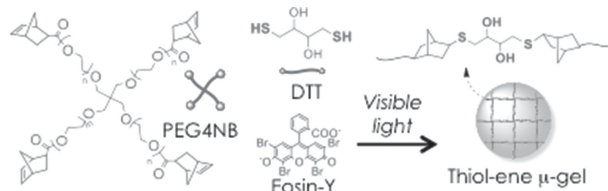


PEG-Based Microgels Formed by Visible-Light-Mediated Thiol-Ene Photo-Click Reactions

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Step-growth PEG-based microgels are produced via three liquid–liquid two-phase suspension polymerization systems: i) hexane with surfactants Span80/Tween80; ii) mineral oil with surfactant Pluronic F-68; and iii) surfactant-free dextran-rich aqueous solution. Following short vortexing to create monomer droplets, microgels are polymerized by a visible-light-initiated thiol-ene photo-click reaction using eosin-Y as the only photoinitiator. The use of hexane as the organic phase and Span-80/Tween-80 as the surfactants leads to PEG microgels with entrapped solvent droplets that dissolve rapidly with time. Microgels polymerized in mineral oil with surfactant Pluronic F-68 contain no entrapped droplets and are more uniform with smaller sizes. Visible-light-cured step-growth thiol-ene microgels can also be photopolymerized in a surfactant-free aqueous two-phase system. The sizes of the microgels formed in aqueous phase are one order of magnitude smaller than those formed in organic solvent. Dual-layer microgels are also prepared using two-step thiol-ene reactions.



1. Introduction

Bulk or macroscale hydrogels have enjoyed tremendous success in many biomedical applications.^[1–5] However, challenges and limitations exist when these bulky matrices were employed to release therapeutically relevant agents or for encapsulation of mammalian cells. Unless an injectable formulation is utilized, invasive surgery is inevitable for the implantation of bulk gels. Even if the gels can be injected and cured *in situ*, the utility of macroscale hydrogels could still be limited, in large part due to reduced permeability of biologically relevant macromolecules.^[6,7] These disadvantages could be overcome by using hydrogel microparticles, or microgels.^[8–10] A convenient and rapid method to produce microgels is through inverse

emulsion or suspension polymerization.^[11] Specifically, an aqueous solution containing macromers, initiators, and payloads (e.g., drugs or cells), is inverse-emulsified or suspended (water-in-oil) in a high volume of organic solvent containing appropriate emulsifiers/surfactants. After the monomer droplets are stabilized by the surfactant, an appropriate polymerization method (e.g., thermal, redox, photopolymerization, etc.) can be used to cross-link the microemulsions or monomer droplets into nanogels or microgels.^[12–14] In one example, Murthy et al.^[12] prepared acid-degradable microgels for delivering protein-based antigens. In such system, hexane was used as the organic phase whereas Tween-80 and Span-80 were employed as emulsifiers. After a short period of sonication, the monomer droplets were polymerized into microgels by radical-mediated chain-growth polymerization. Missirlis et al.^[13] utilized a similar inverse-emulsion photopolymerization of acrylated macromers to form cross-linked nanogels (ca. ≈ 50 –500 nm). Tibbitt et al.^[14] adopted this approach to fabricate photodegradable microgels. Crosslinking of these photodegradable microgels was achieved via a Michael-type conjugation reaction between poly(ethylene glycol) (PEG) tetrathiol and photolabile PEG-diacrylate (PEGDA).

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In addition to hexane, other organic solvents can also be used to prepare inverse emulsion or monomer droplets. For example, Peppas and co-workers^[15] fabricated PEG microspheres by co-polymerizing monomethoxy PEG monomethacrylate with methacrylic acid in silicone oil with tetraethyleneglycol dimethacrylate as a crosslinker, azobisisobutyronitrile as the initiator, and polydimethylsiloxane-*block*-poly(ethylene oxide) as the surfactant. West and co-workers^[16] prepared cell-laden microgels by mixing a PEGDA solution containing cells, surfactant Pluronic F-68, coiniciator triethanolamine (TEA), comonomer *N*-vinyl-pyrrolidinone (NVP), and photoinitiator eosin-Y in mineral oil. The cell-laden microgels were formed by visible-light-mediated chain-growth photopolymerization. In addition to the water-soluble photoinitiator eosin-Y, an additional hydrophobic photoinitiator, Irgacure 651, was included in the organic phase to enhance microgel stability. Due to the use of high concentration of coiniciator (225×10^{-3} M TEA) and comonomer (37×10^{-3} M NVP), the reaction was completed after 25 s and yielded gels with an average diameter of about 100 μm .

Alternatively, microgels can be prepared using aqueous two-phase systems (i.e., water-in-water emulsion). For example, Hennink and co-workers prepared dextran microgels by suspending dextran-methacrylate in PEG-rich aqueous solution.^[17] Microgel cross-linking was achieved at 37 °C for 30 min via a chain-growth polymerization initiated by tetramethylethylenediamine and potassium persulfate. Impellitteri et al.^[18] prepared PEG-based thiol-norbornene microgels via a suspension photopolymerization in a dextran (40%) aqueous solution. The monomer droplets contained multi-arm PEG-norbornene, PEG-dithiol, and a cleavage-type photoinitiator Irgacure 2959. The cross-linking process was achieved via a radical-mediated thiol-norbornene reaction under UV light exposure. This system was also the first to use thiol-ene photochemistry for forming PEG-based microgels for growth factor delivery.

