Macromolecules

ARTICLE

pubs.acs.org/Macromolecules

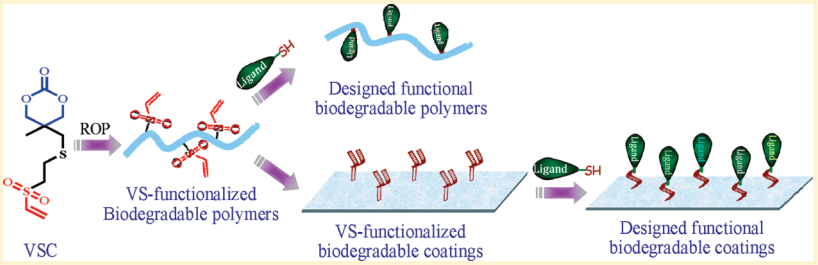
Unprecedented Access to Functional Biodegradable Polymers and Coatings

Rong Wang,† Wei Chen,† Fenghua Meng, Ru Cheng, Chao Deng, Jan Feijen, and Zhiyuan Zhong*

Biomedical Polymers Laboratory, and Jiangsu Key Laboratory of Advanced Functional Polymer Design and Application, Department of Polymer Science and Engineering, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou, 215123, P. R. China

Supporting Information

ABSTRACT:



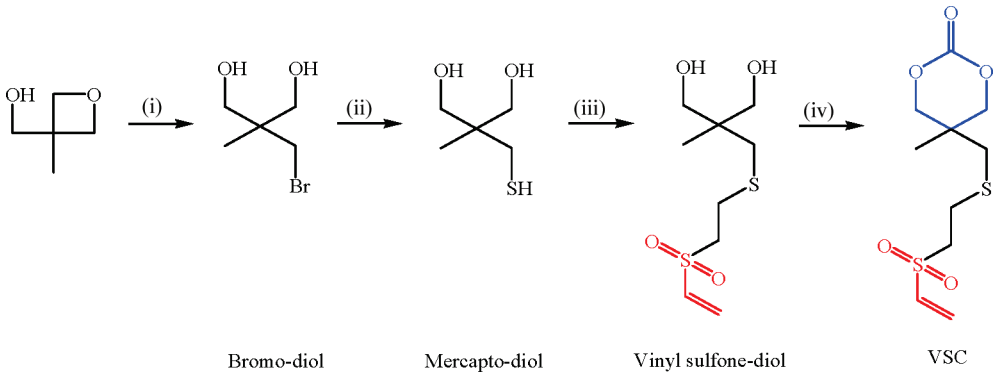
The ever-growing biomedical technology such as tissue engineering, regenerative medicine, and controlled drug release intimately relies on the development of advanced functional biomaterials. Here, we report on versatile and robust synthesis of novel vinyl sulfone (VS)-functionalized biodegradable polymers that offer unprecedented access to advanced functional biodegradable polymers and coatings through selective Michael-type conjugate reaction with thiol-containing molecules. VS-functionalized biodegradable polymers including poly(ε-caprolactone) (PCL), poly(L-lactide) (PLA), and poly(trimethylene carbonate) (PTMC) were conveniently prepared with controlled molecular weights and functionalities through ring-opening copolymerization of ε-caprolactone (ε-CL), L-lactide (LA), or trimethylene carbonate (TMC) with a new cyclic carbonate monomer, vinyl sulfone carbonate (VSC), in toluene at 110 °C using isopropanol as an initiator and stannous octoate as a catalyst. Interestingly, these VS-functionalized biodegradable polymers allowed quantitative modification, without aid of a catalyst, with various thiol-containing molecules including 2-mercaptoethanol, cystamine, cysteine, GRGDC peptide, and thiolated poly(ethylene glycol) (PEG-SH) at a ligand-SH/VS molar ratio of 2/1 in DMF at room temperature, confirming that the Michael-type conjugate addition to VS is highly selective and tolerant to most other functional groups including hydroxyl, carboxyl, and amine. Remarkably, results of contact angle measurements, X-ray photoelectron spectroscopy (XPS), and fluorescence studies showed that biodegradable coatings based on these VS-functionalized polymers allowed direct, efficient, and clean (without catalyst and byproduct) surface functionalization with thiol-containing molecules in aqueous conditions, which is unprecedented and opens a new avenue to surface functionalization of medical implants as well as cell and tissue scaffolds. The preliminary cell culture studies using MG6 cells showed that unmodified VS-functionalized PCL films, similar to tissue culture plate, could well support cell attachment and growth, indicating that VS-functionalized PCL film is nontoxic and biocompatible. The surface of VS-functionalized PCL films could be elegantly engineered with thiolated nonfouling polymers (e.g., PEG and glycol chitosan) or cell adhesive motif (GRGDC peptide) to control cell attachment and growth. We are convinced that these vinyl sulfone-functionalized biodegradable polymers have a tremendous potential in biomedical engineering.

INTRODUCTION

The ever-growing biomedical technology such as tissue engineering, regenerative medicine, and controlled drug release intimately relies on the development of advanced functional biomaterials.[1−3] Aliphatic polyesters and polycarbonates due to their unique biocompatibility, biodegradability, and approved use in biomedical devices by the US food and drug administration (FDA) are the prime synthetic biomaterials.[4] For example, they have been applied for absorbable orthopedic devices,[5] microparticles for controlled protein release,[6,7] cell and tissue scaffolds,[8,9] drug-eluting stents,[10] and nanoparticles for targeted drug release.[11,12] However, common biodegradable polymers are often challenged by their high hydrophobicity, improper degradation profile, and in particular absence of reactive centers for the covalent immobilization of bioactive molecules such as drugs, peptides, and proteins. This "inert" nature has largely hampered

Received: April 8, 2011
Revised: July 5, 2011
Published: July 14, 2011

Scheme 1. Synthetic Pathway for VSC Monomer[a]



[a] Conditions: (i) THF, dropwise addition of 40% HBr at 0 °C, then 25 °C for 5 h; (ii) NaSH, 75 °C, 17 h, DMF; (iii) divinyl sulfone, 30 °C, methanol; (iv) ethyl chloroformate, Et_3N, 0 °C, 4 h, THF.

