Published in final edited form as:

Macromolecules. 2018 May 8; 51(9): 3233–3242. doi:10.1021/acs.macromol.8b00047.

A Vinyl Ether-Functional Polycarbonate as a Template for Multiple Postpolymerization Modifications

Sangho Cho^{†,‡,§,⊥}, Gyu Seong Heo^{†,§,⊥}, Sarosh Khan[†], Jessica Huang[†], David A. Hunstad^{||}, Mahmoud Elsabahy^{†,‡}, and Karen L. Wooley^{*,†}

[†]Department of Chemistry, Department of Chemical Engineering, Department of Materials Science & Engineering, and Laboratory for Synthetic-Biologic Interactions, Texas A&M University, P.O. Box 30012, 3255 TAMU, College Station, Texas 77842-3012, United States

[‡]Materials Architecturing Research Center, Korea Institute of Science and Technology, Seoul 02792, Republic of Korea

[#]Division of Nano & Information Technology, KIST School, Korea University of Science and Technology, Seoul 02792, Republic of Korea

[§]Mallinckrodt Institute of Radiology

^{||}Departments of Pediatrics and Molecular Microbiology, Washington University, St. Louis, Missouri 63110, United States

[⊥]Department of Pharmaceutics, Faculty of Pharmacy and Assiut International Center of Nanomedicine, Al-Rajhy Liver Hospital, Assiut University, 71515 Assiut, Egypt

Abstract

A highly-reactive vinyl ether-functionalized aliphatic polycarbonate and its block copolymer were developed as templates for multiple post-polymerization conjugation chemistries. The vinyl ether-functional six-membered cyclic carbonate monomer was synthesized by a well-established two-step procedure starting from 2,2-bis(hydroxymethyl)propionic acid. An organobase-catalyzed ring-opening polymerization of the synthesized monomer afforded polycarbonates with pendant vinyl ether functionalities (PMVEC). The vinyl ether moieties on the resulting polymers were readily conjugated with hydroxyl- or thiol-containing compounds *via* three different post-polymerization modification chemistries — acetalization, thio-acetalization, and thiol-ene reaction. Acetal-functionalized polycarbonates were studied in depth to exploit their acid-labile acetal functionalities. Acetalization of the amphiphilic diblock copolymer of poly(ethylene glycol) methyl ether (mPEG) and PMVEC, mPEG₁₁₃-*b*-PMVEC₁₃, with the model hydroxyl compound 4-methylbenzyl alcohol resulted in a maximum of 42% acetal and 58% hydroxyl side chain groups. Nonetheless, the amphiphilicity of the block polymer allowed for its self-assembly in

*Correspondence to: (K.L.W.) wooley@chem.tamu.edu Tel.: +1 979 845 4077 Fax: +1 979 862 1137.

[†]These authors contributed equally to this work.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Additional characterization data (Figures S1-S7), thermogravimetric analysis and differential scanning calorimetry traces.

Notes

The authors declare no competing financial interest.

water to afford nanostructures, as characterized *via* dynamic light scattering and transmission electron microscopy. The kinetics of acetal cleavage within the block polymer micelles were examined in acidic buffered solutions (pH 4 and 5). In addition, mPEG-*b*-PMVEC and its hydrolyzed polymer mPEG-*b*-PMHEC (*i.e.*, after full cleavage of acetals) exhibited minimal cytotoxicity to RAW 264.7 mouse macrophages, indicating that this polymer system represents a biologically non-hazardous material with pH-responsive activity.

Graphical abstract

INTRODUCTION

There has been an increasing interest in the development of functional polymers that are accessed at low cost with simple processability and diverse properties. In particular, polymers bearing reactive functional groups provide broad opportunity, since modulation of functionalities at the molecular level by post-polymerization modification offers control over material properties and widens the range of potential applications, for materials originating from a single initial polymer structure. Vinyl ethers are interesting functional groups, as they feature an adjacent π -electron-donating oxygen, which aids in the formation of a stable carbocation at the α -carbon.¹ Although the majority of applications of vinyl ethers are as monomers for cationic^{2–3} and radical⁴ polymerizations, this highly reactive functional group can also participate in a variety of other reactions, including acetalization, thioacetalization, thiol-ene reaction, hydrolysis, *etc.*⁵ One of the advantages of the vinyl ether functionality is its capability to form stable or, alternatively, dynamic covalent bonds. While thiol-ene reactions of vinyl ethers with various thiols yield robust thioether linkages *via* reaction at the β -carbon, reactions with thiols or alcohols in the presence of acid catalysts can instead occur at the α -carbon to generate acid-labile thioacetals or acetals, which may be of use in certain physiological applications. In total, vinyl ether-functional materials offer a variety of potential applications, from radiation-curable coatings *via* formation of poly(vinyl ethers)⁶ to conjugation and stimuli-responsive release of biologically-active molecules *via* acetal or thioacetal linkages.^{7–12}

To date, only few studies have investigated vinyl ether-bearing polymers. In 2011, Frey and Wurm *et al.* developed vinyl ether-functionalized poly(ethylene glycol) (PEG) and demonstrated its functionalization *via* two post-polymerization modifications: thiol-ene reaction and acetal formation.¹³ Meng, Zhong, and coworkers reported conjugation of vinyl ether-functionalized PEG-*b*-poly(acrylic acid) to the anti-proliferative agent paclitaxel (PTX) *via* its acid-labile acetal linkage and demonstrated pH-dependent release of PTX from

self-assembled polymeric micelles.¹⁰ However, the backbones of both of these polymers are non-degradable, limiting their potential utilization in biomedical applications.

We had previously demonstrated the potential utility of degradable polyphosphoester (PPE)-based nanostructures in treatment of epithelial infections, including those of the urinary and respiratory tracts.^{14–15} To expand the chemical functionalization potential of the PPE materials, we had investigated a vinyl ether-functionalized PPE.⁵ However, difficulty in purification of the cyclic phosphotriester monomer as well as the instability of the PPE backbone under acetalization conditions with nucleophiles (alcohols or thiols) and an acid catalyst (*p*-toluenesulfonic acid, PTSA) impeded further application. In the present study, therefore, we introduced the vinyl ether moiety onto polycarbonate (PC) backbones, which are more stable than PPE but still hydrolytically degradable.¹⁶ Well-defined PCs can be prepared *via* metal-free organocatalytic ring-opening polymerization (ROP) of functional cyclic carbonate monomers,^{17–19} and a variety of functionalities can then be introduced by post-polymerization modifications.^{16,20–21} We hypothesized that the stability of the vinyl ether functionality under basic conditions would allow for organobase-catalyzed ROP of functional cyclic carbonate monomers (as previously observed with a vinyl ether-functionalized cyclic phosphotriester monomer).⁵ Hence, we designed and synthesized a novel vinyl ether-functionalized cyclic carbonate monomer, 5-methyl-5-((2-vinyloxy)ethyl)oxycarbonyl-1,3-dioxan-2-one (MVEC). The organobase 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) successfully initiated ROP of this synthesized monomer and yielded well-defined vinyl ether functional homopolymers and (block) copolymers. Multiple conjugation chemistries — acetalization, thio-acetalization, and thiol-ene reactions — were demonstrated as post-polymerization methods. In order to establish its capability for reactivity to generate stimuli-responsive and dynamic nanoassemblies, the amphiphilic block copolymer of ethylene glycol and MVEC (mPEG-*b*-PMVEC) was synthesized, acetalated by a model compound, 4-methylbenzyl alcohol, and investigated as a reactive construct for pH-responsive reactivity, which could trigger assembly and disassembly. After characterizing their self-assembled nanostructures in aqueous solution, the kinetics for cleavage of the acetal bonds were studied using ¹H NMR spectroscopy. Additionally, both the parent polymer (mPEG-*b*-PMVEC) before conjugation and the residual polymer after releasing the model compound demonstrated minimal cytotoxicity against RAW 264.7 mouse macrophages.

EXPERIMENTAL SECTION

Materials

Benzyl alcohol and DBU were dried over CaH₂, distilled, and stored under inert atmosphere. Tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF), and dichloromethane (DCM) were purified by passage through solvent purification system (JC Meyer Solvent Systems) and used as dried solvents. Benzyl 2,2-bis(hydroxymethyl)propanoate (Bn-MPA) was synthesized as reported and dried over P₂O₅ before use.²² All other solvents and chemicals were purchased from Sigma-Aldrich, TCI America, or Fisher Scientific and were used as received.