The objective of this study was to provide an alternative thiol-ene photochemistry for preparing PEG-based microgels without the use of cytotoxic coiniciators, comonomers, or UV light sources. We have recently developed visible-light-mediated thiol-ene photo-click chemistry for forming macroscale hydrogels.^[19–21] Under bright visible light exposure ($\lambda = 400\text{--}700$ nm), gelation could be achieved in seconds to minutes using multi-arm PEG-norbornene (PEGNB) and di-thiol-containing crosslinkers in the presence of photosensitizer eosin-Y. We also found that eosin-Y could be repeatedly excited for sequential initiation of gel cross-linking.^[20] We hypothesized that this simple yet diverse photochemistry could be utilized to prepare microgels with step-growth network structure without using any cytotoxic components. To test this hypothesis, we explored three previously developed liquid-liquid two-phase systems for preparing

PEG-based step-growth microgels: i) hexane and surfactants Span-80/Tween-80,^[12] ii) mineral oil and surfactant Pluronic F-68,^[16] and iii) surfactant-free PEG/dextran aqueous solutions.^[18] The influences of various synthesis parameters (e.g., macromer molecular weight and content, surfactant concentrations, time of emulsion, etc.) on the size distribution of these new thiol-ene microgels were investigated. Finally, we evaluated the feasibility of using this system to prepare multilayer microgels.

2. Experimental Section

2.1. Materials

Four-arm PEG macromers (M.W. 5, 10, and 20 kDa) were purchased from JenKem Technology USA. Eosin-Y disodium salt, mineral oil, and dithiothreitol (DTT) were purchased from Fisher Scientific. Hexane, Tween-80 (polysorbate), and Span-80 (sorbitan monooleate) were obtained from EMD Millipore, BDH, and Tokyo Chemical Industry (TCI), respectively. All other materials were purchased from Sigma-Aldrich unless noted otherwise.

2.2. PEG-tetra-Norbornene (PEG4NB) Synthesis

PEG-tetra-norbornene (PEG4NB) was synthesized following an established protocol.^[22] Briefly, 5-norbornene-2-carboxylic acid was activated by *N,N*-dicyclohexylcarbodiimide (DCC, 2.5 eq.) in anhydrous dichloromethane (DCM) in a fritted reaction vessel. After 2 h of continuous stirring at room temperature under nitrogen, the norbornene anhydride formed was dripped into a two-necked round flask containing four-arm PEG, 4-(dimethylamino)pyridine (DMAP), and pyridine in anhydrous DCM. The reaction was left on ice for overnight in the dark. The PEG4NB product was precipitated in cold ethyl ether and dried in vacuum. Dried PEG4NB was redissolved in ddH₂O and dialyzed for 2 d at room temperature. ¹H NMR (Bruker Avance 500) was used to confirm the degree of PEG4NB functionalization (ca. 80–90%).

2.3. Microgel Fabrication via Inverse Suspension Photopolymerization

A typical prepolymer solution contained macromer PEG4NB, crosslinker DTT, photoinitiator eosin-Y (0.1×10^{-3} M), and surfactants. PEG4NB molecular weight and content were varied while a stoichiometric ratio of thiol (from DTT) to ene (from PEG4NB) was maintained for all prepolymer formulations. Three surfactant conditions were studied: 1) no surfactant; 2) 0.25 wt% Tween-80 and 0.75 wt% Span-80; and 3) Pluronic-F68 (between 0.1 to 5 wt%). An aliquot (100 μL) of prepolymer solution was added to a glass test tube containing 500 μL of either mineral oil or hexane. After vigorously vortexing, the prepolymer solution (between 1 to 60 s at top speed on a Genie2 Vortex), a halogen cold light lamp (AmScope Inc.) was used to initiate visible-light-mediated thiol-ene reaction. Light exposure time was fixed at 2 min unless noted otherwise. Following cross-linking, the microgels were removed from the organic phase by washing

with phosphate buffered saline (PBS) (pH 7.4) once, 2-propanol once, and then PBS once again. At every washing step, supernatant was removed by aspiration after centrifugation at 3000 rpm (Eppendorf Centrifuge 5702) for 10 min.

2.4. Viscometry

The viscosity of prepolymer solution was measured in a Bohlin CVO digital rheometer operating in the viscometry mode using a 4° cone with diameter of 40 mm. The gap size between the cone and plate was set to 50 μm and the shear rate was increased from 100 to 400 Hz over a period of 200 s at 25 °C.

2.5. Characterization of Bulk Hydrogel Swelling

Bulk thiol-ene hydrogels for swelling studies were prepared by radical-mediated thiol-ene photopolymerization as described previously.^[23] Immediately post-gelation, hydrogels were placed in pH 7.4 PBS for 48 h to removed sol fractions while allowing the gels to reach equilibrium swelling. Swollen gel weights were measured gravimetrically and the gels were dried in vacuum desiccator for 24 h prior to measuring dried weight. Equilibrium swelling ratios (q_{eq}) were determined by taking the ratio of the swollen gel weight to dried polymer weight.

2.6. Microgel Fabrication via Aqueous Two-Phase Separation

For aqueous two-phase emulsion, 20 μL prepolymer solution was added to 180 μL of 40 wt% dextran (M.W. 15–25 kDa) solution, which also contained DTT and eosin-Y at the same concentrations as those in the prepolymer solution. The mixture was vortexed for 60 s and the emulsified solution was immediately placed under the visible light source for 4 min. The photopolymerized thiol-ene microgels were washed three times with pH 7.4 PBS.