their design of biologically active biomaterials. In the past decade, significant efforts have been directed to development of functional aliphatic polyesters and polycarbonates,[13] containing e.g. hydroxyl,[14−16] carboxyl,[16−18] amine,[19] allyl,[20−23] alkyne/azide,[24−28] and acryloyl pendant groups.[29−33] The postpolymerization modification based on these functional polymers may provide entry to a variety of sophisticated materials.[34] In our laboratory, we are interested in the development of functional biodegradable polymers in which polymer synthesis does not involve protection and deprotection procedures and postpolymerization modification proceeds quantitatively under mild conditions without aid of any toxic catalyst and without generation of any byproduct. In this way, polymer degradation as well as possible contamination with toxic catalysts and byproducts may be prevented. We and Jerome's group found that acryloyl functional groups amenable to the Michael-type conjugate addition with thiolated molecules appears to be one of the most ideal choices.[30,35] However, acryloyl functional groups tend to undergo radical polymerization during storage, (co)polymerization, work-up, and modification processes. Jerome and co-workers have reported controlled atom transfer radical polymerization of γ-acryloyl-ε-caprolactone.[29] Recently, we reported facile photocross-linking of biodegradable micelles containing poly(acryloyl carbonate) block.[36] We hypothesized that vinyl sulfone (VS) functional group with a significantly higher reactivity and selectivity toward the Michael-type conjugate addition with thiolated molecules,[37] much lower reactivity toward radical polymerization, and highly hydrophilic nature (divinyl sulfone has a water solubility ≥100 mg/mL at 17 °C) might overcome problems associated with acryloyl functional groups and more importantly may enable direct modification of the corresponding polymer films in water. It has been a long challenge to develop a straightforward and efficient modification strategy for biomaterials and coatings that preferably takes place in aqueous conditions without the help of a catalyst.

In this paper, we report on versatile and robust synthesis of VS-functionalized biodegradable polymers that offer unprecedented access to advanced functional biomaterials and coatings through selective Michael-type conjugate reaction with thiol-containing molecules (e.g., 2-mercaptoethanol, cystamine, cysteine, GRGDC, PEG-SH, etc.). This novel functionalization approach presents several remarkable features: (i) VS-functionalized biodegradable polymers with vastly different structures and compositions can be readily prepared without protection and deprotection steps; (ii) postpolymerization modification with thiol-containing molecules proceeds in a quantitative manner under extremely mild conditions (in the absence of catalyst), minimizing possible degradation and contamination; (iii) the Michael-type conjugate addition of VS groups with thiol-containing molecules is highly selective and tolerant to a variety of functional groups including hydroxyl, carboxyl, and amine, enabling facile conjugation of different types of biologically active molecules;[37] and (iv) most spectacularly, biodegradable coatings based on these VS-functionalized polymers allow for the fist time direct, efficient, and clean (without catalyst and byproduct) surface functionalization in aqueous conditions, which opens a new avenue to surface functionalization of medical implants as well as cell and tissue scaffolds.

EXPERIMENTAL SECTION

Materials. 3-Methyl-3-oxetanemethanol (97%, Alfa), hydrobromic acid (40%, SCRC), sodium hydrosulfide hydrate (68%, Acros), divinyl sulfone (95%, Dalian Guanghui, China), triethylamine (Et_3N, 99%, Alfa Aesar), stannous octoate ($Sn(Oct)_2$, 95%, Sigma), 2-mercaptoethanol (>99%, Amresco), 2-mercaptoethylamine hydrochloride (99%, Alfa Aesar), L-cysteine (>99%, Alfa Aesar), and fluorescein isothiocyanate (FITC, 98%, Sigma) were used as received. GRGDC was purchased from Suzhou China Tech Peptide Co., Ltd. Thiolated glycol chitosan (GC-SH, M_n = 80 000, DS = 6.85) and thiolated PEG (PEG-SH, M_n = 5000) were synthesized according to previous reports.[38,39] Ethyl chloroformate (>96%, SCRC) was distilled prior to use. Isopropanol and ε-caprolactone (ε-CL, 99%, Alfa Aesar) were dried over CaH_2 and distilled before use. Toluene was dried by refluxing over sodium wire under an argon atmosphere prior to distillation. L-Lactide (L-LA, >99%, Purac) and trimethylene carbonate (TMC, Jinan Daigang Co. Ltd., China) were recrystallized from dry toluene.

Synthesis of Vinyl Sulfone Carbonate (VSC) Monomer. VSC was synthesized in four steps (Scheme S1). First, HBr (40%, 40 mL) was dropwise added to a solution of 3-methyl-3-oxetanemethanol (10.2 g, 0.10 mol) in THF (100 mL) under stirring at 0 °C. The reaction mixture was warmed to 25 °C and stirred for 5 h. The reaction mixture was then diluted with H_2O (150 mL) and extracted with diethyl ether (4 × 150 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated to give the desired product (bromo-diol) as a white solid (16.38 g, 90%). ^1H NMR (400 MHz, $CDCl_3$): δ 3.68 (s, 4H,

$-C(CH_2OH)_2$), 3.55 (s, 2H, $-CH_2Br$), 2.13 (s, 2H, $-(OH)_2$), 0.93 (s, 3H, $-CH_3$).

To a solution of bromo-diol (16.38 g, 0.09 mol) in DMF (150 mL) under stirring was added NaSH (22.23 g, 0.27 mol). The reaction mixture was stirred at 75 °C for 17 h, cooled to 25 °C, diluted with DI water (1.0 L), and extracted with EtOAc (3 × 250 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated. Residual DMF was removed by distillation under reduced pressure to yield mercapto-diol as a yellowish oil (5.26 g, 43%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.64 (s, 4H, $-C(CH_2OH)_2$), 2.67 (d, 2H, $-CH_2SH$), 2.27 (s, 2H, $-(OH)_2$), 1.31 (t, 1H, $-SH$), 0.85 (s, 3H, $-CH_3$).

To a solution of divinyl sulfone (10 mL, 99.6 mmol) in MeOH (350 mL) under stirring was dropwise added mercapto-diol (5.26 g, 38.7 mmol) at room temperature. The reaction mixture was warmed to 30 °C and stirred overnight in the dark. The solution was concentrated under reduced pressure, and the residue was purified by column chromatography (eluent: ethyl acetate/petroleum ether = 4/1, v/v) to yield vinyl sulfone-diol (5.89 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.23−6.64 (m, 3H, $-CH{=}CH_2$), 3.64 (s, 4H, $-C(CH_2OH)_2$), 3.27 (m, 2H, $-SCH_2CH_2-$), 2.95 (m, 2H, $-SCH_2CH_2-$), 2.75 (s, 2H, $-CCH_2S-$), 2.49 (s, 2H, $-(OH)_2$), 0.85 (s, 3H, $-CH_3$).

