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Physiologically relevant, pH-responsive PEG-based block and statistical copolymers with *N,N*-diisopropylamine units†

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Abstract

In order to impart pH-responsiveness within a physiologically-relevant context to PEG-based biomaterials, a new tertiary amine containing repeat unit, *N,N*-diisopropyl ethanolamine glycidyl ether (DEGE), was developed and incorporated into statistical and block copolymers with ethylene oxide (EO), and allyl glycidyl ether (AGE) *via* anionic ring-opening polymerization. The reactivity of this novel monomeric building block in copolymerizations with EO was investigated by spectroscopy with observed reactivity ratios of $r_{\text{DEGE}} = 1.28 \pm 0.14$ and $r_{\text{EO}} = 0.82 \pm 0.10$. It was further demonstrated that DEGE containing copolymers could serve as building blocks for the formation of new pH-responsive materials with a pK_a of *ca.* 9, which allowed macroscopic hydrogels to be prepared from symmetric triblock copolymers $\text{PDEGE}_{5,3\text{k}}\text{-}b\text{-PEO}_{20\text{k}}\text{-}b\text{-PDEGE}_{5,3\text{k}}$. The triblock copolymers exhibited clear sol-to-gel transitions in a physiologically-relevant critical gelation range of pH 5.8–6.6 and pH-dependent viscoelastic properties. On the nanometer scale, the preparation of pH-responsive micro- or nanogels was demonstrated by crosslinking $\text{P}(\text{DEGE-co-AGE})$ copolymers in miniemulsion droplets stabilized by $\text{PEO-}b\text{-P}(\text{DEGE-co-AGE})$ diblock terpolymers. These nanoparticles exhibited a reversible pH-dependent swelling profile with a volume phase transition at physiological pH 6.5–7.5.

Introduction

Poly(ethylene oxide) (PEO) is an established material in both commodity and specialty applications. For example, PEO is utilized to provide steric shielding, water-solubility, and alter biodistribution of an active pharmaceutical ingredient which is typically conjugated at the polymer terminus, or at the midpoint.^{1,2} This success has driven the need for next generation therapeutic polymer platforms, which overcome many of the limitations of PEO. This includes higher functionality for inclusion of targeting ligands,² the triggered release of therapeutic cargo,³ while allowing for improved biocompatibility and inherent responsiveness to ambient stimuli.^{3–7} In addition, the specific incorporation of functional/

reactive groups at well-defined specific locations (*e.g.* along the backbone, at the termini, or at the midpoint) of the macromolecular architecture is of significant interest.

Based on these considerations, multifunctional and responsive polyethers provide a robust platform for creating the next generation of biomedical materials.⁸ Derived from current therapeutic polymers such as poly(ethylene oxide), these materials not only exhibit excellent biocompatibility as an inherent feature but also can be rendered stimuli-responsive by the incorporation of specific functional groups. Ambient stimuli such as those provided in the vicinity of inflammation, infection, and tumor growth are of particular interest for biomedical applications.^{9,10} An obvious stimulus to exploit in the general design of higher order macromolecular assemblies is the utilization of inherent variations in pH throughout the body as a function of location and health status.⁹ Therefore, pH-responsive polyethers are highly attractive and have been realized by few tertiary amine-containing copolymers with ethylene oxide.^{11–13} The incorporation of pH-sensitive groups enables tuning the cloud-point temperature, and triggering micellization or hydrogelation by changes in pH.^{5,12–19} Specifically for some applications in biomedical materials, transitions with pH must occur within the window of physiologically-relevant pH variation of *ca.* pH 5.0–7.4. For this reason, in a previous report we investigated the incorporation of histamine moieties into PEG-based biomedical materials.¹³ While the histamine-containing materials exhibited a transition between hydrophilic and hydrophobic states at *ca.* pH 6, the associated contrast between the hydrophilic and hydrophobic states was weak. In order to investigate a system with a potentially larger change in hydrophobicity within pH 5.0–8.0, we turn our attention to the *N,N*-diisopropylamine moiety ($pK_a \approx 6.5$).⁵ In this report, a new epoxide monomer based on *N,N*-diisopropylamine was designed and incorporated into block and statistical copolymers with ethylene oxide and functional glycidyl ethers leading to responsive hydrogels and microparticles.