2.7. Dual-Layer Microgel Preparation

Dual-layer microgels were prepared by forming the core microgels first, followed by the formation of an interfacial gel layer using the same visible-light-mediated thiol-ene cross-linking. The prepolymer solution for preparation of core microgels consisted 10 wt% PEG4NB (20 kDa), DTT, and 1 vol% Pluronic-F68. A higher concentration of eosin-Y ($2 \times 10^{-3}\text{M}$) was used. Core microgels were first formed using the mineral oil method described above and were placed in another prepolymer solution containing 15 wt% PEG4NB and DTT. For imaging purpose, 10 vol% of Fluoresbrite blue microparticles (0.1 μm ; Polysciences) were added in the second prepolymer solution. The mixture of microgels and the second prepolymer solution was vortexed for 2 s and exposed to visible light for 5 min. Dual-layer microgels were washed three times in pH 7.4 PBS prior to imaging.

2.8. Image Acquisition and Data Analysis

Prior to imaging, microgels were stored in pH 6.0 PBS to reduce hydrolytic degradation of thiol-ene network.^[23] Microgels were

suspended in PBS and imaged using an inverted microscope (Nikon Eclipse Ti) in phase-contrast mode or epi-fluorescence mode. A DAPI filter was used to image blue nanoparticles and an FITC filter was used to image eosin-Y that has intrinsic fluorescence. Fluorescence intensity plots of dual-layer microgel transverse section were obtained from Nikon imaging software (NIS-Elements). All experiments were conducted independently for at least three times and the results were reported as mean \pm SEM. At least 200 microgels were analyzed for each condition. Data analysis was performed on Prism 5 software.

3. Results and Discussion

3.1. Visible-Light-Initiated Thiol-ene Microgels: Hexane-Based Inverse Suspension Polymerization

UV-light-initiated orthogonal thiol-norbornene photochemistry has been used to fabricate macro-scale hydrogels for cell and protein encapsulation and delivery.^[24–27] Recently, Impellitteri et al.^[18] developed UV-based thiol-ene microscale gels using an immiscible biphasic aqueous system for sequestration, and subsequent delivering, of vascular endothelial growth factor (VEGF). We sought to synthesize orthogonal thiol-norbornene microgels using a visible light source and a re-excitabile photoinitiator. We have previously shown that orthogonal thiol-norbornene photoclick chemistry can be rapidly initiated by visible light using eosin-Y as the only initiator.^[19] The current contribution presents microgel fabrication methods based on this cross-linking scheme. We first prepared step-growth thiol-norbornene microgels using hexane as the organic phase and 1% Span-80/Tween-80 (1:3) as the surfactants.^[12,14] The aqueous prepolymer solution contained 10 wt% macromer PEG4NB (10 kDa), cross-linker DTT (at a stoichiometry ratio to norbornene group), and photoinitiator eosin-Y (100 μM). Water-in-oil inverse suspension was prepared via vortexing the biphasic system while gel crosslinking was achieved within 30 s of visible light exposure (Figure 1A). Polydisperse thiol-ene microgels were formed (10–130 μm) with a mean diameter of $70 \pm 3 \mu\text{m}$ (Figure 1B,C). Interestingly, a close up inspection using a higher magnification microscope objective revealed that many microgels contained small droplets in the core (Figure 1D). These droplet-containing microgels appeared in more than 80% of all microgels following fabrication and the percentage dropped quickly to $\approx 3\%$ after 24 h (Figure 1E). The presence of these small solvent droplets in microgels cross-linked from inverse emulsion has not been reported in previous microgel synthesis procedure. We hypothesized that these droplets were residual hexane or air bubbles that were trapped within aqueous microgel temporarily because of an oil-in-water-in-oil (O/W/O) multi-emulsion process. While these droplets dissolved rapidly, their presence might not be ideal if the