To a solution of vinyl sulfone-diol (5.89 g, 23.2 mmol) and ethyl chloroformate (4.6 mL, 48.7 mmol) in dry THF (200 mL) at 0 °C under stirring was dropwise added a solution of Et_3N (7 mL, 51.1 mmol) in THF (5 mL). The reaction was allowed to proceed for 5 h at 0 °C. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The crude product was recrystallized from THF to yield vinyl sulfone carbonate monomer (4.61 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.23−6.64 (m, 3H, $-CH{=}CH_2$), 4.17−4.26 (s, 4H, $-C(CH_2)_2C-$), 3.27 (m, 2H, $-SCH_2CH_2-$), 2.95 (m, 2H, $-SCH_2CH_2-$), 2.75 (s, 2H, $-CCH_2S$-), 1.10 (s, 3H, $-CH_3$). ^{13}C NMR (400 MHz, $CDCl_3$): δ 145.21, 133.12, 129.27, 72.40, 51.67, 34.65, 30.17, 23.58, 15.75. Anal. Calcd for $C_{10}H_{16}O_5S_2$: C, 42.84; H, 5.75; S, 22.87. Found: C, 43.24; H, 5.70; S, 22.37. TOF-MS (m/z): calcd for $C_{10}H_{16}O_5S_2$ 280.0439; found 280.0231.

Synthesis of VS-Functionalized Polyesters and Polycarbonates. The copolymerizations of VSC with ε-CL, L-LA, and TMC were carried out in toluene at 110 °C for 1 day using isopropanol as an initiator and $Sn(Oct)_2$ as a catalyst. The following is a typical example on synthesis of P(CL-*co*-VSC)4.2% copolymer. In a glovebox under a nitrogen atmosphere, to a solution of ε-CL (1.160 g, 10.18 mmol) and VSC (0.15 g, 0.54 mmol) in toluene (11 mL) under stirring was quickly added the stock solutions of isopropanol (4 mg, 0.06 mmol) and $Sn(Oct)_2$ (22 mg, 0.05 mmol) in toluene. The reaction vessel was sealed and placed in an oil-bath thermostated at 110 °C. After 24 h polymerization, the reaction was terminated by two drops of acetic acid. A sample was taken for the determination of monomer conversion using ^{1}H NMR. The resulting P(CL-*co*-VSC) copolymer was isolated by precipitation in ethanol, filtration, and drying in vacuo. ^{1}H NMR (400 MHz, $CDCl_3$, Figure 2A): δ 6.23−6.64 (m, $-CH{=}CH_2$, PVSC), 5.01 (m, $(CH_3)_2CH-$), 4.05−4.13 (s, $-CH_2O-$, PCL; $C(CH_2)_2O_2-$, PVSC), 3.22 (m, SO_2CH_2-, PVSC), 2.86 (m, $-CH_2S-$, PVSC), 2.75 (s, $-SCH_2C$, PVSC), 2.30 (t, $-COCH_2-$, PCL), 1.64 (m, $-CH_2CH_2CH_2-$, PCL), 1.37 (m, $-CH_2CH_2CH_2-$, PCL), 1.22 (d, $(CH_3)_2C-$), 1.03 (s, $-CH_3$, PVSC).

P(LA-*co*-VSC) and P(TMC-*co*-VSC) copolymers were synthesized in a similar manner. ^{1}H NMR spectra as well as signal assignments of the copolymers are shown in Figure 2B,C. ^{1}H NMR (400 MHz, $CDCl_3$) of P(LA-*co*-VSC): δ 6.23−6.64 (m, $-CH{=}CH_2$, PVSC), 5.16 (m, CH_3CH-, PLA), 4.05 (s, $C(CH_2)_2O_2-$, PVSC), 3.22 (m, SO_2CH_2-, PVSC), 2.86 (m, $-CH_2S-$, PVSC), 2.75 (s, $-SCH_2C$, PVSC), 1.58 (m, $-CHCH_3$, PLA), 1.22 (d, $(CH_3)_2C-$), 1.03 (s, $-CH_3$, PVSC). ^{1}H NMR (400 MHz, $CDCl_3$) of P(TMC-*co*-VSC): δ 6.23−6.64 (m, $-CH{=}CH_2$, PVSC), 4.25 (s, $-CH_2CH_2CH_2-$, PTMC), 4.05 (s, $C(CH_2)_2O_2-$, PVSC), 3.22 (m, SO_2CH_2-, PVSC), 2.86 (m, $-CH_2S-$, PVSC), 2.75 (s, $-SCH_2C$, PVSC), 2.04 (s, $-CH_2CH_2CH_2-$, PTMC), 1.22 (d, $(CH_3)_2C-$), 1.22 (d, $(CH_3)_2C-$), 1.03 (s, $-CH_3$, PVSC).

Postpolymerization Modification of VS-Functionalized Biodegradable Polymers. The postpolymerization modification of VS-functionalized polymers was carried out using Michael-type conjugate addition reaction in DMF at room temperature under a nitrogen atmosphere. Various thiol-containing molecules (R-SH) including 2-mercaptoethanol, 2-mercaptoethylamine hydrochloride, L-cysteine, PEG-SH, GC-SH, or GRGDC were employed. The SH/VS molar ratio was set at 2/1, and the reaction proceeded for 1 day. The modified polymers were isolated by precipitation from cold diethyl ether/ethanol (1/4, v/v) and dried in vacuo at room temperature. The ^{1}H NMR spectra of thus modified P(CL-*co*-VSC)8.7% are given in Figure 3. It could be concluded that the modification was quantitative.

Preparation of VS-Functionalized Biodegradable Films and Direct Modification with Thiol-Containing Molecules. Biodegradable films were prepared on the microscope slides using 0.2 wt % solution of VS-functionalized copolymers in chloroform by dip-coating. The films, after thoroughly dried, were immersed in the phosphate buffered aqueous solution of a thiol-containing molecule (such as 2-mercaptoethanol, 2-mercaptoethylamine hydrochloride, L-cysteine, PEG-SH, GC-SH, and GRGDC) at a concentration of 1 mg/mL for 24 h. The resulting modified films were thoroughly rinsed with deionized water and dried over phosphorus pentoxide under reduced pressure. The contact angles of both modified and unmodified films were determined on an SL-200C optical contact angle meter (Solon Information Technology Co.) using the sessile drop method. For XPS analysis, films were prepared on silicon wafers (0.076 Ω/□).

Characterization. ^{1}H NMR spectra were recorded on the Unity Inova 400 operating at 400 MHz. $CDCl_3$ and DMSO-d_6 were used as solvents, and the chemical shifts were calibrated against residual solvent signals. The molecular weight and polydispersity of the copolymers were determined by a Waters 1515 gel permeation chromatograph (GPC) instrument equipped with two linear PLgel columns (500 Å and Mixed-C) following a guard column and a differential refractive-index detector. The measurements were performed using THF as the eluent at a flow rate of 1.0 mL/min at 30 °C and a series of narrow polystyrene standards for the calibration of the columns. The static water contact angle measurements were performed on an SL-200C optical contact angle meter (Solon Information Technology Co.) using the sessile drop method.