Characterization

^1H and ^{13}C NMR spectra were recorded on Varian Inova 300 or NMRS 500 spectrometers. Chemical shifts were referenced to the solvent resonance signals. IR spectra were recorded on an IR Prestige 21 system (Shimadzu Corp., Japan), equipped with an ATR accessory, and analyzed using IRsolution v.1.40 software. Size-exclusion chromatography (SEC) was conducted on a system equipped with an isocratic pump model 1515, a differential refractometer model 2414, and a three-column set of Polymer Laboratories, Inc. (Amherst, MA) Styragel columns (PLgel 5 μm S3 Mixed C, 500 $\text{\AA}$, and 104 $\text{\AA}$, 300 $\times$ 7.5 mm columns) and a guard column (PLgel 5 μm , 50 $\times$ 7.5 mm) using THF as the eluent (1.00 mL/min) at 40 $^\circ\text{C}$ or a four-column set of Styragel HR 4 (5 μm , 300 $\times$ 7.5 mm), Styragel HR 4E (5 μm , 300 $\times$ 7.5 mm), Styragel HR 2 (5 μm , 300 $\times$ 7.5 mm) and a guard column (PLgel 5 μm , 50 $\times$ 7.5 mm) using DMF (0.05 M LiBr) as the eluent (1.00 mL/min) at 70 $^\circ\text{C}$. The differential refractometers were calibrated with Polymer Laboratories, Inc., polystyrene standards (300–467000 Da) for the THF system and polyethylene oxide standards (106–174000 Da) for the DMF system. Polymer solutions were prepared at a concentration of *ca.* 5 mg/mL with toluene (0.05% *v/v*) as a flow rate marker, and an injection volume of 200 μL was used. Data were analyzed using Empower Pro software from Waters Chromatography, Inc. Glass transition temperatures (T_g) were measured by differential scanning calorimetry (DSC) on a Mettler-Toledo DSC822 (Mettler-Toledo, Inc., Columbus, OH) under N_2 . Measurements of T_g were taken with a heating rate of 10 $^\circ\text{C}/\text{min}$. The measurements were analyzed using Mettler-Toledo STAR[®] v.10.00 software. The T_g was taken as the midpoint of the inflection tangent and T_m was taken as the onset point, upon the third scan. Thermogravimetric analysis (TGA) was performed under Ar atmosphere using a Mettler-Toledo model TGA/DSC 1, with a heating rate of 10 $^\circ\text{C}/\text{min}$. Dynamic light scattering (DLS) measurements were conducted using DelsaNano C (Beckman Coulter, Inc., Fullerton, CA) equipped with a laser diode operating at 658 nm. Size measurements were made in nanopure water ($n = 1.3329$, $\eta = 0.890$ cP at 25 ± 1 $^\circ\text{C}$). Scattered light was detected at 165 $^\circ$ angle and analyzed using a log correlator over 70 accumulations for a 3.0 mL sample in a glass sizing cell (4.0 mL capacity). The samples in a glass sizing cell were equilibrated for 30 min before measurements. The photomultiplier aperture and the attenuator were automatically adjusted to obtain a photon counting rate of *ca.* 10 kcps. Calculations of particle size distributions and distribution averages were performed using CONTIN particle size distribution analysis routines. The ζ -potential of the particles in suspension was obtained by measuring the electrophoretic movement of charged particles under an applied electric field. Scattered light was detected at a 30 $^\circ$ angle at 25 $^\circ\text{C}$. The ζ -potential was measured at five regions in the flow cell, and a weighted mean was calculated. These five measurements were used to correct for electroosmotic flow that was induced in the cell due to the surface charge of the cell wall. Transmission electron microscopy (TEM) images were collected on a JEOL 1200EX operating at 100 kV, and micrographs were recorded at calibrated magnifications using a SIA-15C CCD camera. The samples as aqueous solutions (4 μL) were deposited onto formvar/carbon-coated copper grids. Excess sample was wicked off using filter paper and the grids were allowed to air-dry for 2 min. Then, the grids were stained with 4 μL of a 1% phosphotungstic acid aqueous solution. Excess stain was wicked off using filter paper after 1 min. The sample grids were air-dried overnight before analysis.

Synthesis of (2-Vinyloxy)ethyl 2,2-bis(hydroxymethyl)propanoate (1)

A mixture of 2,2-bis(hydroxymethyl)propionic acid (bis-MPA, 5.0 g, 37 mmol), potassium hydroxide (90%; 2.6 g, 46 mmol), sodium iodide (1.1 g, 7.3 mmol), and DMF (40 mL) was heated to 100 °C for 1 h until a homogeneous solution was formed. 2-Chloroethyl vinyl ether (3.8 mL, 37 mmol) was added to the warm solution, and stirring was continued at 100 °C for 20 h. The reaction was cooled and the solvent was removed *in vacuo*. The residue was extracted with DCM and water twice, and the organic layer was dried over MgSO₄ and evaporated. The yellow concentrate was further purified by column chromatography with ethyl acetate and hexane (1:4) as an eluent to yield **1** as a slightly yellow-colored liquid (5.90 g, 78%). ¹H NMR (500 MHz, CDCl₃): δ 6.47–6.42 (dd, *J* = 6.8 and 14.4 Hz, –OCHCH₂), 4.40–4.37 (m, –C(O)OCH₂CH₂O–), 4.23–4.18 (dd, *J* = 2.3 and 14.2 Hz, –OCHCHH'), 4.06–4.03 (dd, *J* = 2.3 and 6.8 Hz, –OCHCHH'), 3.92–3.89 (m, –C(O)OCH₂CH₂O–), 3.88–3.84 (m, –CH₂OH), 3.72–3.68 (m, –CH₂OH), 3.01 (t, *J* = 6.6 Hz, –CH₂OH), 1.08 (s, –C(CH₃)(CH₂OH)₂). ¹³C NMR (125 MHz, CDCl₃): δ 175.8, 151.5, 87.6, 67.9, 65.8, 63.2, 49.6, 17.3. ESI MS (*m/z*): 211.1169 (experimental), 211.1158 (calculated for C₉H₁₆O₅Li⁺).

Synthesis of 5-Methyl-5-((2-vinyloxy)ethyl)oxycarbonyl-1,3-dioxan-2-one (MVEC) (2)

(2-Vinyloxy)ethyl 2,2-bis(hydroxymethyl)propionate (4.2 g, 21 mmol) and ethyl chloroformate (6.0 mL, 63 mmol) were dissolved in anhydrous THF (80 mL), and the solution was stirred at 0 °C for 30 min under N₂. Subsequently, triethylamine (TEA, 8.5 mL, 61 mmol) was added dropwise over 1 h, after which the reaction mixture was removed from the ice bath and stirred at room temperature overnight. The TEA·HCl precipitate was removed by filtration, and the filtrate was concentrated and purified by column chromatography with ethyl acetate and hexane (1:4) as an eluent to yield **2** as a white solid (2.90 g, 62%). ¹H NMR (500 MHz, CDCl₃): δ 6.44–6.38 (dd, *J* = 6.7 and 14.4 Hz, –OCHCH₂), 4.67 (d, *J* = 10.8 Hz, –OCH₂C(CH₃)(CH₂)–), 4.41–4.38 (m, –C(O)OCH₂CH₂O–), 4.21–4.15 (m, –OCH₂C(CH₃)(CH₂)– and –OCHCHH'), 4.04–4.01 (dd, *J* = 2.3 and 6.7 Hz, –OCHCHH'), 3.89–3.86 (m, –C(O)OCH₂CH₂O–), 1.30 (s, –C(CH₃)(CH₂)–(CH₂)–). ¹³C NMR (125 MHz, CDCl₃): δ 171.2, 151.4, 147.6, 87.5, 73.0, 65.4, 64.2, 40.3, 17.5. ESI MS (*m/z*): 237.0949 (experimental), 237.0950 (calculated for C₁₀H₁₄O₆Li⁺).