Results and discussion

Tertiary amines are well established as pH-responsive moieties in (meth-)acrylate based polymers. While there are a variety of different structures known in this field,⁵ tertiary amines are yet underrepresented in the area of functional polyethers as pH-responsive moieties, particularly those that respond specifically within physiologically relevant pH windows. In order to expand this platform and broaden its versatility, the synthesis, characterization, and the study of the polymerization behavior of new epoxide-based monomers is of high interest. In analogy to 2-(diisopropylamino)ethyl methacrylate, *N,N*-diisopropyl ethanolamine glycidyl ether (DEGE) was investigated as a new pH-responsive epoxide-based monomer for use in anionic ring-opening polymerizations. DEGE was synthesized by the reaction of *N,N*-diisopropyl ethanolamine with epichlorohydrin in the presence of base and obtained in high purity after facile purification allowing for anionic ring-opening polymerization.

In order to examine the polymerization behavior of this new monomer and its suitability for the preparation of more complex polyether architectures, initial experiments examined the homopolymerization of DEGE and its copolymerization with EO. Using anionic ring-opening polymerization, DEGE was polymerized in tetrahydrofuran (THF) at 45 °C using a

potassium alkoxide initiator. Polymerizations were allowed to proceed for 48 hours resulting in quantitative conversion to poly(*N,N*-diisopropyl ethanolamine glycidyl ether) (PDEGE). Copolymerizations (Scheme 1) with ethylene oxide (EO) resulting in poly[(ethylene oxide)-*co*-(*N,N*-diisopropyl ethanolamine glycidyl ether)] (P(EO-*co*-DEGE)) were also carried out under similar conditions leading to quantitative conversion and low polydispersity indices (PDIs) as shown in Table 1. Of special interest for *in vivo* applications is the ability to control the polymeric building blocks to be in the biomedical relevant molecular weight range of 10 to 30 kg mol⁻¹.^{1,20} Moreover, it is vital to be able to predict and tune the composition of the polymers in a facile way. The molecular weights and compositions of each copolymer were determined by ¹H NMR spectroscopy (a representative spectrum is shown in Fig. 1a) and could be controlled by the monomer-to-initiator ratio with the composition of the resulting polymer adjusted by the monomer feed ratio as shown in Table 1. The molar incorporations of DEGE ranged from 10 to 40 mol% in P(EO-*co*-DEGE) and varied less than 8% from the targeted compositions, thus demonstrating the significant versatility of this polyether platform for the preparation of responsive copolymers with controlled architecture and composition.

The ability to control composition, molecular weight and PDI of DEGE containing polyethers bodes well for its suitability in the design of new, PEG-based pH-responsive materials. However, since the solution properties of copolymers can vary drastically based on the specific location of functional groups along the polymer backbone, it is important to gain fundamental insight into the copolymerization process and resultant incorporation statistics. We recently demonstrated that reactivity ratios in copolymerizations of EO and substituted glycidyl ethers can be determined by utilizing ¹H NMR spectroscopy of an end-group dyad.²¹ The chemical shift of the benzylic (2H) protons is sensitive to the sequence of the initial two repeat units of the copolymer and provides a record of the first events that occurred during the copolymerization.²¹ Relating the relative populations of each dyad to reactivity ratios is facilitated by the fact that the dyad is located adjacent to the initiator (see Fig. 1c). By examining the ¹H NMR spectra of the benzyl (2H) signals for the copolymers synthesized at various comonomer compositions shown in Fig. 1b, reactivity ratios were determined for several samples at [EO]₀/[DEGE]₀ = 1.5–9 and averaged over the reactivity ratios for each initial composition [EO]₀/[DEGE]₀. The final averages are reported as the reactivity ratios for the ethylene oxide (*r*_{EO}) and DEGE (*r*_{DEGE}) alkoxide chainends under our reaction conditions. This analysis gives *r*_{DEGE} = 1.28 ± 0.14 and *r*_{EO} = 0.82 ± 0.10 which illustrates that the EO/DEGE system is nearly a random copolymerization, but with a slight gradient favoring DEGE repeat units near the initiator and EO repeat units near the terminus. Based on the evaluated reactivity ratios, copolymerization between DEGE and EO appears *ideal* (i.e., *r*₁ × *r*₂ ≈ 1), which is consistent with other epoxide copolymerizations between EO and glycidyl ethers for which reactivity ratios have been reported,^{6,18,21,22} and with previous work on the copolymerization between ethylene oxide and alkylene oxides.^{23,24}

The preference for DEGE-incorporation during the copolymerization with EO can be interpreted within the mechanistic framework of cation-coordination which was first

introduced experimentally by Chang *et al.*²⁵ and Boileau *et al.*²⁶ with computational insight provided later by Harder *et al.*²⁷

The concept was recently articulated in the context of copolymerization between EO and functional glycidyl ethers by Lee *et al.* in a combined experimental and computational study.²¹ Based on these considerations, we posit that the specific conformational state adopted by the chain-end when a DEGE monomer coordinates to a terminal DEGE or EO repeat unit on the chain terminus must be lower in energy than the configuration of the activated complex adopted by an incoming EO. This is likely due to potential multidentate coordination of DEGE to the counter-ion, and enhanced Lewis basicity of DEGE relative to that of EO. However, the incorporation of DEGE and EO repeat units along the polymer backbone can be characterized as nearly random, and the relative population of dyads agrees well with the polymerization stoichiometry.