X-ray photoelectron spectroscopy (XPS) measurements were carried out on a Kratos AXIS UltraDLD instrument equipped with an evaporation chamber (base pressure <5 × 10^{-10} Torr) and an analysis chamber (3 × 10^{-10} Torr). XPS analysis was undertaken under high vacuum on films prepared on silicon wafers (0.076 Ω/□). The samples were irradiated with monochromatic Al Kα ($h\nu$ = 1486.6 eV, spot size 400 μm × 700 μm) and a takeoff angle of 45° with respect to the sample surface. All spectra were measured at room temperature and calibrated by setting the C 1s (C−C) peak at 284.5 eV.

Fluorescence Observation on Cystamine-Functionalized Films Treated with FITC. To confirm the immobilization of cystamine to VS-functionalized degradable polymer films and to test the chemical reactivity of the amine groups at the surface, cystamine-functionalized PCL film was further treated with FITC in phosphate buffered saline (PBS, 20 mM, pH 9.0) and then visualized with fluorescence microscopy. Briefly, VS-functionalized degradable polymer films following treatment with cystamine as above-described were immersed in 0.5 mg/mL FITC solution in phosphate buffered saline (PBS, 20 mM, pH 9.0, 4 mL) at 37 °C for 24 h in the dark. The films were thoroughly rinsed with deionized water and then visualized using fluorescence microscope (Leica DM4000M).

Cell Culture on Surface Engineered Biodegradable Polymer Films. The influence of surface chemistry on cell adhesion and growth was studied using P(CL-*co*-VSC)8.7% films. The films were prepared and modified with varying thiol-containing molecules including PEG-SH, GC-SH, and GRGDC as above-described. The parent unmodified and modified films were placed into a 6-well tissue culture plate and sterilized by radiation prior to use. MG63 osteoblasts were cultured on the films using Dulbecco's Modified Eagle Medium (DMEM), containing 10% FBS at a density of 1×10^4 cells/well in a humidified 5% CO_2 atmosphere at 37 °C. The culture media was set as 4 mL per well and replaced each day. After 1, 4, and 6 days culture, the cells were observed on an inverted fluorescence microscope (Axiovert 40 CFL microscope equipped with an AxioCam MR3 camera cable).

RESULTS AND DISCUSSION

Synthesis of Vinyl Sulfone Cyclic Carbonate Monomer. To obtain VS-functionalized biodegradable polymers, here we designed and prepared a new cyclic carbonate monomer, vinyl sulfone carbonate (VSC), in four straight steps: (i) 3-methyl-3-oxetanemethanol was treated with HBr to afford bromo-diol; (ii) bromo-diol was reacted with sodium hydrosulfide to yield mercapto-diol; (iii) the Michael-type conjugate addition between mercapto-diol and excess vinyl sulfone gave rise to vinyl sulfone-diol; and (iv) similar to preparation of acid-labile cyclic carbonate monomer,[40] cyclization of vinyl sulfone-diol was performed in the presence of ethyl chloroformate in dilute anhydrous THF solution at 0 °C via dropwise addition of triethylamine (Scheme 1). The resulting VSC monomer was purified by recrystallization from dry THF. ^{1}H NMR spectrum showed clearly signals at δ 6.23−6.64 attributable to VS protons, δ 4.17−4.26 to methylene protons next to the carbonate, δ 3.27 to methylene protons neighboring to the sulfone group, δ 2.95 and 2.75 to methylene protons next to the thiol ether, and δ 1.10 to methyl protons (Figure 1). The integral ratio of resonances at δ 6.23−6.64 (VS protons) and δ 4.17−4.26 (methylene protons next to the carbonate) was close to the theoretical value (3:4), which combined with results of mass and elemental analyses fully confirmed successful synthesis of VSC monomer.

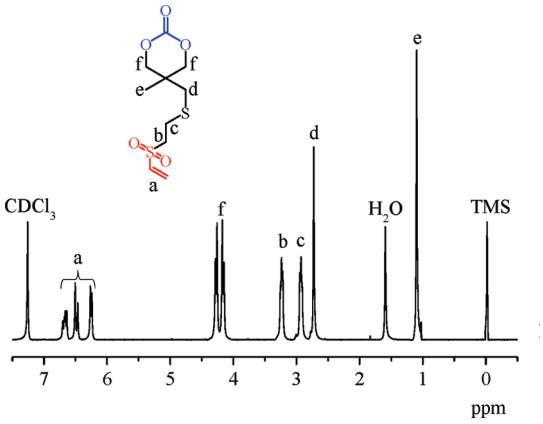
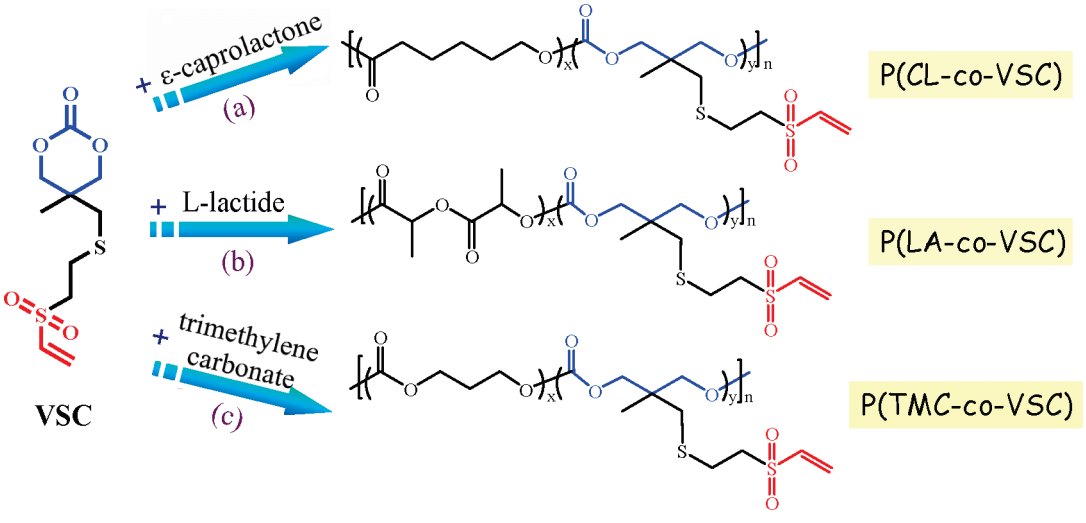


Figure 1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of VSC.

Synthesis of Vinyl Sulfone-Functionalized Biodegradable Polymers. VSC was readily copolymerized with ε-caprolactone (ε-CL) in toluene at 110 °C using isopropanol as an initiator and stannous octoate as a catalyst to afford VS-functionalized poly(ε-caprolactone) (PCL) in good yields (78.6−95.0%) (Scheme 2a). ^{1}H NMR showed resonances of VS protons at δ 6.23−6.64 (Figure 2A), indicating that VS group was intact during copolymerization and subsequent work-up procedures. Notably, copolymer compositions were nicely controlled by the VSC/ε-CL molar feed ratio, in which P(CL-*co*-VSC) copolymers with VSC contents (F_{VSC}) ranging from 4.1 to 34.5 mol % were obtained at VSC monomer in the feed (f_{VSC}) of 5 to 40 mol %, respectively (Table 1). The number-average molecular weights (M_n) estimated from ^{1}H NMR end-group analysis were in good agreement with the theoretical data. The M_n values determined by gel permeation chromatography (GPC) measurements using polystyrene as standards were shown to increase in proportion to the monomer-to-initiator ratios (Table 1, entries 1−3). These P(CL-*co*-VSC) copolymers had moderate polydispersities (PDI) of 1.34−1.81 (Table 1).