Organocatalytic ROP of MVEC (3)

In a glovebox, MVEC and Bn-MPA initiator were dissolved in dry DCM ([MVEC]₀ = 0.7 M). DBU (1 equiv to initiator) was added to the monomer/initiator solution. After the desired time, the polymerization was quenched by addition of a DCM solution of benzoic acid and precipitated into methanol. For example, MVEC (365 mg, 1.6 mmol), Bn-MPA (5.9 mg, 26 μmol), DBU (7.9 μL, 53 μmol), DCM (2.5 mL), benzoic acid solution (9.7 mg, 80 μmol in 300 μL DCM) were used to yield PMVEC₅₀.

PMVEC₅₀. Yield = 185 mg, 65% based upon monomer conversion (80%). *M_n*, NMR = 11700 g/mol; *M* THF n, SEC = 11600 g/mol; PDI = 1.06. ¹H NMR (500 MHz, CDCl₃): δ 7.40–7.29 (m, –OCH₂C₆H₅), 6.48–6.38 (m, –OCHCH₂), 5.13 (br, –C(O)OCH₂C₆H₅), 4.39–4.32 (m, –C(O)OCH₂CH₂O–), 4.32–4.21 (br, –OC(O)OCH₂–), 4.22–4.14 (m, –OCHCHH'), 4.05–4.00 (m, –OCHCHH'), 3.91–3.83 (m, –C(O)OCH₂CH₂O–), 3.74–3.66

(m, $-\text{CH}_2\text{OH}$), 2.48 (m, $-\text{CH}_2\text{OH}$), 1.28–1.23 (s, $-\text{C}(\text{CH}_3)(\text{CH}_2-)_2$), 1.21 (s, $-\text{C}(\text{CH}_3)(\text{CH}_2-)(\text{CH}_2\text{OH})$). ^{13}C NMR (125 MHz, CDCl_3): δ 174.0, 172.2, 155.0, 154.5, 151.6, 135.6, 128.8, 128.6, 128.2, 87.4, 68.7, 65.7, 63.7, 63.3, 48.6, 46.7, 17.6. IR (neat, cm^{-1}): 2965, 1740, 1635, 1620, 1460, 1385, 1234, 1200, 1145, 964, 825, 783, 733. $T_g = -9.0$ °C. TGA in Ar: 270–450 °C, 92% mass loss; 8% mass remaining above 500 °C.

Acetalization of the Vinyl Ether Side Chain Moieties of PMVEC with 4-Methylbenzyl Alcohol (4)

To the solution of PMVEC₇₀ (54 mg, 0.23 mmol for alkene functionalities) and 4-methylbenzyl alcohol (290 mg, 2.4 mmol) in 0.5 mL THF, *p*-toluenesulfonic acid (PTSA, 0.40 mg, 2.3 μmol) in 0.5 mL THF was added at 0 °C. After stirring for 2 h at room temperature, excess TEA was added to quench the reaction. The reaction mixture was purified by dialysis against THF for 24 h and dried *in vacuo* to yield a colorless tacky liquid. Yield = 54 mg, 76%. $M_{n,\text{NMR}} = 19700$ g/mol. ^1H NMR (500 MHz, CD_2Cl_2): δ 7.34–7.30 (br, $-\text{OCH}_2\text{C}_6\text{H}_5$), 7.24–7.10 (m, $-\text{OCH}_2\text{C}_6\text{H}_4\text{CH}_2$), 6.48–6.38 (m, $-\text{OCHCH}_2$), 5.13 (br, $-\text{C}(\text{O})\text{OCH}_2\text{C}_6\text{H}_5$), 4.80 (m, $-\text{OCH}(\text{CH}_3)\text{O}-$), 4.57 (d, $J = 11.5$ Hz, $-\text{OCHH}'\text{C}_6\text{H}_4\text{CH}_3$), 4.43 (d, $J = 11.5$ Hz, $-\text{OCHH}'\text{C}_6\text{H}_4\text{CH}_3$), 4.39–4.12 (br, $-\text{OC}(\text{O})\text{OCH}_2-$, $-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{O}-$, and $-\text{OCHCHH}'$), 4.01 (m, $-\text{OCHCHH}'$), 3.89–3.82 (br, $-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{OCHCH}_2$), 3.80–3.69 (br, $-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{OCH}(\text{CH}_3)\text{O}-$), 3.69–3.59 (br, $-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{OH}$), 3.40 (s, $-\text{CH}_2\text{OH}$), 2.31 (s, $-\text{OCH}_2\text{C}_6\text{H}_4\text{CH}_3$, *para*), 1.30 (d, $J = 5.1$ Hz, $-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{OCH}(\text{CH}_3)\text{O}-$), 1.28–1.20 (s, $-\text{C}(\text{CH}_3)(\text{CH}_2-)_2$), 1.17 (s, $-\text{C}(\text{CH}_3)(\text{CH}_2-)(\text{CH}_2\text{OH})$). ^{13}C NMR (125 MHz, CD_2Cl_2): δ 172.4, 154.8, 151.9, 137.7, 135.7, 129.3, 128.1, 99.6, 87.3, 69.1, 67.5, 66.1, 65.0, 62.7, 60.9, 47.0, 46.9, 21.2, 19.8, 17.6. IR (neat, cm^{-1}): 3700–3300, 3100–2800, 1744, 1458, 1381, 1234, 1134, 1096, 964, 795. $T_g = -20.5$ °C. TGA in Ar: 90–240 °C, 21% mass loss; 240–390 °C, 68% mass loss; 11% mass remaining above 500 °C.

Kinetic Study of Acetalization of the Vinyl Ether Side Chain Moieties of PMVEC

PMVEC₅₀ (52 mg, 0.23 mmol of vinyl ether moieties) and 4-methylbenzyl alcohol (280 mg, 2.3 mmol) were dissolved in 1 mL THF-*d*₈. After addition of PTSA solution (380 μg , 2.3 μmol) in THF-*d*₈ (200 μL), the solution was transferred to a NMR tube and the reaction was monitored *via* ^1H NMR while spinning in the spectrometer (19 °C).

Thio-Acetalization of the Vinyl Ether Side Chain Moieties of PMVEC with 4-Methylbenzyl Mercaptan (5)

To the solution of PMVEC₇₀ (78 mg, 0.34 mmol for alkene functionalities) and 4-methylbenzyl mercaptan (0.46 mL, 3.4 mmol) in 1.0 mL THF, PTSA (0.58 mg, 3.4 μmol) in 0.50 mL THF was added at 0 °C. After stirring for 2 h at room temperature, an excess amount of TEA was added to quench the reaction. The reaction mixture was precipitated into methanol and collected by centrifugation followed by drying *in vacuo* to yield a colorless tacky liquid. Yield = 61 mg, 67%. $M_{n,\text{NMR}} = 18900$ g/mol. ^1H NMR (500 MHz, CDCl_3): δ 7.37–7.28 (br, $-\text{OCH}_2\text{C}_6\text{H}_5$), 7.22–7.07 (m, $-\text{OCH}_2\text{C}_6\text{H}_4\text{CH}_2$), 6.46–6.39 (m, $-\text{OCHCH}_2$), 5.15 (br, $-\text{C}(\text{O})\text{OCH}_2\text{C}_6\text{H}_5$), 4.79–4.64 (m, $-\text{OCH}(\text{CH}_3)\text{S}-$), 4.42–4.12 (m, $-\text{OC}(\text{O})\text{OCH}_2-$, $-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{O}-$, and $-\text{OCHCHH}'$), 4.02 (br, $-\text{OCHCHH}'$), 3.89–3.83

(br, $-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{OCHCH}_2$), 3.80–3.73 (br, $-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{OCH}(\text{CH}_3)\text{S}-$), 3.73–3.69 (m, $-\text{SCH}_2\text{C}_6\text{H}_4\text{CH}_3$), 3.69–3.56 (br, $-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{OH}$), 2.31 (s, $-\text{SCH}_2\text{C}_6\text{H}_4\text{CH}_3$, *para*), 1.49 (br, $-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{OCH}(\text{CH}_3)\text{S}-$), 1.30–1.20 (s, $-\text{C}(\text{CH}_3)(\text{CH}_2-)_2$). ^{13}C NMR (125 MHz, CDCl_3): δ 172.1, 154.5, 151.6, 136.7, 135.5, 129.5, 129.3, 128.9, 128.0, 99.7, 87.3, 80.8, 68.7, 65.7, 64.3, 63.7, 63.6, 62.6, 46.7, 32.4, 29.8, 21.9, 21.2, 19.5, 17.6. IR (neat, cm^{-1}): 3700–3300, 3100–2800, 2361, 1744, 1458, 1381, 1234, 1142, 1103, 972, 918, 864, 787, 733, 648. $T_g = 7.3$ °C. TGA in Ar: 205–410 °C, 95% mass loss; 4% mass remaining above 500 °C.