Being able to control composition and architecture of DEGE/EO copolymers is an important first step towards expanding PEG-based platforms with this new monomeric building block. However, in order to be suitable for biomedical applications, a well-defined pH-response within a specific physiologically-relevant pH range of 5.0 to 7.4 is required as this transition is associated with many disease states or endocytosis pathways.^{5,7}

In order to establish the pH-responsiveness of DEGE-containing copolymers with EO, a series of base (NaOH) and acid (HCl) titrations of 1.0 wt% aqueous solutions of P(EO_{0.9-co}-DEGE_{0.1}), P(EO_{0.8-co}-DEGE_{0.2}), P(EO_{0.7-co}-DEGE_{0.3}), and P(EO_{0.6-co}-DEGE_{0.4}) were carried out. The titration curves are shown in Fig. 2a and b as a function of mmol of base and acid added. As the incorporation of DEGE repeat units increases from 10 to 40 mol%, a plateau in the titration curve around pH 9 becomes evident. The P(EO-*co*-DEGE) copolymers possess some degree of buffering capacity and resist changes to pH where $\text{pH} \approx \text{p}K_a$. Based on these results, the $\text{p}K_a$ of DEGE repeat units is approximately 9, and a rapid change in solution pH occurs within pH 4–8, which encompasses the targeted physiologically relevant window of pH 5–7.4. This analysis could not be carried out on a homopolymer of DEGE repeat units, *i.e.*, P(EO_{0.0-co}-DEGE_{1.0}) in Table 1, due to the complete insolubility of the material in basic aqueous solutions.

Responsive hydrogels have a range of potential applications within a biomedical context: controlled drug release, cell carriers, and tissue-engineering matrices.^{28–31} Having established the pH-responsiveness of DEGE-containing materials, we set out to explore the use of poly(*N,N*-diisopropyl ethanolamine glycidyl ether) (PDEGE) domains as the responsive element within the context of pH-triggered hydrogelation from symmetric PDEGE-*b*-PEO-*b*-PDEGE triblock copolymers. A symmetric DEGE-containing triblock copolymer was synthesized using a 20 kg mol^{−1} PEO-diol as a macroinitiator as shown in Scheme 2. PDEGE end-blocks with 5.3 kg mol^{−1} (~26 repeat units) were synthesized yielding the targeted triblock architecture PDEGE_{5.3k}-*b*-PEO_{20k}-*b*-PDEGE_{5.3k} with ~35 wt % PDEGE, and overall M_n of 31 kg mol^{−1}. The resultant molecular weights were in excellent agreement with those defined by the reaction stoichiometry, and the yield was nearly quantitative.

To assess the pH-responsive hydrogel-formation of PDEGE_{5.3k}-*b*-PEO_{20k}-*b*-PDEGE_{5.3k}, 10 wt% gels/solutions were prepared in buffer solutions with pH from 5.8 to 8.0. The PDEGE-containing triblock copolymers were expected to be soluble in acidic solutions due to the charged state of the protonated diisopropylamine moieties (Scheme 3), with deprotonation under basic conditions triggering hydrophobic collapse of the PDEGE end blocks and phase-separation from the surrounding aqueous medium. The resulting hydrophobic PDEGE-rich meso-domains form physical crosslinks resulting in a hydrogel. The pH-dependent viscoelasticity of the hydrogels was evaluated both visually and quantitatively using rheometry as shown respectively in Fig. 3a and b. For the triblock copolymer dissolved in buffer at pH 5.8, the viscous solution underwent flow which is in direct contrast to the behavior at pH 6.6 where flow was severely retarded when tilted or inverted as shown in Fig. 3a. This suggests that the critical transition from a viscous polymer solution to hydrogel occurs within a pH range of 5.8–6.6. To quantitatively assess the pH-dependent hydrogelation, rheometry was carried out on the triblock copolymer solutions. At pH 5.8, the loss-modulus G'' was larger than the storage-modulus G' which is consistent with a viscous polymer solution. However, the frequency dependence of the rheological response suggested that the system was near the critical gelation transition between solution and hydrogel.^{32,33} At pH 6.6 and above the hydrogels displayed solid-like viscoelastic properties with nearly frequency-independent storage moduli G' which were higher than the corresponding loss moduli G'' . Increasing the pH from 6.6 to 7.4 and then 8.0 results in stiffening of the gel as the observed values for G' are increased. This is a result of changing degree of protonation of the amines. However, the amines are sufficiently neutral to maintain the hydrophobically-driven continuous network structure of the gel. Further protonation of the amines with decreasing pH results in disruption of the hydrophobic interactions that maintain the network structure underlying the solid-like mechanical properties of the hydrogel. Our initial investigations of hydrogel formation of PDEGE_{5.3k}-*b*-PEO_{20k}-*b*-PDEGE_{5.3k} demonstrate the potential utility of DEGE as a functional monomer for the development of polyether materials in advanced biomedical applications.