In the same way, we synthesized VS-functionalized poly(L-lactide) (PLA) (Scheme 2b) and VS-functionalized

Scheme 2. Synthesis of VS-Functionalized Biodegradable Polymers Using Isopropanol as an Initiator and Stannous Octoate as a Catalyst in Toluene at 110 °C: (a) VS-Functionalized Poly(ε-caprolactone), (b) VS-Functionalized Poly(L-lactide), and (c) VS-Functionalized Poly(trimethylene carbonate)



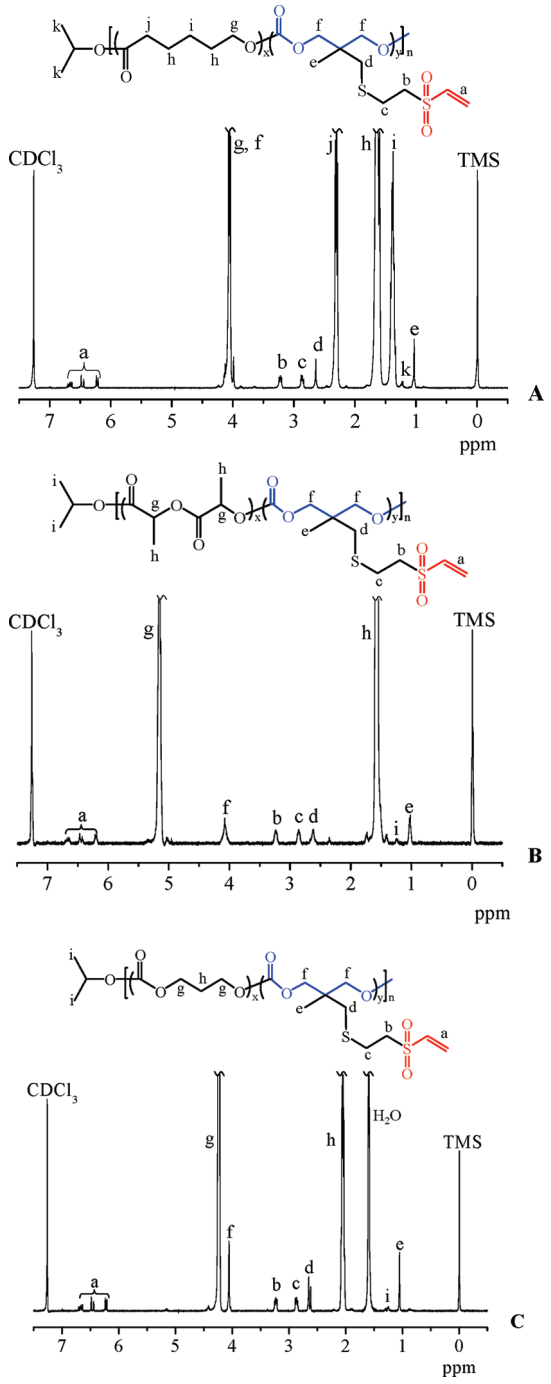


Figure 2. ^{1}H NMR spectra (400 MHz, $CDCl_3$) of VS-functionalized biodegradable polymers: (A) P(CL-*co*-VSC), (B) P(LA-*co*-VSC), and (C) P(TMC-*co*-VSC).

poly(trimethylene carbonate) (PTMC) (Scheme 2c). ^{1}H NMR spectra corroborated successful incorporation of VSC moieties into PLA and PTMC (Figure 2B,C). The VSC contents in P(LA-*co*-VSC) copolymers were determined to be about 3.5 and 7.4 mol % at f_{VSC} of 5 and 10 mol %, respectively (Table 2, entries 1−3). Very similarly, for P(TMC-*co*-VSC) copolymer, a VSC content of 3.5 mol % was obtained at f_{VSC} of 5 mol % (Table 2, entry 4). The M_n data determined by GPC were in general higher than those determined by ^{1}H NMR (Table 2), which is most likely due to use of polystyrene standards for molecular weight

Table 1. Synthesis of VS-Functionalized Biodegradable PCL through Ring-Opening Copolymerization of ε-CL and VSC[a]

				$M_n \times 10^{-3}$				
		f_{VSC}[c]	F_{VSC}[d]	^{1}H			PDI	yield
entry	M/I[b]	(%)	(%)	theory	NMR[e]	GPC[f]	GPC[f]	(%)
1	100/1	5	4.8	12.2	11.3	17.7	1.78	84.8
2	160/1	5	4.2	20.9	19.8	26.4	1.34	91.5
3	320/1	5	4.1	39.1	36.4	46.7	1.66	95.0
4	150/1	10	8.7	19.6	18.1	21.0	1.50	80.0
5	150/1	20	16.3	22.0	19.4	21.3	1.62	81.4
6	120/1	40	34.5	21.6	19.5	24.0	1.80	78.6

[a]The copolymerization was carried out in toluene at 110 °C using isopropanol as an initiator and $Sn(Oct)_2$ as the catalyst for 1 day. [b] Total monomer-to-initiator molar ratio. [c] Molar fraction of VSC monomer in the feed. [d] Molar fraction of VSC units in the resulting copolymer determined by ^{1}H NMR. [e] Estimated by ^{1}H NMR end-group analysis. [f] Determined by GPC (eluent: THF, flow rate: 1.0 mL/min, standards: polystyrene).

calibration in our GPC measurements. Therefore, through copolymerization with VSC monomer, a range of VS-functionalized biodegradable polymers can be conveniently prepared with controlled functionalities.

Modification of VS-Functionalized Biodegradable Polymers or Coatings. The VS groups in the biodegradable polymers allowed facile modification, without aid of a catalyst, with various thiol-containing molecules including 2-mercaptoethanol, cystamine, cysteine, GRGDC, and PEG-SH at a ligand-SH/VS molar ratio of 2/1 under mild conditions (Scheme 3). ^{1}H NMR analysis displayed complete vanishing of signals assignable to the VS protons and appearance of new peaks due to the conjugated ligands with corresponding intensities (Figure 3), indicating quantitative conjugation. These results confirm that the Michael-type conjugate addition to VS is highly selective and tolerant to most other functional groups including hydroxyl, carboxyl, and amine.

