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Versatile Synthesis of Functional Biodegradable Polymers by Combining Ring-Opening Polymerization and Postpolymerization Modification via Michael-Type Addition Reaction

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ABSTRACT: Various functional biodegradable polymers were readily prepared based on novel cyclic carbonate monomers, acryloyl carbonate (AC) and methacryloyl carbonate (MAC), by combining ring-opening polymerization (ROP) and Michael-type conjugate addition. AC and MAC monomers were synthesized in four straightforward steps from 1,1,1-tris(hydroxymethyl)ethane with good overall yields (ca. 40%). AC and MAC were able to copolymerize with ε-caprolactone (ε-CL) and D,L-lactide (LA) in toluene at 110 °C using stannous octoate as a catalyst, yielding biodegradable copolymers with controlled (meth)acryloyl functional groups and molecular weights. The acryloyl groups were amenable to the Michael-type conjugate addition with varying thiol-containing molecules such as 2-mercaptoethanol, 3-mercaptopropanoic acid, cysteamine, cysteine, and arginine-glycine-aspartic acid-cysteine (RGDC) peptide under mild conditions, to provide biodegradable materials with vastly different functionalities (e.g., hydroxyl, carboxyl, amine, amino acid, and peptides) and properties (e.g., hydrophilicity, cell adhesion). Notably, 100% functionalization was achieved with 2-mercaptoethanol, cysteamine and cysteine. Initial cell culture studies demonstrated enhanced cell adhesion and growth on films containing functional RGDC peptides as compared to those of the parent copolymer. Therefore, combination of ROP and Michael-type conjugate addition provides a versatile access to diverse types of functional biodegradable materials.

Introduction

Aliphatic polyesters and polycarbonates, such as poly(ε-carpolactone) (PCL), polylactide (PLA), poly(lactide-*co*-glycolide) (PLGA), and poly(trimethylene carbonate) (PTMC), are among the most important synthetic biodegradable materials.[1−7] These polymers are biocompatible, mechanically viable, degradable in vivo into nontoxic products, and readily processable to fibers, films, rods, microparticles, nanoparticles, and porous three-dimensional constructs.[8−10] In addition to applications as resorbable sutures and in various medical devices, degradable polyesters and polycarbonates are one of the key biomaterials used and/or currently investigated for controlled drug delivery, tissue engineering and regenerative medicine.[11−16]

These degradable polymers are, nevertheless, not ideal. In practice, very often they can not meet the requirements of particular applications, due to their high hydrophobicity, improper degradation profile, and/or lack of reactive centers in the polymer chain for the covalent immobilization of bioactive molecules such as drugs, peptides and proteins.[17,18] While, the ever advancing biomedical technology demands development of complex biologically active biomaterials.[19,20] In the past decade, various functional aliphatic polyesters and polycarbonates containing, e.g., hydroxyl,[21−30] carboxyl,[31−36] amine,[27,32,37,38] and cyclohexene[39] pendant groups have been reported. These functional polymers on one hand show improved physiochemical properties such as enhanced hydrophilicity and biodegradability, and on the other hand facilitate drug conjugation or further derivatization. Their synthesis is, however, usually a multistep process involving protection and deprotection of the functional groups before and after the polymerization, which may result in low overall yields as well as degradation.[3]

Notably, there are several reports on degradable polymers presenting acryloyl,[40−42] allyl,[43−45] or alkyne/azide[46,47] functional groups, in which no protection/deprotection steps is needed and they can readily be transformed into diverse functionalities through postpolymerization modification.[48] In particular, functional PCL containing acryloyl groups reported by Jerome and co-workers is of immense interest in that (1) further derivatization through the Michael-type conjugate addition with thiol-containing molecules is highly selective and is tolerant to a variety of functional groups including hydroxyl, carboxyl, and amine; (2) reaction takes place under very mild conditions, so degradation is minimized; and (3) no catalyst is needed and no side products is produced, thereby precluding possible contaminations.[49] It has been reported, nevertheless, that polymerization of γ-acryloyl-ε-caprolactone (ACL) is associated with significant side reactions.[50,51] The other drawback is that ACL is not able to copolymerize with lactide (LA) monomers.

In this paper, we report on synthesis of a variety of functional biodegradable polymers based on two novel cyclic carbonate monomers, acryloyl carbonate (AC) and methacryloyl carbonate (MAC), by combining ROP and Michael-type conjugate addition. AC and MAC monomers were readily synthesized and copolymerized with ε-CL or LA. The versatile postpolymerization

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modification by Michael-type conjugate addition yielded a range of functional biodegradable materials. The combination of ROP and Michael-type conjugate addition has appeared to be an efficient and practical approach to developing biologically active biomaterials.