To demonstrate utilization of the highly amphiphilic, pH-responsive character of the PEO-*b*-PDEGE motif in combination with other functional comonomers, we explored the design and synthesis of pH-responsive PEG-based microgel particles. A general requirement for good overall performance of microgel nanomaterials is the correct choice of the polymeric building blocks with well-defined stimuli-responsive behavior and good biocompatibility being critical features. The well-defined pH-responsive behaviour of DEGE in combination with the reactivity of allyl glycidyl ether (AGE) renders the PEG-based P(DEGE-*co*-AGE) co- and ter-polymers ideal candidates for exploring new concepts in microgel synthesis. The crosslink density and degree of swelling as a response to ambient pH may therefore be tuned *via* the stoichiometry of the polymeric constituents and the specific particle formulation. Leveraging the versatility of the polyether toolkit enables the design of microgel particles with triggered embedding and release of functional compounds rendering these materials highly attractive for drug delivery applications.^{34–36}

We synthesized two functional polymeric components for microgel preparation. A stimuli-responsive network-forming copolymer, P(DEGE₁₃₃-*co*-AGE₁₅), was synthesized

consisting of pH-responsive DEGE moieties and AGE units distributed along the copolyether backbone for crosslinking with a multifunctional thiol *via* photoinitiated thiol-ene *click* reactions. An amphiphilic diblock terpolymer, PEO-*b*-[P(DEGE-*co*-AGE)], was synthesized by copolymerization of DEGE with AGE using a monofunctional 10 kg mol⁻¹ PEO as macroinitiator, in order to serve as a functional surfactant for the preparation of microgel nanoparticles. The incorporation of AGE units in the hydrophobic P(DEGE-*co*-AGE) block, under basic conditions, allowed for the covalent integration of the PEO-*b*-[P(DEGE-*co*-AGE)] surfactant molecules into the polymeric network that would form the core of the hydrogel particle, resulting in the permanent attachment of a PEO corona to the microgel-surface.

Microgel particles were produced by crosslinking P(DEGE-*co*-AGE) and surface-active PEO-*b*-[P(DEGE-*co*-AGE)] within the miniemulsion droplets. The continuous phase was prepared by dissolving the PEO-*b*-[P(DEGE-*co*-AGE)] surfactant polymer in pH 9 carbonate buffer to ensure the amphiphilic nature of the diblock terpolymer. The dispersed phase was a solution of P(DEGE-*co*-AGE) in toluene, pentaerythritol tetrakis(3-mercaptopropionate) (PTMP) as a tetrafunctional crosslinker, and 2,2-dimethoxy-2-phenylacetophenone (DMPA) as a radical photoinitiator. The continuous phase and dispersed phase were mixed and the miniemulsion was prepared by ultrasonication followed by irradiation with 365 nm UV light which initiated crosslinking.

After gel formation, the particles were purified by washing with acidic water in order to remove excess non-crosslinked PEO-*b*-[P(DEGE-*co*-AGE)] surfactant and P(DEGE-*co*-AGE). Freeze-drying of the purified dispersions yielded the microgels as white powders, which could be stored in the dried state and reconstituted by simple dispersal and swelling in aqueous solution. The resulting aqueous dispersions were stable without any additional surfactant due to the steric stabilization provided by the covalently attached PEO corona. This is highly advantageous in comparison to approaches using physically adsorbed non-reactive surfactants, since the surface functionalization of nanoscale objects with PEO is known to increase blood circulation time significantly thus enhancing retention time and thereby the potential for drug delivery applications.

The prepared microgels were designed to exhibit a pH-dependent swelling behavior due to the cationic network-forming P(DEGE-*co*-AGE) copolymer core. Protonation of the tertiary amine groups increases the hydrophilicity of the network, thus inducing an increase in the degree of swelling of the particles from 290 nm at pH 10.5 to 800 nm at pH 4.5. The increase in size of the microgel particles is further enhanced by electrostatic repulsion of the protonated amines at lower pH values. The pH-dependent change in microgel size was investigated by varying the pH of the 0.125 wt% microgel dispersions from pH 10.5 to pH 4.5 by adding HCl_(aq). The hydrodynamic diameters (d_h) of the particles were measured by dynamic light scattering at each pH value and the resulting plot of d_h against pH is shown in Fig. 4a. Fig. 4b depicts the size distribution of a microgel dispersion at pH 10.5.