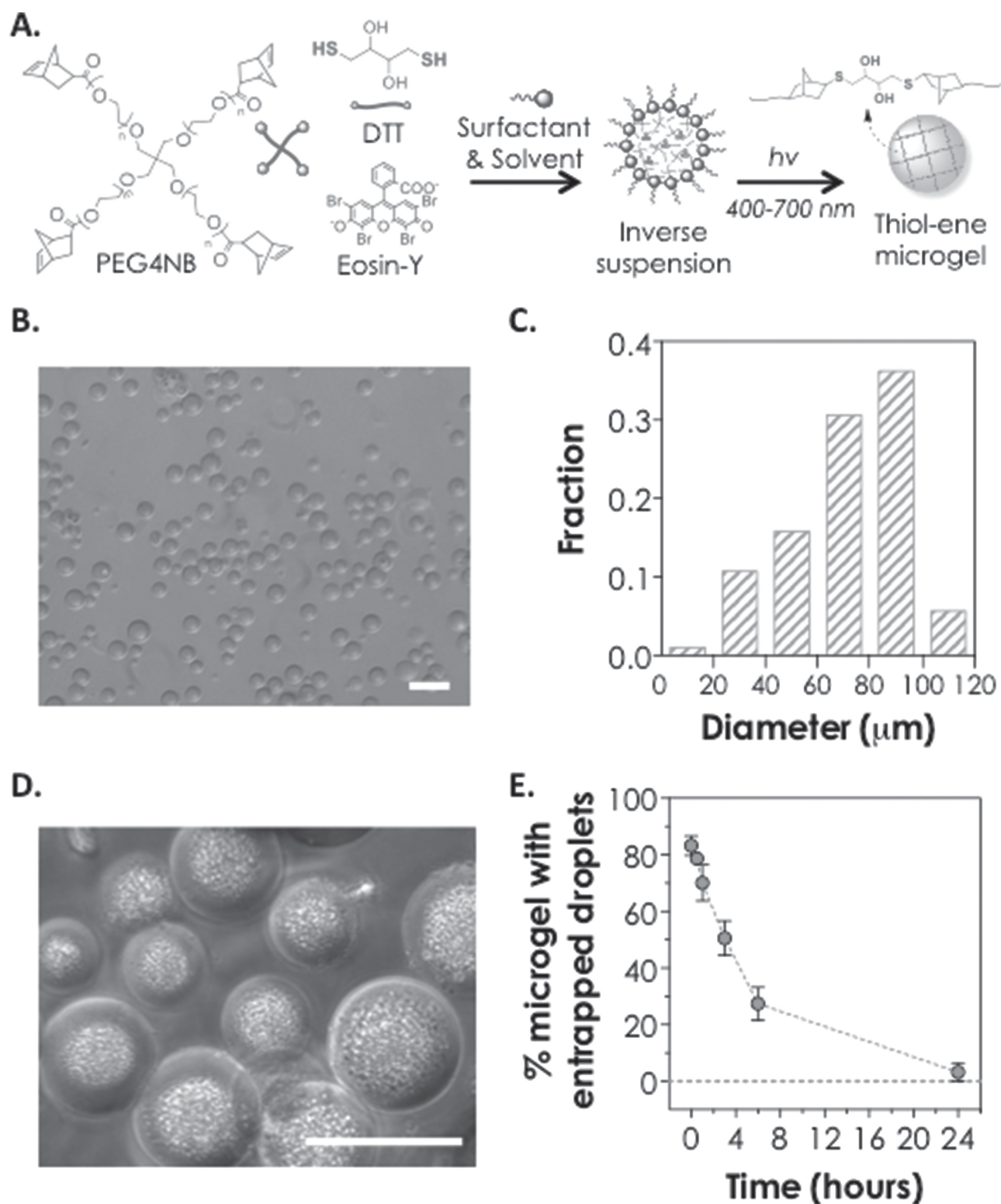


Figure 1. A) Schematic of inverse suspension and visible-light-initiated thiol-ene reaction for forming step-growth microgels. B) Representative image and C) size distribution of microgels formed by visible-light-mediated thiol-ene reaction using hexane as an organic phase and 1% Span-80/Tween-80 (3:1) as the surfactants. D) Higher magnification of microgels showing the entrapment of small droplets of organic solvent. Images were taken immediately after gel cross-linking. E) Percentage of microgels with entrapped droplets as a function of time (PEG4NB: 10 kDa, 10 wt%; Mean \pm SEM; all scales: 100 μm).

microgels were to be used for encapsulating biologics. It may be possible to eliminate the formation of these droplets by using different ratios or concentrations of the two surfactants and future work is required for this optimization.

3.2. Visible-Light-Initiated Thiol-ene Microgels: Mineral Oil-Based Inverse Suspension Polymerization

To improve the quality of these visible-light-cured microgels, we explored a second inverse suspension polymerization method using mineral oil as the organic phase and nonionic cell culture grade Pluronic F-68 as the surfactant. We found that microgels cross-linked in mineral oil contained no small droplets as seen in those formed in hexane (Figure 2A). Without the use of any surfactant, microgels could still be polymerized from the unstable inverse-suspension following 20 s of vortexing (Figure 2B). These microgels, however, had a wide range of sizes and a relatively larger average diameter ($122 \pm 21 \mu\text{m}$, Figure 2B). More than 12% of these microgels formed without emulsifier had diameters higher than $190 \mu\text{m}$ (data not shown). Since many studies have shown that increasing surfactant concentration decreases the size of the emulsified particles, we further studied the influence of Pluronic F-68 concentration on thiol-ene microgel size. Figure 2B reveals that 1% of Pluronic F-68 was sufficient to yield small microgels for this system. When 1% Pluronic F-68 was added as the emulsifier, the inverse suspension was stabilized and afforded smaller microgels with an average diameter of $47 \pm 1 \mu\text{m}$ (Figure 2B). Further increasing surfactant concentration to 2% did not yield substantially smaller microgels (Figure 2C). We also evaluated the influence of vortexing time on the resulting microgel diameter. We found that although increasing vortexing time from 1 s to 60 s did not change the average diameter of the thiol-ene microgels, a longer vortexing time did decrease the polydispersity of the microgels (data not shown). It is also worth noting that the use of either Span-80/Tween-80 mixture in mineral oil or Pluronic F-68 in hexane did not form stable monomer droplets and hence no microgels were obtained.

