biomaterials. For example, the plasticizer 2-ethylhexyl diphenyl phosphate which is used in blood-bags and dialysis equipment that need to be sterilized, can undergo decomposition at 170 °C that leads to the acceleration of plasticizer migration and corrosion of the parent network [Wypych

2012; Tuzum Demir and Ulutan 2015]. To study the impact of nanogels on the thermal stability of the E9 network, TGA of E9NG films was studied (Fig. 5). The TGA curves indicate that the thermal stability of the parent E9 was maintained with no significant weight loss (~ 1 wt %) observed up to 200 °C and that the addition of nanogels did not significantly alter the degradations characteristics of the parent E9 network. In fact, at 300 °C, the E9NG-20 networks recorded < 3 % weight loss while the parent E9 polymer network recorded a 7 % weight loss, indicating enhanced thermal stability whereas the plasticized polymer networks currently available in the market are seen to degrade at temperatures 200 °C. This observation warrants further investigation as it indicates that reactive thiol-functionalized nanogel plasticizers have the ability to stabilize polymer networks up to 300 °C, possibly via the enhanced radical scavenging properties of the thiol-functionality within nanogels [Starnes et al., 2006; Ge et al., 2007].

The hydrolytic stability of the plasticized polymer-nanogel matrices were studied by implementing a published protocol by Navarro *et al.* in which the weight loss of the E9NG samples immersed in DI water at 70 °C for 28 days was observed [Navarro et al., 2008; Navarro et al 2016]. As shown in the Fig. 6, the presence of the nanogels increases the hydrolytic stability of the E9 polymer network with only 2 % weight loss detected for E9NG-20 at 28 days treatment, whereas 3.3 % weight loss was detected in E9 polymer network indicating additional stability imparted as the nanogels bind to the polymer matrix. As significant migration of plasticizers is typically observed in hydrolytic tests with a weight loss of up to ~50 % [Bernard et al., 2015], it is of crucial significance that the E9NG networks had the ability to mitigate plasticizer migration while maintaining a hydrolytically stable polymer network.

As our primary interest was in designing plasticizers that could then be used in biomaterials such as dental adhesives, linings and blood bags, the cytocompatibility of the networks is of significance. The parent E9 and E9NG networks were studied using the direct contact test. The morphology of the cultured mouse fibroblast cells L929 in contact with the different polymer films for 48 h is shown in the Fig. 7. Typically, when in contact with a toxic surface, the spindle-shaped appearance of the L929 mouse fibroblast cells is altered and the cell takes on a spherical morphology. As seen in the images, the cells in contact with the E9NG network films demonstrate no significant change from its spindle-shaped morphology when compared with that of a negative control of media only (Fig. 7A), thereby suggesting good cytocompatibility of the parent E9 network film and E9NG network at different nanogel mass fraction.

Conclusion

The ability of thiol-functionalized nanogels to function as efficient reactive plasticizers that can selectively alter the thermomechanical properties of highly crosslinked photopolymerized methacrylate networks while preserving the optical properties, thermal stability, hydrolytic stability and biocompatibility of the bulk matrix is evaluated. Thiol-functionalized nanogels form a highly homogenous crosslinked polymer network with PEG400 UDMA parent matrix in which the glass transition temperature is seen to decrease as a function of increasing nanogel content. As evidenced from thermogravimetric analysis

and percentage weight loss tests, thermally and hydrolytically stable E9NG networks are maintained up to 300° C and a maximum 2 % weight loss observed at the end of 28 days. The fundamental approach outlined in this study to generate highly specific, efficient, functionalized nanoscale plasticizers via a one-pot synthetic protocol can be adapted widely as a simple, cost-efficient methodology to achieve targeted properties within networks.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig 1.

The glass transition temperature (T_g) of the of E9NG films at different nanogel mass fraction estimated from the peak of the tan delta curve shows a decrease in the T_g as a function of nanogel loading when compared to the E9 parent matrix (A). As the nanogel content within the parent matrix increases, an increase in the elongation of the material is also observed (B).

Fig 2.

The decrease in glass transition temperature (T_g) of the E9NG formulations as a function of increasing viscosity can be used as a formulation-aid to estimate the T_g vs Viscosity measurements in designing polymer networks.

Fig 3.

(A) The ability of nanogels to alter hydrophilicity of the parent polymer network is shown as a measure of the equilibrium water content (EWC) measured as a function of nanogel mass fractions (B). The contact angle of a water drop measured on the surface of the polymer films with different nanogel mass fractions indicate an increased surface hydrophilicity at 20 % NG loading.

Fig 4.

Refractive Index (RI) of polymer networks remain unchanged at different nanogel mass fractions. No significant changes in RI as a function of nanogel content is observed in the wet and dry RI of E9NG matrix (A). Light transmittance of the E9NG polymer network at different nanogel mass fractions follow a similar trend indicating that the bulk network maintains its translucency (B).

Fig 5.

Thermogravimetric analysis of the E9NG networks indicate that the thermal stability of the parent E9 networks was largely unaltered up to 200 °C.

Fig 6.

The hydrolytic stability tests of the E9NG networks in DI water at 70 °C for 28 days captures the enhanced stability of plasticized E9NG networks.

Fig 7. Cell morphology after 48 h incubation with (A) media only (control), (B) E9 film (C) E9NG-10 film and (D) E9NG-20 film. The spindle morphology of L929 mouse fibroblast cells are retained after incubating with E9NG at different nanogel mass fraction, indicating the cytocompatibility of these materials.

Scheme 1.

Thiol-functionalized nanogels are synthesized and dispersed within the PEG400 UDMA matrix and subsequently photopolymerized. Polymer networks generated from different mass fractions of nanogels (0, 5, 10, 20 and 30 %) within the PEG400 UDMA matrix are characterized to study the plasticizing effect of the nanogels.

Scheme 2.

Schematic representing the monomers used in this study and the synthesis of the thiol-functionalized nanogels via thiol-methacrylate Michael-addition reaction using three methacrylate monomers, PEG400 UDMA, GMA, and UDMA in the molar ratio of 30:30:40. The step-growth reaction with a thiol to methacrylate functional group ratio of 1.5:1 was catalyzed by a mixture of trimethylamine and triphenylphosphine in a solution polymerization reaction with 4× excess of solvent to yield thiol-functionalized nanogels.

Table 1

The full width at half height (FWHH) and the crosslinking density of the different networks decreases as a function of different nanogel mass fraction.

Formulation	FWHH (°C)	Rubbery Modulus (MPa); $T_g + 40$ °C
E9	54.2 ± 2.0	23.9 ± 3.9
E9NG-5	47.4 ± 2.2	15.3 ± 1.4
E9NG-10	47.1 ± 1.0	13.7 ± 1.8
E9NG-20	32.8 ± 3.4	10.5 ± 0.5
E9NG-30	26.5 ± 1.0	7.5 ± 0.5