All microgels exhibited pH-dependent swelling properties with critical swelling transitions from the collapsed to the swollen state at pH values between 7.4 and 6.5. The PEG-based microgel particles exhibited a nearly 20-fold change in volume from the completely

collapsed state to the fully swollen state. The swelling behavior was fully reversible with three cycles of pH variation from pH 4.5 to pH 9 leading to the same size changes. This variation is assumed to correspond to a change in the mesh size of the network as the DEGE repeat units undergo hydrophobic coalescence at higher pH. The prepared microgels exhibit potential for the loading and release of sequestered functional compounds triggered by decreases in the ambient pH.

Conclusions

In order to leverage the advantageous properties of poly-(ethylene glycol) in new and responsive biomedical material contexts, a novel pH-responsive building block *N,N*-diisopropyl ethanolamine glycidyl ether (DEGE) was copolymerized with ethylene oxide in both statistical and block configurations. We demonstrated the near-randomness ($r_{\text{DEGE}} = 1.28 \pm 0.14$ and $r_{\text{EO}} = 0.82 \pm 0.10$) of the statistical copolymerization by examining the relative end-group dyad populations by ^1H NMR spectroscopy, and used the resultant copolymers to establish the pH-responsiveness of the materials within a physiologically-relevant pH window. Symmetric triblock copolymers were synthesized in order to explore the reversibly hydrophilic/hydrophobic transition within the context of pH-responsive hydrogels. Aqueous solutions of the symmetric triblock copolymer at pH 6.6 and above formed robust hydrogels. At pH 5.8 and below, the triblock copolymer solution was an unstructured viscous fluid. A copolymer of DEGE and allyl glycidyl ether (AGE) and a diblock terpolymer of PEO-*b*-P(DEGE-*co*-AGE) were synthesized as functional and reactive surfactants for the synthesis of novel pH-responsive microgels, which exhibited reversible pH-dependent swelling behavior. Finally, a significant advantage of DEGE as a pH-responsive building block its ready availability combined with facile incorporation into polyether materials for various applications.

Experimental

Materials

Chemicals were obtained from Sigma-Aldrich unless specified as otherwise. THF was obtained from a dry solvent system and used immediately afterwards. Epichlorohydrin and ethylene glycol monovinyl ether were obtained from TCI-America, Inc. Potassium naphthalenide was prepared from potassium metal and recrystallized naphthalene in dry THF and allowed to stir with a glass-coated stir-bar for 24 hours at room temperature. Deuterated chloroform (CDCl_3) was obtained from Cambridge Isotope Laboratories, Inc.

Measurements/characterization

Gas chromatography (GC) was carried out in a Shimadzu GC-2014 equipped with a flame ionization detector and a Restek column (SHRXL-5MS). Gel permeation chromatography (GPC) was carried out with a Waters 2690 Separation Module using a Waters 2414 differential refractometer and 2998 photodiode array detectors using chloroform with 0.1% triethylamine as the mobile phase. ^1H and ^{13}C NMR spectroscopy were carried out on a Varian VNMRs 600 MHz spectrometer in deuterated chloroform (CDCl_3). Differential scanning calorimetry (DSC) was performed using a TA Instruments Q2000 MDSC equipped

with an autosampler. The temperature ranged from -100 to 100 °C and was controlled by heating and cooling at 10 K min^{-1} under nitrogen.

Synthesis of *N,N*-diisopropyl ethanolamine glycidyl ether (DEGE)