3.3. Effect of PEG4NB Molecular Weight on Microgel Size

We further examined the influence of PEG4NB macromer compositions (e.g., molecular weight and weight content) on the sizes of thiol-ene microgels using mineral oil as the organic phase and Pluronic F-68 as the surfactant. As shown in Figure 3A, the use of higher molecular weight PEG4NB at the same weight content (i.e., 10 wt%) resulted in larger microgels. The majority of thiol-ene microgels formed with 5 kDa PEG4NB were smaller with diameters ranging from 10–30 μm and averaged to $27 \pm 3 \mu\text{m}$. On the other hand, almost all microgels formed with 20 kDa PEG4NB were larger than 50 μm and had an average diameter of $96 \pm 5 \mu\text{m}$. Larger microgels obtained at a higher

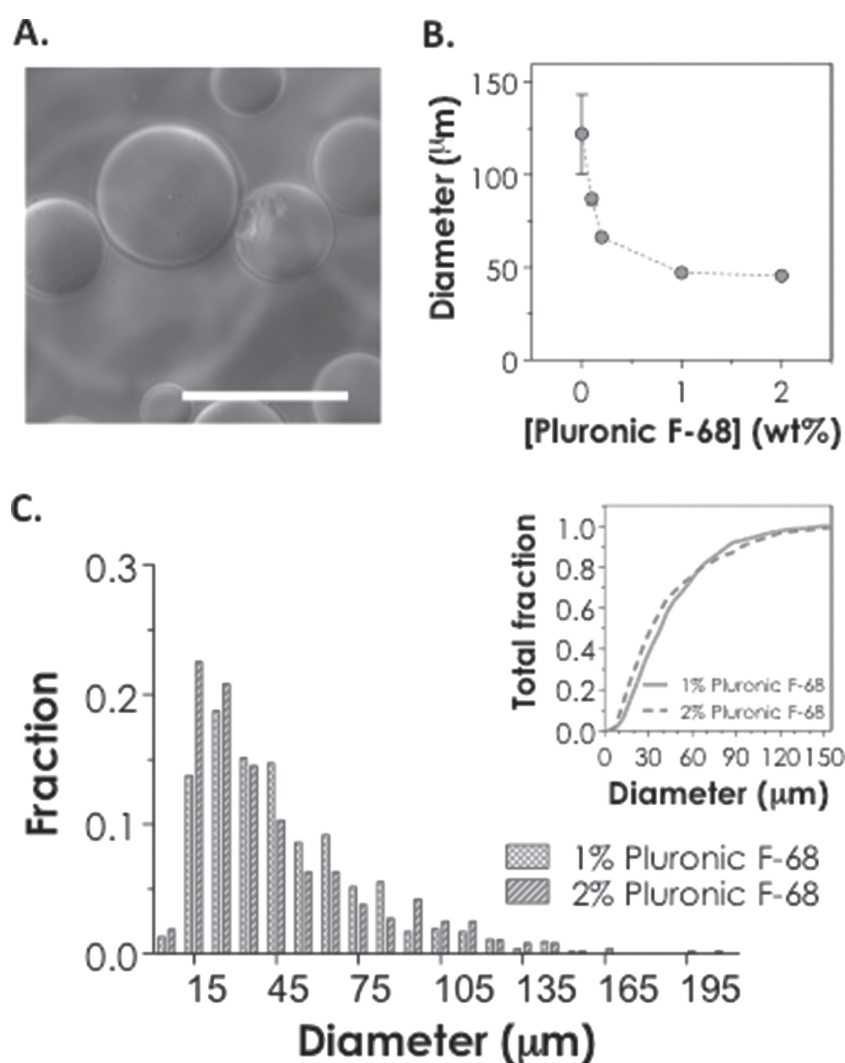


Figure 2. A) Photograph of microgels formed by visible-light-mediated thiol-ene reaction using mineral oil as the organic phase and Pluronic F-68 as the surfactant (scale: 100 μm). B) Effect of Pluronic F-68 concentration on average diameter of thiol-ene microgels (vortex time: 20 s; Mean \pm SEM). C) Effect of 1% and 2% Pluronic F-68 on the distribution of thiol-ene microgel diameter. Inset figure shows the accumulative fraction. All microgels were prepared from 10 kDa, 10 wt% PEG4NB.