Thiol-Ene “Click” Reaction of the Vinyl Ether Side Chain Moieties of PMVEC with 3-Mercaptopropionic Acid (6)

PMVEC₇₀ (50 mg, 220 μmol for alkene functionalities), 2,2-dimethoxy-2-phenylacetophenone (DMPA, 11 mg, 43 μmol), and 3-mercaptopropionic acid (190 μL , 2.2 mmol) were dissolved in 5 mL DMF. The solution was bubbled with N_2 for 10 min and irradiated by UV (365 nm, 6 W) for 2 h. The reaction mixture was precipitated into ether/pentane (v/v = 1/4) and dried *in vacuo* to yield a colorless tacky liquid. Yield = 46 mg, 63% based on 100% conversion. $M_{n,\text{NMR}} = 23800$ g/mol. ^1H NMR (500 MHz, $\text{CD}_2\text{Cl}_2/\text{MeOD}$): δ 7.36–7.27 (m, $-\text{OCH}_2\text{C}_6\text{H}_5$), 5.13 (br, $-\text{C}(\text{O})\text{OCH}_2\text{C}_6\text{H}_5$), 4.32–4.25 (br, $-\text{OC}(\text{O})\text{OCH}_2-$), 4.25–4.21 (m, $-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{O}-$), 3.65–3.59 (m, $-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{O}-$ and $-\text{OCH}_2\text{CH}_2\text{S}-$), 2.77 (t, $J = 7.3$ Hz, $-\text{SCH}_2\text{CH}_2\text{C}(\text{O})\text{OH}$), 2.68 (t, $J = 6.6$ Hz, $-\text{OCH}_2\text{CH}_2\text{S}-$), 2.57 (t, $J = 7.3$ Hz, $-\text{SCH}_2\text{CH}_2\text{C}(\text{O})\text{OH}$), 1.23 (s, $-\text{C}(\text{CH}_3)(\text{CH}_2-)_2$). ^{13}C NMR (125 MHz, $\text{CD}_2\text{Cl}_2/\text{MeOD}$): δ 174.8, 172.8, 155.0, 71.4, 69.3, 69.0, 64.9, 47.1, 35.3, 32.0, 27.9, 17.6. IR (neat, cm^{-1}): 3700–2800, 1728, 1458, 1389, 1234, 1119, 964, 787, 710, 633. $T_g = -14.8$ °C. TGA in Ar: 220–395 °C, 82% mass loss; 18% mass remaining above 500 °C.

Synthesis of mPEG113-*b*-PMVEC13 (7)

MVEC (276 mg, 1.2 mmol) and poly(ethylene glycol) methyl ether (mPEG, 5 kDa, 300 mg, 60 μmol) were dissolved in dry DCM (2.4 mL, $[\text{MVEC}] = 0.5$ M). DBU (9.0 μL , 60 μmol) was added to initiate the polymerization. After 30 min, the polymerization was quenched by addition of a DCM solution of benzoic acid, precipitated into diethyl ether, and collected by centrifugation followed by drying *in vacuo* to yield a white powder. Yield = 487 mg, >98%. $M_{n,\text{NMR}} = 8000$ g/mol. $M_{n,\text{SEC}}^{\text{DMF}} = 11800$ g/mol. PDI = 1.09. ^1H NMR (500 MHz, CDCl_3): δ 6.46–6.37 (m, $-\text{OCHCH}_2$), 4.36–4.31 (m, $-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{O}-$), 4.31–4.22 (br, $-\text{OC}(\text{O})\text{OCH}_2-$), 4.21–4.13 (m, $-\text{OCHCHH}'$), 4.04–3.99 (m, $-\text{OCHCHH}'$), 3.89–3.83 (m, $-\text{C}(\text{O})\text{OCH}_2\text{CH}_2\text{O}-$), 3.62 (s, $-\text{OCH}_2\text{CH}_2\text{O}-$ for PEG), 3.35 (s, $-\text{OCH}_3$), 1.24 (s, $-\text{C}(\text{CH}_3)(\text{CH}_2-)_2$), 1.19 (s, $-\text{C}(\text{CH}_3)(\text{CH}_2-)(\text{CH}_2\text{OH})$). ^{13}C NMR (125 MHz, CDCl_3): δ 172.04, 154.4, 151.5, 87.3, 71.3, 70.6, 70.0, 69.0, 68.6, 68.3, 65.6, 63.5, 46.6, 17.5. IR (neat, cm^{-1}): 3050–2700, 1751, 1628, 1466, 1342, 1273, 1242, 1103, 957, 841. $T_g = -20.4$ °C; $T_m = 44.5$ °C. TGA in Ar: 190–350 °C, 33% mass loss; 350–430 °C, 62% mass loss; 5% mass remaining above 500 °C.

Acetalization of the Vinyl Ether Side Chain Moieties of mPEG₁₁₃-*b*-PMVEC13 with 4-Methylbenzyl Alcohol (**8**)

To the solution of mPEG₁₁₃-*b*-PMVEC13 (300 mg, 0.49 mmol for alkene functionalities) and 4-methylbenzyl alcohol (0.60 g, 4.9 mmol) in 1.0 mL THF, PTSA (1.7 mg, 9.8 μ mol) in 0.50 mL THF was added at room temperature. After stirring for 4 h at room temperature, an excess amount of triethylamine was added to quench the reaction. The reaction mixture was precipitated into diethyl ether twice and dried *in vacuo* to yield a white powder. Yield = 296 mg, 93%. $M_{n,NMR}$ = 8400 g/mol. $M_{n,SEC}^{DMF}$ = 12700 g/mol. PDI = 1.11. 1H NMR (500 MHz, CD₂Cl₂): δ 7.22–7.09 (m, $-OCH_2C_6H_4CH_2-$), 6.46–6.37 (m, $-OCHCH_2-$), 4.85–4.76 (m, $-OCH(CH_3)O-$), 4.87 (d, J = 11.5 Hz, $-OCHH'C_6H_4CH_3$), and 4.44 (d, J = 11.5 Hz, $-OCHH'C_6H_4CH_3$), 4.39–4.17 (br, $-OC(O)OCH_2-$, $-C(O)OCH_2CH_2O-$), 3.80–3.50 (m, $-C(O)OCH_2CH_2O-$ and $-OCH_2CH_2O-$ for PEG), 2.31 (s, $-OCH_2C_6H_4CH_3$, *para*), 1.32 (d, J = 5.1 Hz, $-C(O)OCH_2CH_2OCH(CH_3)O-$), 1.25 (s, $-C(CH_3)(CH_2-)_2$), 1.19 (s, $-C(CH_3)(CH_2-)(CH_2OH)$). ^{13}C NMR (125 MHz, CD₂Cl₂): δ 172.4, 154.5, 135.0, 129.1, 127.7, 99.2, 71.3, 70.6, 69.9, 60.6, 46.7, 19.6, 17.5. IR (neat, cm⁻¹): 3700–3200, 2882, 1749, 1466, 1387, 1343, 1277, 1238, 1144, 1103, 1061, 961, 841, 787, 444, 420, 409. T_g = –9.6 °C; T_m = 45.1 °C.

Self-Assembly of Functionalized Diblock Copolymers

The functionalized diblock copolymers were suspended into nanopure water (5 mg/mL) and sonicated for 10 min.

Monitoring Cleavage of Acetal Moieties of **8**

The hydrolytic cleavage of the acetal moieties of **8** (100 mg), dissolved in 20 mL of 0.1 M acetate buffers (pH 4 and pH 5, final concentrations = 5 mg/mL), was monitored by 1H NMR spectroscopy. After incubation of polymer solutions at 37 °C for specified times, an aliquot (2 mL) of the solution was collected, dialyzed against nanopure water for 1 day, and lyophilized. The resulting polymer powder was dissolved in CDCl₃, and the 1H NMR spectrum was collected.