A solution of 2-(diisopropylamino)ethanol (60.0 g, 413 mmol) and epichlorohydrin (115 g, 1.23 mol) were cooled to 0 °C and stirred with a magnetic stir-bar. Sodium hydroxide (16.5 g, 413 mmol) was slowly added and the flask was vented. The solution was left to stir and allowed to warm to room temperature overnight. Acetone was added to the solution and the insoluble salts were filtered *via* vacuum filtration. Excess epichlorohydrin was removed *in vacuo*. Excess alcohol was removed by distillation from BuMgCl and the final product was obtained by distillation under reduced pressure at 65 °C to yield a clear liquid (yield, 56%, purity 99.8% by GC). In the following NMR spectroscopic data, the epoxide ring of the glycidyl moiety is represented by $-\text{CH} \langle \text{O} \rangle \text{CH}_2$. ^1H NMR (600 MHz, CDCl_3): δ 0.97 (d, 12H, $-\text{N}-(\text{CH}(\text{CH}_3)_2)_2$), δ 2.59 (overlapping m, 2H + 1H, $\text{HHC} \langle \text{O} \rangle \text{CH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{CH}_2-\text{N}-(\text{CH}(\text{CH}_3)_2)_2$), δ 2.75 (t, 1H), δ 2.95 (hept, 2H), δ 3.10 (ddt, 1H), δ 3.40 (overlapping m, 2H + 1H, $-\text{CH}_2-\text{O}-\text{CHH}-\text{CH} \langle \text{O} \rangle \text{CH}_2$), δ 3.68 (dd, 1H, $-\text{O}-\text{CH}_2-\text{CH} \langle \text{O} \rangle \text{CH}_2$). ^{13}C NMR (150 MHz, CDCl_3): δ 20.86 ($-\text{N}-(\text{CH}(\text{CH}_3)_2)_2$), δ 44.43 ($-\text{O}-\text{CH}_2-\text{CH} \langle \text{O} \rangle \text{CH}_2$), δ 44.78 ($-\text{CH}_2-\text{N}-(\text{CH}(\text{CH}_3)_2)_2$), δ 49.51 ($-\text{O}-\text{CH}_2-\text{CH} \langle \text{O} \rangle \text{CH}_2$), δ 50.87 ($-\text{CH}_2-\text{N}-(\text{CH}(\text{CH}_3)_2)_2$), δ 71.84 ($-\text{O}-\text{CH}_2-\text{CH} \langle \text{O} \rangle \text{CH}_2$), δ 73.17 ($-\text{CH}_2-\text{O}-\text{CH}_2-\text{CH} \langle \text{O} \rangle \text{CH}_2$).

P(EO-co-DEGE) copolymer synthesis

Polymerizations of EO and DEGE were carried out on a Schlenk line in custom thick-walled dry glass reactors. The reaction chamber was filled with argon and evacuated by vacuum four times. The reactor was left under static argon above ambient pressure and isolated from the Schlenk line for the remainder of the synthesis. Dry THF was released into the reactor and benzyl alcohol initiator was added using a gas-tight syringe. The potassium alkoxide initiator was formed by titrating the benzyl alcohol solution with potassium naphthalenide (0.3 M in THF) until the solution remained light green in color indicating complete deprotonation. Polymerizations were carried out at 45 °C for 48 hours and terminated with degassed methanol. The polymers were purified by dialysis in methanol, filtered, and dried *in vacuo*. PDEGE homopolymer ($\text{P}(\text{EO}_{0.0}\text{-co-DEGE}_{1.0})$ in Table 1) was purified by precipitation in diethyl ether and dried *in vacuo*. ^1H NMR (600 MHz, CDCl_3): δ 0.98 (d, $-\text{N}-(\text{CH}(\text{CH}_3)_2)_2$), δ 2.57 (t, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{N}-(\text{CH}(\text{CH}_3)_2)_2$), δ 2.96 (m, $-\text{N}-(\text{CH}(\text{CH}_3)_2)_2$), δ 3.35–3.75 (bm, $[-\text{CH}_2-\text{CH}(\text{CH}_2-\text{O}-\text{CH}_2-\text{CH}_2-\text{N}-(\text{CH}(\text{CH}_3)_2)_2-\text{O}-], [-\text{CH}_2-\text{CH}_2-\text{O}-]$), δ 4.53–4.55 (four singlets, $\text{Ph}-\text{CH}_2-\text{O}-$), δ 7.31–7.33 (bm, $\text{Ph}-\text{CH}_2-\text{O}-$). ^{13}C NMR (150 MHz, CDCl_3): δ 20.94 (s, $-\text{N}-\text{CH}(\text{CH}_3)_2$), δ 44.86 ($-\text{CH}_2-\text{N}-\text{CH}(\text{CH}_3)_2$), δ 49.53 ($-\text{CH}_2-\text{N}-(\text{CH}(\text{CH}_3)_2)_2$), δ 69.77–71.48 (m, EO/DEGE triads), δ 73.31 (s, EO triad), δ 78.53–78.93 (DEGE methine *mm*, *rm*, *mr*, and *rr* triads), δ 110.15, 127.70 ($\text{Ph}-\text{CH}_2-\text{O}-$), and 128.43 ($\text{Ph}-\text{CH}_2-\text{O}-$).