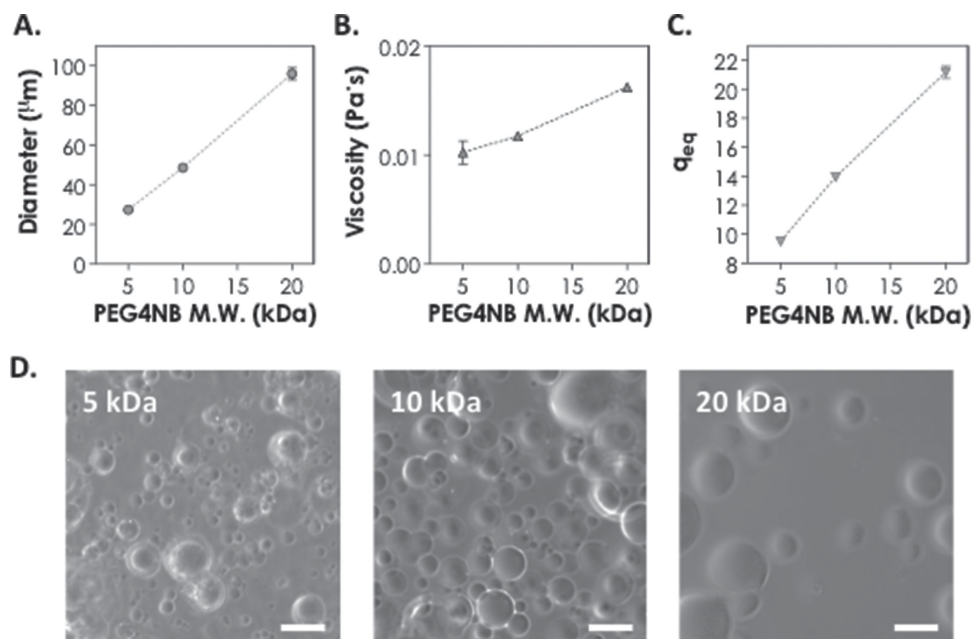


Figure 3. Effect of PEG4NB molecular weight on: A) thiol-ene microgel average diameter; B) prepolymer solution viscosity; and C) bulk hydrogel equilibrium swelling ratio (q_{eq}). D) Representative images of thiol-ene microgels with different PEG4NB M.W. (at 10 wt% using mineral oil as an organic phase and 0.1% Pluronic-F68 as a surfactant. Vortex time: 20 s. Mean \pm SEM. Scale bars: 100 μm).

molecular weight PEG4NB could be a result caused by increased prepolymer solution viscosity (Figure 3B). Specifically, increasing PEG4NB macromer molecular weight from 5 to 20 kDa led to a 60% increase in prepolymer solution viscosity (from ca. 0.01 to ca. 0.016 Pa s). A higher prepolymer solution viscosity produces stronger cohesive forces between polymer chains and hence results in microgels with larger sizes during inverse suspension and following cross-linking. The positive correlation between PEG4NB molecular weight and microgel size could also be explained by hydrogel cross-linking efficiency and swelling. At the same macromer content (i.e., 10 wt%), prepolymer solution prepared from higher molecular weight PEG4NB yielded lower total functionality (e.g., 80×10^{-3} M, 40×10^{-3} M, 20×10^{-3} M norbornene groups for

5 kDa, 10 kDa, and 20 kDa PEG4NB, respectively) and produced hydrogels or microgels with lower cross-linking density and higher equilibrium swelling.^[23] This effect could be easily observed from the equilibrium swelling ratios of bulk hydrogels with the same macromer compositions as that used in microgel cross-linking (Figure 3C). Specifically, equilibrium swelling ratios were 9.5 ± 0.2 , 14 ± 0.2 , and 21 ± 0.4 for thiol-ene bulk hydrogels prepared from 5, 10, and 20 kDa PEG4NB, respectively.

3.4. Effect of PEG4NB Weight Content on Microgel Size

Next, we examined the effect of PEG4NB (at 10 kDa) concentration in the aqueous phase on microgel size. As shown in Figure 4A, microgel diameter decreased with

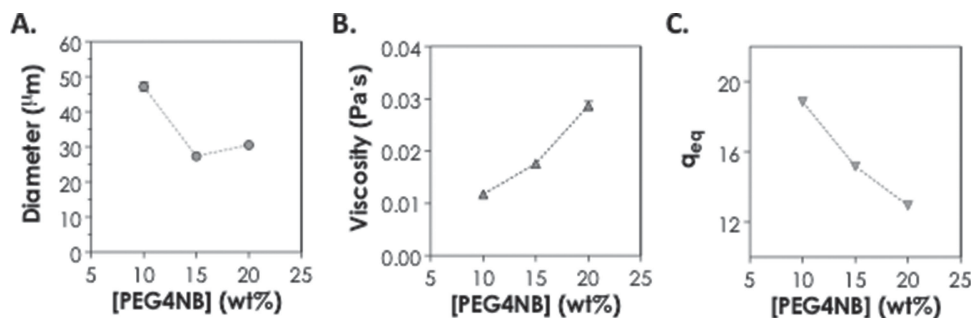


Figure 4. Effect of 10 kDa PEG4NB weight content on: A) thiol-ene microgel average diameter; B) prepolymer solution viscosity; and C) bulk hydrogel equilibrium swelling ratio (q_{eq}). Mineral oil was used as an organic phase and 0.1% Pluronic-F68 as a surfactant (Vortex time: 20 s. Mean \pm SEM).

increasing macromer concentration up to 15 wt% (with 1% Pluronic F-68). The average diameter of 15 wt% PEG4NB microgels ($27 \pm 1 \mu\text{m}$) was 42% smaller than the diameter of 10 wt% PEG4NB microgels ($47 \pm 1 \mu\text{m}$). Further increasing the PEG4NB weight content to 20 wt% did not decrease the size of the microgels (dia. $31 \pm 1 \mu\text{m}$). We believe that this was due to a counter-balance between prepolymer viscosity and microgel cross-linking density. Although increasing macromer concentration in the prepolymer solution increases viscosity of the prepolymer solution (Figure 4B), an effect that would have resulted in larger microgels, this effect also improves step-growth hydrogel cross-linking efficiency, which causes a reduction in hydrogel swelling. We verified the enhancement of gel cross-linking density by measuring the equilibrium swelling ratios of bulk thiol-ene hydrogels with identical gel formulations (Figure 4C). Although equilibrium swelling ratio data shown in Figure 4C indicate an improvement in gel cross-linking density when PEG4NB weight content was increased from 15 to 20 wt%, the drastic increases in prepolymer solution viscosity in a concentrated prepolymer solution (from 0.012 to 0.029 Pa s) likely yielded a larger droplets prior to photo-crosslinking. Comparing results from Figure 3 and 4, it is clearly that using macromer with different molecular weight produced microgels with more predictable sizes.