In the next, we tried to prepare functional biodegradable coatings by directly treating VS-functionalized PCL films with thiol-containing water-soluble molecules under aqueous conditions. Remarkably, results of the contact angle measurements demonstrated that VS-functionalized PCL films following treatment with 2-mercaptoethanol, cystamine, cysteine, thiolated glycol chitosan, GRGDC, or thiolated PEG all became significantly more hydrophilic, in which significant reduction of contact angles by 13°−24° was observed depending on the nature of thiol-containing molecules (Figure 4). In contrast, no change of contact angles was detected for the control films of acryloyl-functionalized PCL under otherwise the same conditions (data not shown). These results strongly suggest that VS-functionalized biodegradable coatings are amenable to facile derivatization with thiol-containing ligands.

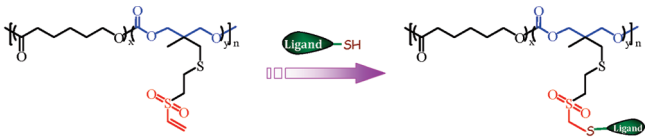
To confirm the covalent immobilization of thiol-containing molecules, VS-functionalized PCL films fabricated on a supporting silicon substrate before and after treatment with cystamine were analyzed with X-ray photoelectron spectroscopy (XPS). The XPS spectrum of VS-functionalized PCL films after treating with cystamine displayed besides prominent carbon (around 284.5 eV) and oxygen signals (around 532.4 eV) also two sets of peaks at around 399.5, 401.5 eV and around 163.7, 164.4, 167.5 eV due to N−(C,H) nitrogen unique to the cystamine moieties

Table 2. Synthesis of VS-Functionalized PLA and PTMC through Ring-Opening Copolymerization of LA or TMC with VSC[a]

					$M_n \times 10^{-3}$				
entry	copolymer	M/I[b]	f_{VSC}[c] (%)	F_{VSC}[d] (%)	theory	^{1}H NMR[e]	GPC[f]	PDI GPC[f]	yield (%)
1	P(LA-*co*-VSC)	140/1	5	3.5	21.1	20.2	58.3	1.67	91.3
2	P(LA-*co*-VSC)	300/1	5	3.4	45.2	39.8	86.2	1.56	70.6
3	P(LA-*co*-VSC)	130/1	10	7.4	20.5	18.5	40.1	1.71	88.1
4	P(TMC-*co*-VSC)	180/1	5	3.5	19.9	15.1	39.7	1.87	79.0

[a]The copolymerization was carried out in toluene at 110 °C using isopropanol as an initiator and $Sn(Oct)_2$ as the catalyst for 1 day. [b]Total monomer-to-initiator molar ratio. [c]Molar fraction of VSC monomer in the feed. [d]Molar fraction of VSC units in the resulting copolymer determined by ^{1}H NMR. [e]Estimated by ^{1}H NMR end-group analysis. [f]Determined by GPC (eluent: THF, flow rate: 1.0 mL/min, standards: polystyrene).

Scheme 3. Postpolymerization Modifications of P(CL-*co*-VSC)8.7% Copolymer by Michael-Type Addition with Varying Thiol-Containing Molecules (Ligand-SH/VS = 2/1 mol/mol, DMF, rt, 1 day)[a]



Functional Copolymer	Ligand-SH	Functionality (%)[a]
P(CL-co-VSC)(OH)	HS, OH	8.7
P(CL-co-VSC)(NH_2)	HS, NH_2	8.7
P(CL-co-VSC)(cys)	HS, COOH, NH_2	8.7
P(CL-co-VSC)(PEG_{5k})	PEG-SH	8.7
P(CL-co-VSC)(GRGDC)	GRGDC	8.7

[a]Functionality was defined as mole percentage of functional groups relative to CL and VSC units in total.

and S−(C,O) sulfur atom, respectively (Supporting Information, Figure S1A). In comparison, nitrogen atom was not detected for the untreated VS-functionalized PCL control (Supporting Information, Figure S1B). It is evident, therefore, that cystamine has successfully immobilized to the surface.

To test the reactivity of the amine groups at the surface, cystamine-functionalized PCL film was further treated with fluorescein isothiocyanate (FITC) in phosphate buffered saline (PBS, 20 mM, pH 9.0) and then visualized with fluorescence microscopy. Interestingly, strong green fluorescence was observed throughout the whole film (Supporting Information, Figure S2). Notably, the fluorescence intensities were shown to increase with increasing the initial VS contents in the P(CL-*co*-VSC) copolymers (Supporting Information, Figure S2A−C). In contrast, no fluorescence was detected for the VS-functionalized PCL film treated with FITC directly (Supporting Information, Figure S2F). Similarly, the treatment of VS-functionalized PLA or PTMC films with cystamine and FITC resulted in apparent fluorescence (Supporting Information, Figure S2D,E). It appears that PTMC film has stronger fluorescence than PCL and PLA films at comparable VS contents. This is likely due to that the amorphous nature of PTMC facilitates exposure of VS groups to the surface. The above results conclude that biodegradable

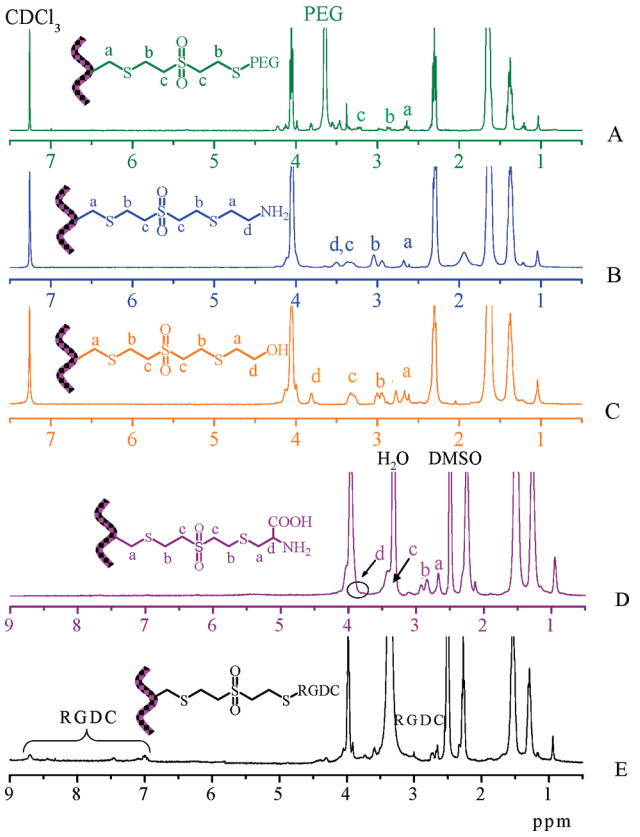


Figure 3. ^{1}H NMR spectra (400 MHz) of P(CL-*co*-AC)8.7% copolymer after modification with thiol-containing molecules: (A) PEG-SH ($CDCl_3$), (B) 2-mercaptoethylamine ($CDCl_3$), (C) 2-mercaptoethanol ($CDCl_3$), (D) L-cysteine (DMSO-d_6), and (E) RGDC (DMSO-d_6).

coatings based on VS-functionalized polymers enable versatile and efficient surface modification under aqueous conditions, likely due to the fact that (i) VS groups are hydrophilic, rendering them readily available at the surface in the aqueous environment, and (ii) VS groups are highly reactive toward the Michael-type conjugate addition reaction under aqueous conditions. Notably, unlike amine- or carboxyl-functionalized counterparts in which the presence of amine or carboxyl groups in the bulk material largely alters their physicochemical properties, VS-functionalized biodegradable polymers showed similar solubility and filming characteristics to the parent polymers without VS functional groups.