PDEGE-*b*-PEO-*b*-PDEGE triblock copolymer synthesis

Poly(ethylene oxide) (PEO, 20 kg mol^{-1}) was melted at 74 °C inside of a thick-walled glass reactor with a glass-coated stir-bar and dried under vacuum. The reactor was cooled and

charged with positive argon pressure and dry THF was added to dissolve the PEO. At 45 °C, the solution was titrated with potassium naphthalenide solution (0.3 M in THF) until a green color persisted indicating complete deprotonation of the hydroxylterminal PEO. Finally, DEGE was added and the reaction took place for two days and was terminated with degassed methanol. The product was purified by dialysis in methanol and dried *in vacuo*. ¹H NMR (600 MHz, CDCl₃): δ 0.98 (d, -N-[CH-(CH₃)₂]₂), δ 2.57 (t, -O-CH₂-CH₂-N-[CH-(CH₃)₂]₂), δ 2.96 (m, -N-[CH-(CH₃)₂]₂), δ 3.35–3.75 (bm, [-CH₂-CH(-CH₂-O-CH₂-CH₂-N-[CH-(CH₃)₂]₂)-O-], [-CH₂-CH₂-O-]).

P(DEGE-co-AGE) copolymer synthesis

The initiator, 2-(tetrahydro-2H-pyran-2-yloxy)ethanol (THP) (20.2 mg, 0.138 mmol), was added to a thoroughly dried and argon flushed thick-walled glass reactor with a glass-coated stir-bar. This was titrated with potassium naphthalenide solution (0.3 M in THF) until a green color remained. Subsequently, DEGE (5.00 g, 24.8 mmol) and AGE (276 mg, 2.8 mmol) were added. The polymerization was carried out at 45 °C for eight days. Finally, the copolymer was terminated with degassed methanol and purified by extensive dialysis in methanol. ¹H NMR (600 MHz, CDCl₃): δ 0.98 (d, -N-[CH-(CH₃)₂]₂), δ 2.57 (t, -O-CH₂-CH₂-N-[CH-(CH₃)₂]₂), δ 2.96 (m, -N-[CH-(CH₃)₂]₂), δ 3.35–3.75 (bm, [-CH₂-CH(-CH₂-O-CH₂-CH₂-N-[CH-(CH₃)₂]₂)-O-], [-CH₂-CH-O-(CH₂-O-CH₂-CH=CH₂)], δ 3.98 (bd, [-O-CH₂-CH=CH₂]), δ 5.14 (d, [-O-CH₂-CH=CH₂]), δ 5.23 (d, [-O-CH₂-CH=CH₂]), δ 5.88 (dqt, [-O-CH₂-CH=CH₂]).

PEO-*b*-P(DEGE-co-AGE) diblock terpolymer synthesis

Monohydroxy-functionalized poly(ethylene oxide) (PEO, 10 kg mol⁻¹, 2.00 g) was added to a thick-walled glass reactor equipped with a glass-coated stir-bar. The polymer was molten and dried at 75 °C under high vacuum overnight. Afterwards, the reactor was cooled down to room temperature and flushed with dry argon, the polymer was dissolved by adding degassed anhydrous THF (200 mL). The hydroxyl end groups of the PEO macroinitiator were titrated with potassium naphthalenide solution (0.3 M in THF) until a green color remained. In the next step, DEGE (1.127 g, 5.6 mmol) and AGE (0.060 g, 0.6 mmol) were added. The polymerization was carried out at 45 °C for eight days until being terminated with degassed methanol. The resulting diblock terpolymer was purified by repeated precipitation from diethyl ether and finally dried *in vacuo*. ¹H NMR (600 MHz, CDCl₃): δ 0.98 (d, -N-[CH-(CH₃)₂]₂), δ 2.57 (t, -O-CH₂-CH₂-N-[CH-(CH₃)₂]₂), δ 2.96 (m, -N-[CH-(CH₃)₂]₂), δ 3.30–3.75 (bm, [-CH₂-CH(-CH₂-O-CH₂-CH₂-N-[CH-(CH₃)₂]₂)-O-], [-CH₂-CH-O-(CH₂-O-CH₂-CH=CH₂)], [-CH₂-CH₂-O-]), δ 3.98 (bd, [-O-CH₂-CH=CH₂]), δ 5.14 (d, [-O-CH₂-CH=CH₂]), δ 5.23 (d, [-O-CH₂-CH=CH₂]), δ 5.88 (dqt, [-O-CH₂-CH=CH₂]).