Cytotoxicity Assays

RAW 264.7 mouse macrophages (2×10^4 cells/well) were plated in 96-well plates in DMEM medium with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO₂ for 24 h to adhere. Medium was then replaced with fresh medium 1 h prior to the addition of 20 μ L of polymer stock solutions to 100 μ L of the medium (final concentrations ranged from 1–3000 μ g/mL). The cells were incubated with the various formulations for 72 h; then, the medium was replaced with 100 μ L of fresh medium prior to the addition of 20 μ L of MTS combined reagent to each well (Cell Titer 96® Aqueous Non-Radioactive Cell Proliferation Assay, Promega Co., Madison, WI). The cells were incubated with the reagent for 2 h at 37 °C in a humidified atmosphere containing 5% CO₂ and protected from light. Absorbance was measured at 490 nm using SpectraMax M5 (Molecular Devices Co., Sunnyvale, CA). Cell viability was calculated based on the absorbance relative to untreated control cells.

RESULTS AND DISCUSSION

Monomer Design and Synthesis

Six-membered cyclic carbonate monomers, the most commonly used monomers for the preparation of PCs by ROP, can be prepared by cyclization of 1,3-diols in various ways.¹⁷ Among them, 2,2-bis(hydroxymethyl)propionic acid (bis-MPA)-based cyclic carbonate monomers have been well studied, due to facile introduction of functionalities to the carboxylic acid, for instance through ester or amide linkages. A vinyl ether-functionalized cyclic carbonate monomer, 5-methyl-5-((2-vinyloxy)ethyl)oxycarbonyl-1,3-dioxan-2-one (MVEC), **2**, was synthesized by a well-established two-step procedure from bis-MPA.^{22–23} Esterification of bis-MPA with 2-chloroethyl vinyl ether, followed by cyclization using ethyl chloroformate, yielded cyclic carbonate monomer with vinyl ether functionality, MVEC, **2**, in 48% overall yield (Scheme 1). At the early stage of development, the esterification step suffered from low reaction yields (~20%) due to the low reactivity of the alkyl chloride. Sodium iodide was added to compensate for the low reactivity by *in situ* halogen exchange reaction, which improved reaction yields to *ca.* 78%. Although the cyclization step was straightforward in moderate yield (~60%), careful purification was necessary since small amounts of impurities hindered crystallization of MVEC. The crude MVEC was a yellowish liquid and was not readily crystallized or solidified. After isolation by flash chromatography, the resulting MVEC crystallized slowly *in vacuo*. Drying MVEC was easier in the solid state than in the viscous oily state. Removal of water is imperative to obtain well-defined PCs *via* ROP of cyclic carbonate monomers, since hydroxyl chain-ends produced from decarboxylation following initiation by water can yield telechelic PCs. ¹H and ¹³C NMR spectra (Figures S1 and S2) and electrospray ionization mass spectrometry (ESI-MS) results were consistent with the structure of the monomer.

MVEC, **2**, is an interesting molecule due to versatile reactivity derived from both its vinyl ether and cyclic carbonate functionalities. It can participate in ROP through the cyclic carbonate moiety, and also in cationic^{2–3} and radical⁴ polymerizations through the vinyl ether moiety. In addition, both functional groups can undergo post-polymerization modifications: crosslinking reaction^{24–25} of cyclic carbonates, thiol-ene “click” reaction, (thio)acetalization, and hydrolysis of vinyl ethers,⁵ *etc.* In this study, we exploited the pendant vinyl ether functionality for post-polymerization modification after ROP of the cyclic carbonate.

Organocatalytic ROP of MVEC, **2**

Vinyl ether-functional PCs [poly(5-methyl-5-((2-vinyloxy)ethyl)oxycarbonyl-1,3-dioxan-2-one), PMVEC, **3**] were synthesized by organobase-catalyzed ROP of the functionalized six-membered cyclic carbonate monomer, **2** (Scheme 2). The ROP of MVEC was conducted in a glovebox under similar conditions as previously reported.^{16, 20} The living characteristics of organobase-catalyzed ROP were demonstrated by a kinetic study in dichloromethane (DCM) solution of MVEC using benzyl-protected bis-MPA (Bn-MPA) as a bi-functional initiator and DBU as a catalyst ([MVEC]₀ = 0.7 M in DCM, [MVEC]₀ : [Bn-MPA] : [DBU] = 75:1:2). The semi-logarithmic plot of [M]₀/[M] *vs.* time displayed pseudo-first-order kinetic behavior of ROP (Figure S3b). Also, a linear relationship between number average

molecular weight (M_n) and monomer conversion indicated that the degrees of polymerization were pre-determinable (Figure S3c). The consistent low dispersities (M_w/M_n 1.10) up to 83% monomer conversion evidenced minimal degree of transesterification of the PC backbone during ROP (Figure S3a and c). After the polymerizations were quenched by addition of a DCM solution of benzoic acid, residual monomer, DBU, and benzoic acid were removed by repeated precipitations into methanol. By ^1H NMR spectroscopy, the ratios of the integrals of the vinyl protons (b, f, and g) and those of the PC backbone (e and j) to those of the aromatic and benzylic protons (a and c) from the initiator were consistent, which indicates that the vinyl ether functionalities remained intact during the ROP and after quenching with benzoic acid (Figure 1a).

The chemical availability of the vinyl ether functionalities for post-polymerization modifications was verified using three types of reactions: acetalization, thio-acetalization, and thiol-ene “click” reaction (Scheme 2).

Acetalization and Thio-acetalization of the Vinyl Ether Side Chain Moieties of PMVEC with 4-Methylbenzyl Alcohol and 4-Methylbenzyl Mercaptan

Post-polymerization modifications were demonstrated by conjugation between the electron-rich vinyl ether moiety of PMVEC and hydroxyl- or thiol-bearing model compounds *via* formation of acetal or thioacetal linkages. First, initial acetalization studies were conducted in THF- d_8 to monitor reaction progress by ^1H NMR spectroscopy. ^1H NMR spectra were collected at a specified time while a mixture of 4-methylbenzyl alcohol, PMVEC, and PTSA in THF- d_8 in an NMR tube was spinning in the NMR spectrometer at 19 °C (Figure S4). Formation of acetal linkages was monitored by disappearance of vinyl resonances (a and b) at 6.49 and 4.02 ppm, respectively, of PMVEC and appearance of methine proton (d) at 4.83 ppm and methyl protons (g) at 1.33 ppm of the acetal group, as well as benzylic protons (e) at 4.62 and 4.49 ppm of the installed 4-methylbenzyloxy groups, using ^1H NMR spectroscopy (Figure S4). Also, the resonance frequency of the methylene protons next to the oxygen of the vinyl ether group of PMVEC shifted upfield from 3.85 to 3.75 ppm upon conversion to the acetal groups (from c to f in Figure S4). In addition, consumption of vinyl ether groups, calculated by integration of the vinyl resonances at 6.52 ppm (a in Figure S4) at corresponding times divided by that of the initial polymer were plotted against reaction time, which showed first order kinetics. With an exponential fit, the half-life of the vinyl ether moieties was estimated at *ca.* 70.6 min under these conditions (Figure S5). Because PMVEC, other reagents, and the solvent were not dried before the acetalization, water impurities could cause hydrolysis of vinyl ether and resulting acetal functionalities. In addition, acetal exchange reactions were possible due to the large excess of alcohol or thiol reagents. Therefore, consumption of the vinyl ether groups was not directly correlated to the formation of acetal linkages. Considering the competition of acetalization, acetal exchange and hydrolysis, monitoring of the reaction time was needed to control the desired amounts of vinyl, acetal, and hydroxyl moieties. The reaction was quenched by adding excess TEA; subsequently, unreacted 4-methylbenzyl alcohol, acid catalyst, and TEA were removed by dialysis against THF. Figure 1b shows the ^1H NMR spectrum of PMVEC after acetalization with 4-methylbenzyl alcohol for 2 h at room temperature and purification by dialysis. The conversion of vinyl ethers to acetals was calculated by chain-end analysis using ^1H NMR

spectroscopy. The conversion of vinyl ethers to acetal linkages after 2 h reaction was 51%, with 16% intact vinyl ethers and 33% hydroxyl groups (Figure 1b). It is noteworthy that all polymers and reagents were not dried for post-polymerization modifications, and conjugations were not conducted in an inert atmosphere, demonstrating the suitability of this chemistry for practical applications. The acetal conversion efficiency (*ca.* 51%) for PC-based polymers, **4**, was superior to that in PPE-based systems (*ca.* 18%),⁵ since the more stable PC backbone allowed for prolonged reaction time. Precipitation of the resulting polymers into organic solvents, such as methanol or diethyl ether, did not ensure the complete removal of acid catalyst, which led to insoluble materials after concentration *in vacuo*. Residual PTSA likely catalyzed cross-linking reactions *via* trans-acetalization, or acetalization of remaining vinyl ethers with hydroxyl groups that were introduced along the polymer backbone *via* acetal exchange and hydrolysis of vinyl ethers and/or acetals.^{5, 13} When the vinyl ether moieties of the resulting acetalized polymers were fully consumed, insolubility was not encountered, even after long-term storage in ambient conditions.