Influence of Surface Modification on Cell Culture. It is known that the surface chemistry of biomaterials is of critical

dx.doi.org/10.1021/ma200824k |*Macromolecules* 2011, 44, 6009–6016

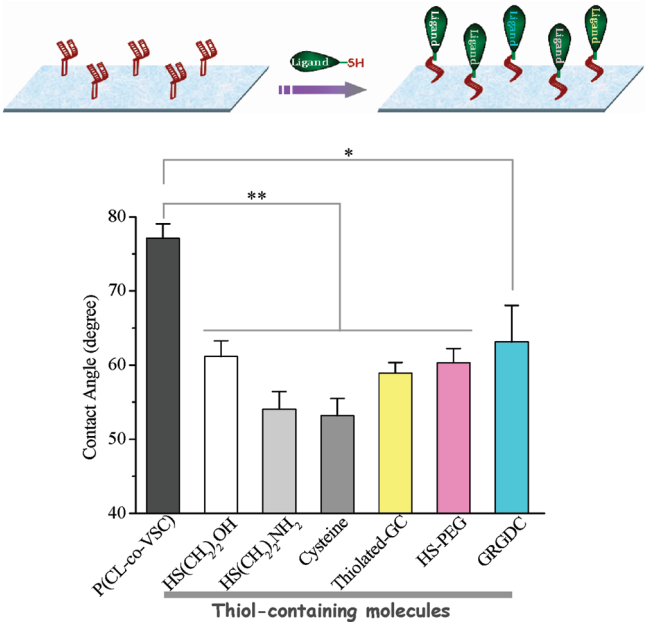


Figure 4. Contact angles of P(CL-*co*-VSC)8.7% copolymer films following postpolymerization modification with varying thiol-containing molecules (Student's *t* test, $^{*}p < 0.05$, $^{**}p < 0.01$).

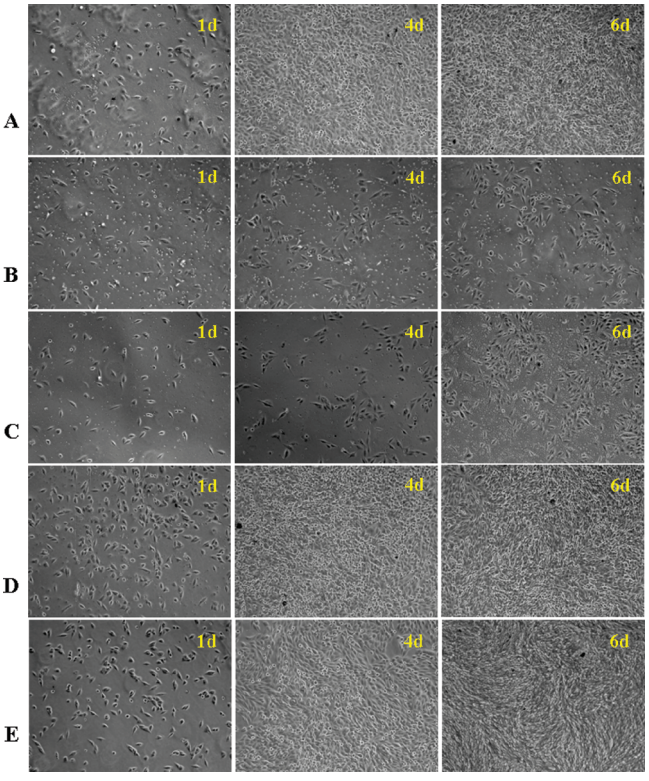


Figure 5. Images (×50) of MG63 cells cultured on the unmodified and modified P(CL-*co*-VSC)8.7% films for 1, 4, and 6 days: (A) unmodified P(CL-*co*-VSC)8.7% film, (B) PEG-SH modified film, (C) GC-SH modified film, (D) RGDC modified film, and (E) tissue culture plastic (blank control).

importance to the *in vivo* performance of medical coatings and devices.[41] Our preliminary cell culture studies using MG6 cells showed that unmodified VS-functionalized PCL films, similar to tissue culture plate, could well support cell attachment and growth (Figure 5A,B), indicating that VS-functionalized PCL film is nontoxic and biocompatible. The VS-functionalized PCL films following treatment with thiolated nonfouling polymers such as PEG and glycol chitosan, however, revealed diminished cell adhesion and growth as compared to the unmodified control (Figure 5C,D). Notably, excellent cell adhesion and growth were observed for VS-functionalized PCL films treated with GRGDC peptide (Figure 5E). These initial results show that the surface of VS-functionalized biodegradable polymer-based biomaterials can be elegantly engineered to meet the demands of a particular application.

CONCLUSIONS

We have demonstrated that novel vinyl sulfone-functionalized biodegradable polymers, which can be readily prepared with vastly different structures and compositions by ring-opening copolymerization with vinyl sulfone cyclic carbonate monomer, provide an unprecedented and robust access to advanced functional biomaterials as well as coatings. To the best of our knowledge, this represents a first report on direct, efficient, and clean (without catalyst and byproduct) modification of biodegradable coatings under aqueous conditions. These vinyl sulfone-functionalized biodegradable polymers open a brand-new avenue to engineering the surface chemistry of biomedical devices and coatings. We are convinced that these vinyl sulfone-functionalized biodegradable polymers have a tremendous potential in biomedical engineering.

ASSOCIATED CONTENT

Supporting Information. XPS data and fluorescence images. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: +86-512-65880098. E-mail: zyzhong@suda.edu.cn.

Author Contributions

†R.W. and W.C. made equal contributions to this work.

ACKNOWLEDGMENT

This work is financially supported by research grants from the National Natural Science Foundation of China (NSFC 50703028, 20974073, 50973078, and 20874070), a Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions, and the Program of Innovative Research Team of Soochow University.