Microgel synthesis

The continuous phase was prepared by first dissolving PEO-*b*-P(DEGE-co-AGE) block copolymer (50 mg) in acetone (2 mL) followed by adding this solution to pH 9 sodium carbonate buffer (5 mL, 1 mM) and evaporating the acetone *via* stirring the mixture in an open glass vial for two days. The volume of the continuous phase was adjusted to a total of

7.5 mL by adding the respective amount of pH 9 buffer. The dispersed phase consisted of a solution of PDEGE-*co*-PAGE (250 mg, 0.13 mmol of AGE units), PTMP as tetrafunctional crosslinker (11 mg, 0.044 mmol of thiol groups) and DMPA as photoinitiator (1 mg). After mixing the two phases and preemulsification *via* vortexing, the mixture was homogenized using an ultrasonic tip (Branson Emulsifier, 1/8" tip, 70% amplitude, total time two min, 15 s pulse, 10 s pause). Crosslinking was then performed by irradiating the emulsion with 365 nm ($\sim 1 \text{ mW cm}^{-2}$) for 8 h. Subsequently, coagulates were removed by filtration and the resulting dispersions were centrifuged at 5000 rpm for 30 min to collect the particles. The supernatant was removed and replaced by deionized water. Re-dispersion was carried out using a vortexer. In order to remove excess surfactant, the dispersions were further washed two times with water, three times with methanol and two additional times with water following the procedure described above. Freeze drying yielded the crosslinked gel particles as white powders. The purified particles were redispersed in water of the respective pH by swelling at room temperature for 6 h at the desired concentration. Particle sizes and distributions were determined by means of dynamic light scattering (DLS).

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Fig. 1.

(a) ^1H NMR spectrum of $\text{P}(\text{EO}_{0.6}\text{DEGE}_{0.4})$. (b) Array of benzyl singlet end-group dyad signals are shown for the copolymers $\text{P}(\text{EO}_{0.6}\text{DEGE}_{0.4})$, $\text{P}(\text{EO}_{0.7}\text{DEGE}_{0.3})$, $\text{P}(\text{EO}_{0.8}\text{DEGE}_{0.2})$, and $\text{P}(\text{EO}_{0.9}\text{DEGE}_{0.1})$. The relative integral of each end-group dyad represents the fractional population of copolymers containing end-group dyads shown in (c). Reactivity ratios from the relative end-group dyads were calculated: $r_{\text{DEGE}} = 1.28 \pm 0.14$ and $r_{\text{EO}} = 0.82 \pm 0.10$.²¹

Fig. 2. Base (a) and acid (b) titration curves of 1 wt% solutions of P(EO-co-DEGE) with 10–40 mol % DEGE incorporation. The buffering capacity at pH 9 increases with higher incorporation of DEGE.

Fig. 3.

(a) Tilted solutions of hydrogels made in different pH buffers. (b) Storage (G' , purple) and loss (G'' , orange) moduli of hydrogels. The samples at and above pH 6.6 show $G' > G''$, and a nearly frequency-independent G' consistent with solid-like viscoelasticity. At pH 5.8, $G' \approx G''$ which is consistent with the behavior of a viscous liquid.

Fig. 4. Summary of dynamic light scattering measurements on the pH-responsive crosslinked miniemulsion particles are shown above as a function of pH: (a) hydrodynamic diameter vs. pH, and (b) size distribution of microgels at pH 10.5.

Scheme 1.

Copolymerization of DEGE with EO using a potassium alkoxide initiator.

Scheme 2.
Synthesis of PDEGE-*b*-PEO-*b*-PDEGE.

Scheme 3.

Protonation of the DEGE units underlies the pH-responsive transition between hydrophobic (left) and hydrophilic states (right).

Table 1

Molecular and thermal characterization of P(EO-co-DEGE)

Sample	$\frac{[\text{EO}]}{[\text{DEGE}]}$ ^a	x_{DEGE} ^b	M_n^{stoich} ^c	M_n^{obs} ^d	PDI ^e	T_g^f
P(EO _{0.9} -co-DEGE _{0.1})	9.0	0.09	20 000	20 000	1.12	-66
P(EO _{0.8} -co-DEGE _{0.2})	4.0	0.18	20 000	20 000	1.13	-66
P(EO _{0.7} -co-DEGE _{0.3})	2.3	0.31	30 000	29 000	1.13	-64
P(EO _{0.6} -co-DEGE _{0.4})	1.5	0.40	20 000	19 000	1.14	-63
P(EO _{0.0} -co-DEGE _{1.0})	0.0	1.00	10 000	9000	1.14	-57

^a Initial feed ratio of EO to DEGE.

^b Final mole fraction (xDEGE) of DEGE repeat units by ¹H NMR spectroscopy (see Fig. 1a).

^c (Total mass of monomer)/(moles of benzyl alcohol).

^d Molecular weight as measured by ¹H NMR spectroscopy using the benzyl end-group resonance (2H) as a reference for end-group analysis.

^e Polydispersity index measured by size exclusion chromatography relative to polystyrene standards.

^f Glass transition temperature measured by differential scanning calorimetry.