Thio-acetalization with the model thiol compound, 4-methylbenzyl mercaptan, was also conducted on small NMR scale under conditions similar to the acetalization. The conversion of vinyl ethers to thioacetals after 2 h reaction was 36%, with 28% intact vinyl ethers and 36% hydroxyl groups, again determined *via* chain-end analysis of ¹H NMR spectra (Figure 1c). The observed higher efficiency of acetalization (*ca.* 51%) compared with thioacetalization (*ca.* 36%) might be due to the stronger nucleophilicity of alcohols than thiols in THF, a polar aprotic solvent. The apparent issues with crosslinking upon storage and insolubility of the polymer products was not studied in detail; however, it was not observed to be as pronounced as for the acetalization reaction systems. Although we did not optimize reaction conditions for thio-acetalization of PC, conversion was still higher than in the corresponding PPE-based system (*ca.* 8%).⁵

Thiol–Ene “Click” Reaction of the Vinyl Ether Side Chain Moieties of PMVEC with 3-Mercaptopropionic Acid

The thiol-ene “click” reaction was also tested as a post-polymerization modification method to convert the vinyl ether functionalities of PMVEC into stable and robust thioether-ether linkages. Previous reports have demonstrated that radical-mediated thiol-ene reaction conditions are compatible with PC backbones.^{18–20} Conjugation between vinyl ether groups on PMVEC and a model thiol, 3-mercaptopropionic acid, was initiated by UV irradiation (365 nm, 6 W) in the presence of a photoinitiator, 2,2-dimethoxy-2-phenylacetophenone (DMPA). An excess amount (10 equiv. relative to the vinyl ether functionalities) of the thiols was applied to avoid radical-mediated cross-linking of polymers. Formation of conjugate, **6**, was confirmed by disappearance of vinyl resonances (b, f, and g in Figure 1a) at 6.40, 4.18, and 4.02 ppm, respectively, of PMVEC and appearance of protons *α* to the carboxylic acid (w in Figure 1d) at 2.57 ppm and methylene protons u and v (Figure 1d) at 2.77 and 2.68 ppm, respectively, using ¹H NMR spectroscopy. Also, the signal for the methylene proton resonances of the ethyl vinyl ether side chain shifted upfield from (d) at 4.35 ppm and (h) at 3.87 ppm to (r) at 4.23 ppm and (s) at 3.62 ppm, respectively (Figure 1a and d). The ¹H NMR integration indicated that the conjugation yield was quantitative. In addition, the thiol-ene reaction was confirmed by FTIR spectroscopy. The disappearance of C=C vibration

bands [$\nu(\text{C}=\text{C})$] of **3** at 1635 and 1620 cm^{-1} represented the consumption of the vinyl groups. The broadening of the C=O stretching vibration indicated the addition of the carboxylic acid functionality (of 3-mercaptopropionic acid), which is further supported by the appearance of a broad O-H peak (Figure S6).

Synthesis of Block Copolymers and Their Post-polymerization Modifications

The amphiphilic diblock copolymer, α -methoxy poly(ethylene glycol)-*block*-PMVEC (mPEG-*b*-PMVEC), **7**, was synthesized using mPEG-OH (5 kDa, mPEG₁₁₃-OH) as a macroinitiator and DBU as an organobase catalyst ([mPEG_{5K}-OH] : [DBU] : [MVEC] = 1:1:20, [MVEC]₀ = 0.5 M in DCM, Scheme 3). After the desired time, the polymerization was quenched by adding a DCM solution of benzoic acid, and the resulting diblock copolymer was isolated by precipitation into diethyl ether. The ¹H NMR spectrum of mPEG-*b*-PMVEC showed proton resonances of PMVEC (as in Figure 1a) as well as those of mPEG (Figure S7a). The degree of polymerization (DP_n) calculated by chain-end analysis by ¹H NMR spectroscopy was in agreement with the monomer conversion (65%) calculated by ¹H NMR spectroscopy. The distinct peak shift on SEC profiles of the macroinitiator and the block copolymer verified successful growth of a PMVEC block from mPEG₁₁₃-OH (Figure 2). The small high-molecular-weight shoulder, observed at shorter retention time, is expected to arise from a fraction of the mPEG macroinitiator containing hydroxyl groups at both chain ends. Subsequent acetalization of the resulting block copolymer with 4-methylbenzyl alcohol afforded the amphiphilic polymer with pendant acetal functionalities, **8**. The high-molecular-weight shoulder of polymer **8** was more prominent compared to polymer **7**, which might be attributed to chain-chain coupling *via* transacetalization. After quenching the reaction with TEA, the polymer was isolated by dialysis and precipitation. Its ¹H NMR spectrum (Figure S7b) was consistent with that of acetal-functionalized PMVEC, **4** (Figure 1b). Based upon literature examples of the synthesis of block copolymers *via* chain extension with other cyclic monomers for ROP such as cyclic carbonates,^{16, 26} lactide,^{27–28} and cyclic esters,^{29–30} it is anticipated that a variety of PMVEC-based polymers could be synthesized by this approach. Also, post-polymerization modifications of the resulting block copolymers would further increase the diversity of PMVEC-based macromolecular structures.

Self-Assembly of Amphiphilic Block Polymers

The self-assembled polymeric nanostructures were readily prepared by direct dissolution of the synthesized amphiphilic polymers in suitable buffers. First, the self-assembly behaviors of mPEG-*b*-PMVEC (**7**), acetal-functionalized mPEG-*b*-PMVEC (**8**), and mPEG-*b*-poly(5-methyl-5-(2-hydroxyethoxy)carbonyl-1,3-dioxan-2-one (PMHEC) (**9**) were studied by dynamic light scattering (DLS), ξ -potential measurements, and transmission electron microscopy (TEM) (Figure 3). PMHEC is the hydrolyzed block of PMVEC or acetal-functionalized PMVEC. The number-averaged hydrodynamic diameters of the micellar nanostructures of **7**, **8**, and **9**, in buffered solutions measured by DLS were 28 ± 7 , 16 ± 5 , and 9 ± 3 nm, respectively, in acetate buffer (pH 5.0), and 28 ± 8 nm (micelles of **7**) and 16 ± 3 nm (micelles of **8**) in 1× PBS buffer (pH 7.4) (Figure 3a). The hydrodynamic diameters decreased depending on the amount of hydroxyl functionalities, which is attributed to the increased hydrophilicity of the core-forming PC blocks by hydrolysis.^{31–32} DLS

measurement of micelles of **9** was not feasible in 1× PBS buffer even at the relatively high concentration of 5 mg/mL. On the other hand, hydrodynamic diameters of micelles of **7** could be measured at 0.25 mg/mL. These results suggest that the critical micelle concentration (CMC) of **9** is higher than those of **7** and **8**, as would be expected. In addition, we were unable to obtain quality TEM images of assemblies from **8** or **9**, whereas the globular, uniform sizes of micelles from **7** were confirmed by TEM, with number-averaged diameters of *ca.* 20 nm in the dry state, after deposition from either 1× PBS or acetate buffer (Figure 3b and c). The PEG corona made the ξ -potentials almost neutral: -3.2 ± 0.3 (micelles of **7**), -2.6 ± 0.5 (micelles of **8**), and 0.23 ± 1.1 mV (micelles of **9**) in acetate buffer (pH 5.0); and -9.6 ± 2.3 mV (micelles of **7**) and -6.6 ± 2.9 mV (micelles of **8**) in 1× PBS (pH 7.4).