REFERENCES

(1) Huebsch, N.; Mooney, D. J. *Nature* **2009**, *462*, 426–432.
(2) Place, E. S.; Evans, N. D.; Stevens, M. M. *Nature Mater.* **2009**, *8*, 457–470.
(3) Langer, R.; Tirrell, D. A. *Nature* **2004**, *428*, 487–492.
(4) Nair, L. S.; Laurencin, C. T. *Prog. Polym. Sci.* **2007**, *32*, 762–798.
(5) Middleton, J. C.; Tipton, A. J. *Biomaterials* **2000**, *21*, 2335–2346.
(6) Ye, M. L.; Kim, S.; Park, K. *J. Controlled Release* **2010**, *146*, 241–260.

(7) Kim, H. K.; Chung, H. J.; Park, T. G. *J. Controlled Release* **2006**, *112*, 167–174.
(8) Lutolf, M. P.; Hubbell, J. A. *Nature Biotechnol.* **2005**, *23*, 47–55.
(9) Lutolf, M. P.; Gilbert, P. M.; Blau, H. M. *Nature* **2009**, *462*, 433–441.
(10) Nakazawa, G.; Finn, A. V.; Kolodgie, F. D.; Virmani, R. *Expert Rev. Med. Devices* **2009**, *6*, 33–42.
(11) Vasir, J. K.; Labhasetwar, V. *Adv. Drug Delivery Rev.* **2007**, *59*, 718–728.
(12) Soppimath, K. S.; Aminabhavi, T. M.; Kulkarni, A. R.; Rudzinski, W. E. *J. Controlled Release* **2001**, *70*, 1–20.
(13) Jerome, C.; Lecomte, P. *Adv. Drug Delivery Rev.* **2008**, *60*, 1056–1076.
(14) Leemhuis, M.; van Nostrum, C. F.; Kruijtzer, J. A. W.; Zhong, Z. Y.; ten Breteler, M. R.; Dijkstra, P. J.; Feijen, J.; Hennink, W. E. *Macromolecules* **2006**, *39*, 3500–3508.
(15) Zhang, X. J.; Mei, H. J.; Hu, C.; Zhong, Z. L.; Zhuo, R. X. *Macromolecules* **2009**, *42*, 1010–1016.
(16) Trollsas, M.; Lee, V. Y.; Mecerreyes, D.; Lowenhielm, P.; Moller, M.; Miller, R. D.; Hedrick, J. L. *Macromolecules* **2000**, *33*, 4619–4627.
(17) Noga, D. E.; Petrie, T. A.; Kumar, A.; Weck, M.; Garcia, A. J.; Collard, D. M. *Biomacromolecules* **2008**, *9*, 2056–2062.
(18) Seow, W. Y.; Yang, Y. Y. *J. Controlled Release* **2009**, *139*, 40–47.
(19) Zhou, Y.; Zhuo, R. X.; Liu, Z. L. *Macromol. Rapid Commun.* **2005**, *26*, 1309–1314.
(20) Hu, X. L.; Chen, X. S.; Liu, S.; Shi, Q.; Jing, X. B. *J. Polym. Sci., Polym. Chem.* **2008**, *46*, 1852–1861.
(21) He, F.; Wang, Y. P.; Liu, G.; Jia, H. L.; Feng, J.; Zhuo, R. X. *Polymer* **2008**, *49*, 1185–1190.
(22) Pratt, R. C.; Nederberg, F.; Waymouth, R. M.; Hedrick, J. L. *Chem. Commun.* **2008**, 114–116.
(23) Tempelaar, S.; Mespouille, L.; Dubois, P.; Dove, A. P. *Macromolecules* **2011**, *44*, 2084–2091.
(24) Riva, R.; Schmeits, S.; Jerome, C.; Jerome, R.; Lecomte, P. *Macromolecules* **2007**, *40*, 796–803.
(25) Lu, C. H.; Shi, Q.; Chen, X. S.; Lu, T. C.; Xie, Z. G.; Hu, X. L.; Ma, J.; Jing, X. B. *J. Polym. Sci., Polym. Chem.* **2007**, *45*, 3204–3217.
(26) Lecomte, P.; Riva, R.; Jerome, C.; Jerome, R. *Macromol. Rapid Commun.* **2008**, *29*, 982–997.
(27) Xu, J. W.; Prifti, F.; Song, J. *Macromolecules* **2011**, *44*, 2660–2667.
(28) Zhang, X. J.; Zhong, Z. L.; Zhuo, R. X. *Macromolecules* **2011**, *44*, 1755–1759.
(29) Mecerreyes, D.; Humes, J.; Miller, R. D.; Hedrick, J. L.; Detrembleur, C.; Lecomte, P.; Jerome, R.; San Roman, J. *Macromol. Rapid Commun.* **2000**, *21*, 779–784.
(30) Chen, W.; Yang, H. C.; Wang, R.; Cheng, R.; Meng, F. H.; Wei, W. X.; Zhong, Z. Y. *Macromolecules* **2010**, *43*, 201–207.
(31) Vaida, C.; Mela, P.; Keul, H.; Moller, M. *J. Polym. Sci., Polym. Chem.* **2008**, *46*, 6789–6800.
(32) Vaida, C.; Takwa, M.; Martinelle, M.; Hult, K.; Keul, H.; Moller, M. *Macromol. Symp.* **2008**, *272*, 28–38.
(33) Mecerreyes, D.; Lee, V.; Hawker, C. J.; Hedrick, J. L.; Wursch, A.; Volksen, W.; Magbitang, T.; Huang, E.; Miller, R. D. *Adv. Mater.* **2001**, *13*, 204–208.
(34) Gauthier, M. A.; Gibson, M. I.; Klok, H. A. *Angew. Chem., Int. Ed.* **2009**, *48*, 48–58.
(35) Rieger, J.; Van Butsele, K.; Lecomte, P.; Detrembleur, C.; Jerome, R.; Jerome, C. *Chem. Commun.* **2005**, 274–276.
(36) Xiong, J.; Meng, F.; Wang, C.; Cheng, R.; Liu, Z.; Zhong, Z. *J. Mater. Chem.* **2011**, *21*, 5786–5794.
(37) Mather, B. D.; Viswanathan, K.; Miller, K. M.; Long, T. E. *Prog. Polym. Sci.* **2006**, *31*, 487–531.
(38) Cai, S. S.; Liu, Y. C.; Shu, X. Z.; Prestwich, G. D. *Biomaterials* **2005**, *26*, 6054–6067.
(39) Sun, H. L.; Guo, B. N.; Cheng, R.; Meng, F. H.; Liu, H. Y.; Zhong, Z. Y. *Biomaterials* **2009**, *30*, 6358–6366.
(40) Chen, W.; Meng, F.; Li, F.; Ji, S.-J.; Zhong, Z. *Biomacromolecules* **2009**, *10*, 1727–1735.
(41) Ratner, B. D.; Bryant, S. J. *Annu. Rev. Biomed. Eng.* **2004**, *6*, 41–75.