3.4. Microgels Prepared from Aqueous Two-Phase Separation

Another liquid–liquid two-phase approach to prepare microgels for biomedical applications is aqueous two-phase separation. In this process, two immiscible aqueous solutions are mixed together to form discontinuous prepolymer droplets (e.g., PEG-based macromers) in a continuous aqueous solution (e.g., dextran solution). Surfactant-free PEG/dextran aqueous two-phase systems have been developed for preparing PEG or dextran microgels depending on the relative solution ratio and monomer functionality (i.e., cross-linkable PEG or dextran).^[17,18] PEG and dextran are immiscible and based on thermodynamic principles, phase separation occurs when the change in Gibbs free energy is positive or:

$$\Delta G_{\text{mix}} = \Delta H_{\text{mix}} - T\Delta S_{\text{mix}} > 0 \quad (1)$$

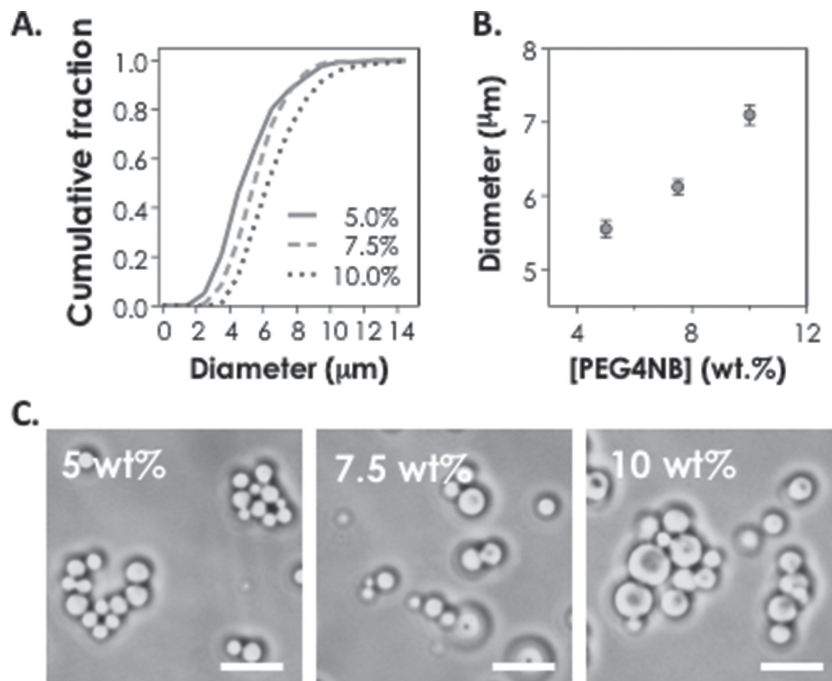


Figure 5. Effect of PEG4NB (10 kDa) weight content on size distribution of thiol-ene microgels formed by aqueous two-phase emulsion using 40% dextran as the second aqueous phase. A) Cumulative fraction of microgel diameter. B) Average diameter of microgels (Mean \pm SEM). C) Representative images of thiol-ene microgels formed with 5, 7.5, and 10 wt% PEG4NB-10 kDa. Vortex time: 20 s (scales: 20 μm).

When the gain in entropy upon mixing ($T\Delta S_{\text{mix}}$) is not large enough to compensate for the repulsive dextran/PEG interaction enthalpy (ΔH_{mix}), mixing of the two polymer solutions is thermodynamically unfavorable.^[17] Consequently, phase separation of the two solutions occurs. In this study, we used 40% dextran solution as the continuous aqueous phase, from which to generate PEG4NB macromer droplets. After vortexing for 60 s, PEG-based monomer droplets formed in dextran continuous phase and the system was placed under visible light exposure for 4 min. This aqueous two-phase separation is stable (at least stable enough for the time needed to achieving thiol-ene photogelation) without surfactant because of the highly viscous continuous phase (i.e., 40% dextran solution). In another word, the high viscosity in the dextran phase slows down the coalescence and sedimentation of the discontinuous PEG-based droplets.

Compared with the inverse suspension formed in organic solvents, we found that the microgels formed from this aqueous two-phase system were much smaller (Figure 5). Furthermore, increasing PEG4NB content in the prepolymer solution increased the average diameter of the microgels (Figure 5A,B). This trend was opposite to that obtained from using inverse suspension photopolymerization (Figure 3C), even though the sizes of the thiol-ene microgels formed from these two systems

were different in one order of magnitude (Figure 5C). The cross-linking efficiency of thiol-ene microgels cured in this aqueous two-phase system was potentially different from those cured in the pure inverse suspension polymerization (i.e., mineral oil + Pluronic F-68). Because some macromer components (i.e., DTT & eosin-Y) were also soluble in the disperse phase (i.e., dextran solution), the cross-linking was likely achieved in a mixed suspension-interfacial photopolymerization. Previous work on aqueous two-phase separation has also revealed a dependency of microgel size on solution viscosity. For example, Stenekes et al.^[17] showed that a higher viscosity in the disperse phase increased the size of the microgels. Since increasing PEG4NB concentration increases the viscosity of prepolymer solution (Figure 4B), the positive correlation between thiol-ene microgel size and PEG4NB wt. content could be attributed to the influence of disperse phase viscosity.