Monitoring Cleavage of Acetal Moieties

Hydrolysis of the acetal functionalities under acidic conditions was monitored using ^1H NMR spectroscopy for quantitative analysis of cleavage kinetics. During this process, the residual vinyl ether functionalities (in the context of partial acetalization of mPEG-*b*-PMVEC) could undergo further acetalization with hydrolyzed hydroxyl groups on the PC side chain and/or cleaved 4-methylbenzyl alcohol in acidic conditions, which could complicate the analysis. Therefore, available vinyl ether groups were completely consumed during acetal formation of mPEG₁₁₃-*b*-PMVEC₁₃ with 4-methylbenzyl alcohol, even though it could diminish the final acetal contents *via* the competing hydrolysis reaction over a longer time (4 h). Since the acetal-functionalized mPEG-*b*-PMVEC, **8**, self-assembles into micelles in aqueous solution, acetal moieties may reside in various domains within the micelles, which would complicate *in situ* ^1H NMR monitoring of acetal protons in D₂O. Therefore, aliquots of micellar solution were collected at specified times, purified by dialysis against nanopure water, and analyzed by ^1H NMR spectroscopy in CDCl₃ to observe proton signals along the entire block polymer chains (Figure 4a). Two samples were prepared in acetate buffers (pH 4 and 5) and kept under shaking at 37 °C. As expected, the decrease of acetal content was faster in the more acidic solution. The half-lives of acetal cleavage were *ca.* 10 days at pH 4 and *ca.* 27 days at pH 5, respectively (Figure 4b). As a control experiment, no significant decrease of acetal content was observed in PBS buffer (pH 7.4) during the same timeframe (data not shown). Interestingly, our systems showed slower cleavage rates compared to previously-reported systems.^{10, 33–34} Even though further investigations are required to fully characterize the behavior of micelles during the course of acetal cleavage, the slow cleavage rates may enable sustained and controlled release of polymeric nanostructure-conjugated therapeutics compared to physical encapsulation, which could reduce patient side effects related to premature release of payload and decrease the frequency of administration for better patient compliance and lower cost of therapy.³⁵ In addition, after the release of compounds under acidic conditions, remaining hydroxyl groups could further enhance hydrophilicity of the residual polymer, which might aid in the clearance of polymers from the body.

Cytotoxicity of Micelles of mPEG₁₁₃-*b*-PMVEC₁₃ and mPEG₁₁₃-*b*-PMHEC₁₃

The cytotoxicities of mPEG₁₁₃-*b*-PMVEC₁₃ (**7**) and mPEG₁₁₃-*b*-PMHEC₁₃ (**9**) were examined against RAW 264.7 mouse macrophages as a function of concentration (Figure 5).

Both the parent micelles and the polymer solutions after hydrolysis of the side chain acetals exhibited minimal cytotoxicity over the range of the tested concentrations (5 to 3000 µg/mL after incubation for 72 h). Hence, mPEG-*b*-PMVEC has the potential to be exploited as a biocompatible building block for stimuli-responsive drug delivery or other technological applications.

CONCLUSIONS

A vinyl ether-functional degradable polycarbonate (PMVEC) was synthesized *via* organocatalytic ROP of the corresponding cyclic carbonate monomer. Post-polymerization modifications on pendant vinyl ethers were demonstrated by acetalization, thio-acetalization, and thiol-ene reaction of PMVEC with hydroxyl- or thiol-containing compounds. These facile functionalizations are expected to contribute to improving the diversity of functional polymers. Among the resulting functionalities, acetal linkage was further examined to utilize its acid-labile characteristics. Self-assembly of acetal-functionalized amphiphilic block polymers yielded nanostructures with size and other characteristics suitable for biomedical applications. Cleavage of acetal groups within the polymeric micelles in acidic buffered solutions (pH 4 and 5) demonstrated the potential of PMVEC as a promising component for a stimuli-responsive drug delivery system.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge financial support from the National Heart Lung and Blood Institute of the National Institutes of Health as a Program of Excellence in Nanotechnology (HHSN268201000046C) and from the National Institute of Diabetes and Digestive and Kidney Diseases (R01-DK082546). The Welch Foundation is gratefully acknowledged for support through the W. T. Doherty-Welch Chair in Chemistry, Grant No. A-0001.

References

1. Login, RB. Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley & Sons, Inc; 2000. Vinyl Ether Monomers and Polymers.
2. Kawamura M, Kanazawa A, Kanaoka S, Aoshima S. Sequence-controlled degradable polymers by controlled cationic copolymerization of vinyl ethers and aldehydes: precise placement of cleavable units at predetermined positions. *Polym Chem.* 2015; 6(22):4102–4108.
3. Kanazawa A, Hashizume R, Kanaoka S, Aoshima S. Design of Benign Initiator for Living Cationic Polymerization of Vinyl Ethers: Facile in Situ Generation of Vinyl Ether–Hydrogen Halide Adducts and Subsequent Controlled Polymerization without a Lewis Acid Catalyst. *Macromolecules.* 2014; 47(5):1578–1585.
4. Sugihara S, Kawamoto Y, Maeda Y. Direct Radical Polymerization of Vinyl Ethers: Reversible Addition–Fragmentation Chain Transfer Polymerization of Hydroxy-Functional Vinyl Ethers. *Macromolecules.* 2016; 49(5):1563–1574.
5. Lim YH, Heo GS, Rezenom YH, Pollack S, Raymond JE, Elsabahy M, Wooley KL. Development of a Vinyl Ether-Functionalized Polyphosphoester as a Template for Multiple Postpolymerization Conjugation Chemistries and Study of Core Degradable Polymeric Nanoparticles. *Macromolecules.* 2014; 47(14):4634–4644. [PubMed: 25601803]
6. Glöckner P. Radiation Curing: Coatings and Printing Inks ; Technical Basics, Applications and Trouble Shooting. Vincentz Network. 2008

7. Li M, Gao M, Fu Y, Chen C, Meng X, Fan A, Kong D, Wang Z, Zhao Y. Acetal-linked polymeric prodrug micelles for enhanced curcumin delivery. *Colloids Surf B*. 2016; 140:11–18.
8. Schlossbauer A, Dohmen C, Schaffert D, Wagner E, Bein T. pH-Responsive Release of Acetal-Linked Melittin from SBA-15 Mesoporous Silica. *Angew Chem Int Ed*. 2011; 50(30):6828–6830.
9. Wang H, He J, Cao D, Zhang M, Li F, Tam KC, Ni P. Synthesis of an acid-labile polymeric prodrug DOX-acetal-PEG-acetal-DOX with high drug loading content for pH-triggered intracellular drug release. *Polym Chem*. 2015; 6(26):4809–4818.
10. Gu Y, Zhong Y, Meng F, Cheng R, Deng C, Zhong Z. Acetal-Linked Paclitaxel Prodrug Micellar Nanoparticles as a Versatile and Potent Platform for Cancer Therapy. *Biomacromolecules*. 2013; 14(8):2772–2780. [PubMed: 23777504]
11. Tardy A, Honoré J-C, Tran J, Siri D, Delplace V, Bataille I, Letourneur D, Perrier J, Nicoletti C, Maresca M, Lefay C, Gigmes D, Nicolas J, Guillauneuf Y. Radical Copolymerization of Vinyl Ethers and Cyclic Ketene Acetals as a Versatile Platform to Design Functional Polyesters. *Angew Chem Int Ed*. 2017; 56(52):16515–16520.
12. Huang D, Zhuang Y, Shen H, Yang F, Wang X, Wu D. Acetal-linked PEGylated paclitaxel prodrugs forming free-paclitaxel-loaded pH-responsive micelles with high drug loading capacity and improved drug delivery. *Mater Sci Eng C*. 2018; 82:60–68.
13. Mangold C, Dingels C, Obermeier B, Frey H, Wurm F. PEG-based Multifunctional Polyethers with Highly Reactive Vinyl-Ether Side Chains for Click-Type Functionalization. *Macromolecules*. 2011; 44(16):6326–6334.
14. Lim YH, Tiemann KM, Heo GS, Wagers PO, Rezenom YH, Zhang S, Zhang F, Youngs WJ, Hunstad DA, Wooley KL. Preparation and in Vitro Antimicrobial Activity of Silver-Bearing Degradable Polymeric Nanoparticles of Polyphosphoester-block-Poly(L-lactide). *ACS Nano*. 2015; 9(2):1995–2008. [PubMed: 25621868]
15. Zhang F, Smolen JA, Zhang S, Li R, Shah PN, Cho S, Wang H, Raymond JE, Cannon CL, Wooley KL. Degradable polyphosphoester-based silver-loaded nanoparticles as therapeutics for bacterial lung infections. *Nanoscale*. 2015; 7(6):2265–2270. [PubMed: 25573163]
16. Cho S, Heo GS, Khan S, Gonzalez AM, Elsbahy M, Wooley KL. Functionalizable Hydrophilic Polycarbonate, Poly(5-methyl-5-(2-hydroxypropyl)aminocarbonyl-1,3-dioxan-2-one), Designed as a Degradable Alternative for PHPMA and PEG. *Macromolecules*. 2015; 48(24):8797–8805.
17. Tempelaar S, Mespouille L, Coulembier O, Dubois P, Dove AP. Synthesis and post-polymerisation modifications of aliphatic poly(carbonate)s prepared by ring-opening polymerisation. *Chem Soc Rev*. 2013; 42(3):1312–1336. [PubMed: 23151841]
18. Tempelaar S, Mespouille L, Dubois P, Dove AP. Organocatalytic Synthesis and Postpolymerization Functionalization of Allyl-Functional Poly(carbonate)s. *Macromolecules*. 2011; 44(7):2084–2091.
19. Tempelaar S, Barker IA, Truong VX, Hall DJ, Mespouille L, Dubois P, Dove AP. Organocatalytic synthesis and post-polymerization functionalization of propargyl-functional poly(carbonate)s. *Polym Chem*. 2013; 4(1):174–183.
20. Heo GS, Cho S, Wooley KL. Aldehyde-functional polycarbonates as reactive platforms. *Polym Chem*. 2014; 5(11):3555–3558. [PubMed: 25580163]
21. Thomas AW, Dove AP. Postpolymerization Modifications of Alkene-Functional Polycarbonates for the Development of Advanced Materials Biomaterials. *Macromol Biosci*. 2016; 16(12):1762–1775. [PubMed: 27654885]
22. Pratt RC, Nederberg F, Waymouth RM, Hedrick JL. Tagging alcohols with cyclic carbonate: a versatile equivalent of (meth)acrylate for ring-opening polymerization. *Chem Commun*. 2008; 1:114–116.
23. Al-Azemi TF, Bisht KS. Novel Functional Polycarbonate by Lipase-Catalyzed Ring-Opening Polymerization of 5-Methyl-5-benzoyloxycarbonyl-1,3-dioxan-2-one. *Macromolecules*. 1999; 32(20):6536–6540.
24. Campagne B, David G, Améduri B, Jones DJ, Rozière J, Roche I. New semi-IPN PEMFC membranes composed of crosslinked fluorinated copolymer bearing triazole groups and sPEEK for operation at low relative humidity. *Int J Hydrogen Energy*. 2015; 40(46):16797–16813.

25. Suzuki A, Nagai D, Ochiai B, Endo T. Facile synthesis and crosslinking reaction of trifunctional five-membered cyclic carbonate and dithiocarbonate. *J Polym Sci, Part A: Polym Chem*. 2004; 42(23):5983–5989.
26. Naumann S, Thomas AW, Dove AP. Highly Polarized Alkenes as Organocatalysts for the Polymerization of Lactones and Trimethylene Carbonate. *ACS Macro Lett*. 2016; 5(1):134–138.
27. Kalelkar PP, Alas GR, Collard DM. Synthesis of an Alkene-Containing Copoly lactide and Its Facile Modification by the Addition of Thiols. *Macromolecules*. 2016; 49(7):2609–2617.
28. MacDonald JP, Shaver MP. An aromatic/aliphatic polyester prepared via ring-opening polymerisation and its remarkably selective and cyclable depolymerisation to monomer. *Polym Chem*. 2016; 7(3):553–559.
29. Pepels MPF, van der Sanden F, Gubbels E, Duchateau R. Catalytic Ring-Opening (Co)polymerization of Semiaromatic and Aliphatic (Macro)lactones. *Macromolecules*. 2016; 49(12):4441–4451.
30. Wilson JA, Hopkins SA, Wright PM, Dove AP. Dependence of Copolymer Sequencing Based on Lactone Ring Size and ϵ -Substitution. *ACS Macro Lett*. 2016; 5(3):346–350.
31. Zhang L, Eisenberg A. Multiple Morphologies of “Crew-Cut” Aggregates of Polystyrene-*b*-poly(acrylic acid) Block Copolymers. *Science*. 1995; 268(5218):1728–1731. [PubMed: 17834990]
32. Zhang L, Eisenberg A. Multiple Morphologies and Characteristics of “Crew-Cut” Micelle-like Aggregates of Polystyrene-*b*-poly(acrylic acid) Diblock Copolymers in Aqueous Solutions. *J Am Chem Soc*. 1996; 118(13):3168–3181.
33. Gao AX, Liao L, Johnson JA. Synthesis of Acid-Labile PEG and PEG-Doxorubicin-Conjugate Nanoparticles via Brush-First ROMP. *ACS Macro Lett*. 2014; 3(9):854–857. [PubMed: 25243099]
34. Gillies ER, Goodwin AP, Fréchet MJ. Acetals as pH-Sensitive Linkages for Drug Delivery. *Bioconjugate Chem*. 2004; 15(6):1254–1263.
35. Elsabahy M, Heo GS, Lim S-M, Sun G, Wooley KL. Polymeric Nanostructures for Imaging and Therapy. *Chem Rev*. 2015; 115(19):10967–11011. [PubMed: 26463640]

Figure 1.

^1H NMR (300 MHz) spectra of (a) PMVEC, **3** (in CDCl_3), (b) after acetalization with 4-methylbenzyl alcohol, **4** (in CD_2Cl_2), (c) after thio-acetalization with 4-methylbenzyl mercaptan, **5** (in CDCl_3), (d) after thiol-ene reaction with 3-mercaptopropionic acid, **6** (in $\text{CD}_2\text{Cl}_2/\text{MeOD}$).

Figure 2. SEC traces (DMF as eluent, 1 mL/min) of macroinitiator (mPEG₁₁₃-OH), **7** (mPEG₁₁₃-*b*-PMVEC₁₃), and **8** (the acetal-functionalized mPEG₁₁₃-*b*-PMVEC₁₃).

Figure 3. Self-assembly of amphiphilic polymers. (a) Number-averaged (D_h (num, nm)) hydrodynamic diameters and ζ -potential values of **7** (mPEG₁₁₃-*b*-PMVEC₁₃), **8** (the acetal-functionalized mPEG₁₁₃-*b*-PMVEC₁₃), and **9** (acetal-cleaved block copolymer, mPEG₁₁₃-*b*-PMHEC₁₃). Error bars represent the standard deviation. (b, c) Bright-field TEM images of the block copolymer, **7**, micelles following deposition of pH 7.4 (b, 1× PBS) and pH 5 (c, acetate) buffered solutions, stained negatively with 1% phosphotungstic acid.

Figure 4.

Cleavage of acetal moieties within the micelles of acetal functionalized mPEG₁₁₃-*b*-PMVEC₁₃, **8**, with 4-methylbenzyl alcohol in 0.1 M acetate buffered solutions at 37 °C. (a) Time-dependent ¹H NMR (300 MHz) spectra (CDCl₃) of the sample at pH 4. At each specified time, an aliquot of the reaction solution was collected, purified by dialysis against nanopure water, and freeze-dried for NMR analysis. (b) Consumption of acetal groups vs time during the acetal cleavage reaction at pH 4 and pH 5.

Figure 5. Cytotoxicity of the micelles of mPEG₁₁₃-b-PMVEC₁₃ (black circles) and mPEG₁₁₃-b-PMHEC₁₃ (red triangles) in RAW 264.7 mouse macrophages.

Scheme 1. Synthesis of Vinyl Ether-Functionalized Cyclic Carbonate Monomer, MVEC, 2

Scheme 2. Synthesis of PMVEC and its Functionalizations

Scheme 3. Synthesis of mPEG-*b*-PMVEC and its Functionalization via Acetal Formation with a Model Hydroxyl Compound, 4-Methylbenzyl Alcohol