3.5. Interfacial Thiol-ene Photopolymerization for Forming Multilayer Microgels

Microgels with complex structures such as multilayers, patches, or hollow compartments are promising materials for biomedical applications.^[28–31] As a proof-of-concept, we demonstrated here the feasibility of using our visible-light-initiated thiol-ene photochemistry to prepare dual-layer microgels. This method combines the interfacial thiol-ene photopolymerization that we reported previously^[20] and the inverse suspension polymerized thiol-ene microgels developed in this report. As shown in Figure 6, dual-layer microgels were successfully synthesized (bottom panels represent fluorescence intensity profiles obtained from image analysis). Green fluorescence (in the core gel) shown in Figure 6 indicates the presence of residual eosin-y trapped in the core microgel, while blue fluorescence (on the peripheral area of the gel) was from the blue micro-particles entrapped in the newly polymerized outer layer. Without light exposure (Figure 6A), no noticeable blue fluorescence was present around the peripheral of the core microgel. After 2 min of visible light exposure, however, a noticeable blue fluorescence layer (20–30 μm thick) was observed around the core microgel, indicating the formation of an interfacial cross-linked hydrogel (Figure 6B). It should be noted that no additional initiator component was used in the second prepolymer solution to prepare the gel coating and that the gelation process was initiated from the surface of the core gel. The secondary light exposure time was critical in the final thickness of the coating because a bulk gel containing all microgel gels was obtained when the secondary light exposure time was increased to 5 min (data not shown). It is worth noting that we used a higher concentration (i.e., 15 wt%) of the secondary prepolymer solution primarily because it not only

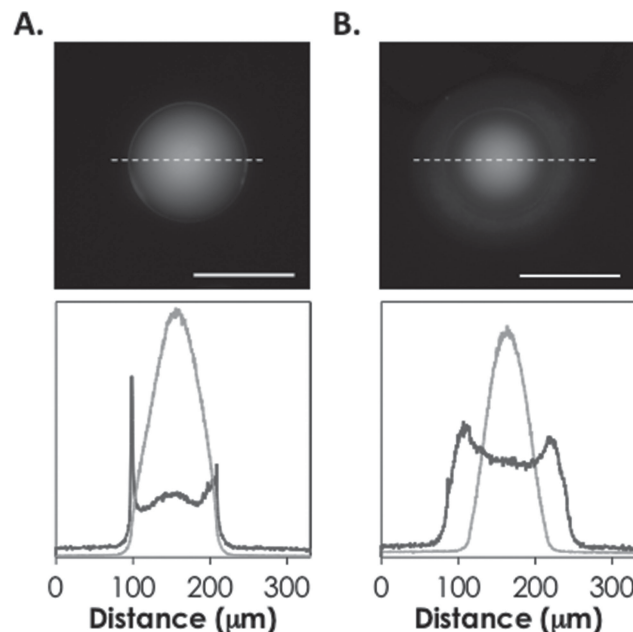


Figure 6. Fluorescent images and corresponding intensity plots of thiol-ene microgels with conformal coating. Core microgels (10 wt% PEG4NB-20 kDa) were formed in mineral oil/Pluronic-F68 inverse suspension thiol-ene photopolymerization. Conformal gel coating was formed with secondary interfacial thiol-ene photopolymerization in 15 wt% PEG4NB-20 kDa macromer solution containing DTT. Green fluorescence was from residual eosin-y in the core gel, while blue fluorescence was from blue micro-particles incorporated in the outer layer prepolymer solution. Secondary photopolymerization time: A) 0 min, B) 2 min (scale bars: 100 μm).

accelerated the coating polymerization, but also prevented the coalescence and sedimentation of the core microgels. Although not the focus of this study, the addition of a hydrogel coating greatly expands the utility of these microgels in controlled delivery applications. An outer coating layer can serve as a diffusion barrier that delays the release of therapeutic agent contained in the core microgel. The coating modification can also conceivably be rendered responsive to certain external stimuli such as solution pH, temperature, and proteases. Additional optimization and characterization of the coating properties are required to fully exploit the utilities of this new microgel platform.

4. Conclusion

We have successfully synthesized step-growth thiol-ene microgels using visible-light-initiated inverse suspension or aqueous two-phase photopolymerization. We found that mineral oil with Pluronic F-68 surfactant affords thiol-ene microgels with smaller and more uniform sizes. We further systematically studied the influence of various processing parameters (i.e., suspension type, surfactant concentration,

vortexing time, macromer molecular weight, and concentration, etc.) on thiol-ene microgel size. We also evaluated the relationship between microgel size, prepolymer solution viscosity, and hydrogel cross-linking efficiency. Lastly, we show that dual-layer step-growth microgels could be readily obtained using a unique two-step visible light cross-linking without the presence of additional initiator in the secondary macromer solution.

Acknowledgements: This project was supported by the Department of Biomedical Engineering at IUPUI, Indiana CTIS and Indiana Diabetes Research Center in IU School of Medicine, IUPUI Biomechanics and Biomaterials Research Center (BBRC), and an IUPUI UROP grant. The authors thank Han Shih for providing PEG4NB macromers and technical support.

Received: November 23, 2013; Revised: January 12, 2014; Published online: February 12, 2014; DOI: 10.1002/macp.201300731

Keywords: aqueous two-phase; microgels; photopolymerization; suspension polymerization; thiol-ene

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