Experimental Section

Materials. 1,1,1-Tris(hydroxyl methyl)ethane (THME, Alfa Aesar, 99%), triethylamine (Et_3N, Alfa Aesar, 99%), acryloyl chloride (Alfa Aesar, 96%), and stannous octoate ($Sn(Oct)_2$, 95%, Sigma), 3-mercaptopropanoic acid (Alfa Aesar, 99%), 2-mercaptoethanol (Amresco, >99%), 2-mercaptoethylamine hydrochloride (Alfa Aesar, 99%), and L-cysteine (Alfa Aesar, >99%) were used as received. Benzaldehyde (>99%) and *p*-toluenesulfonic acid monohydrate (TsOH, >99%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (SCRC) and used as received. Methacryloyl chloride was purchased from Wuxi Chemical Reagent Co., Ltd. (Jiangsu, China). RGDC was purchased from Suzhou ChinaTech Peptide Co., Ltd. ε-Caprolactone (ε-CL, Alfa Aesar, 99%) was dried over CaH_2 and distilled under reduced pressure prior to use. D,L-lactide (LA, Purac, >99%) was recrystallized from dried toluene. Tetrahydrofuran (THF) and toluene were dried by refluxing over sodium wire under an argon atmosphere prior to distillation. Dichloromethane (CH_2Cl_2) and isopropanol (SCRC, >99%) were dried by refluxing over CaH_2 under an argon atmosphere. Ethyl chloroformate (SCRC, >96%) was freshly distilled before use.

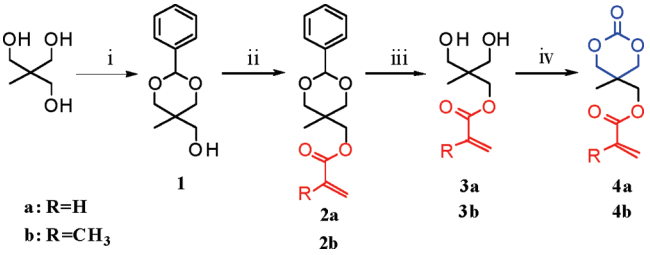
Preparation of Acryloyl Carbonate (AC, 4a) and Methacryloyl Carbonate (MAC, 4b). AC and MAC monomers were synthesized in four steps. The following is a typical example of synthesis of **4a**. To a stirred solution of THME (24 g, 0.2 mol) and TsOH (1.2 g, 6.3 mmol) in THF (375 mL) at room temperature was added dropwise benzaldehyde (21.4 mL, 0.21 mol). After 16 h of reaction, the reaction mixture was neutralized with aqueous ammonia, the solvent was evaporated under reduced pressure, and the residues were dissolved in 150 mL of CH_2Cl_2 and extracted twice with 150 mL of phosphate buffer (pH 7.4). The organic phase was concentrated to yield 39 g (94%) of a colorless powder (**1**). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.81 (s, 3H), 3.66 (d, 2H), 3.91 (s, 2H), 4.06 (d, 2H), 5.44 (s, 1H), 7.35−7.49 (m, 5H).

To a stirred solution of **1** (10 g, 48 mmol) and Et_3N (12 mL, 86.4 mmol) in 150 mL of anhydrous CH_2Cl_2 at 0 °C was added dropwise acryloyl chloride (5.8 mL, 72 mmol) dissolved in CH_2Cl_2. After 4 h reaction at 0 °C, the reaction mixture was filtered. The filtrate was washed twice with phosphate buffer (pH 7.4) and then concentrated to yield crude **2a**. **2a** was dissolved in 160 mL of CH_3OH/1.0 M HCl (v/v 1/1) and stirred at room temperature for 2 h. The solution pH was then adjusted to 7.0 using 2 M NaOH. The solution was concentrated and extracted with ethyl acetate. The organic phase was concentrated to yield crude product **3a**, which was purified by column chromatography (eluent: ethyl acetate/petroleum ether = 1/1, v/v). Yield: 5.07 g (61%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 086 (s, 3H), 2.96 (s, 2H), 3.56 (q, 2H), 4.26 (s, 2H), 5.88−6.42 (m, 3H).

To a stirred solution of **3a** (4 g, 22.9 mmol) and ethyl chloroformate (4.6 mL, 48.09 mmol) in dried THF (150 mL) at 0 °C was added dropwise Et_3N (7 mL, 50.49 mmol) dissolved in THF. After 4 h reaction at 0 °C, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residues were crystallized in diethyl ether to yield **4a**. Yield: 2.94 g (64%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14 (s, 3H), 4.17 (d, 2H), 4.19 (s, 2H), 4.33 (d, 2H), 5.91−6.45 (m, 3H). Anal. Calcd for $C_9H_{12}O_5$: C, 54.00; H, 6.04. Found: C, 53.87; H, 6.06.

In a similar way, **4b** was prepared with an overall yield of 40%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12 (s, 3H), 1.96 (s, 3H), 4.16 (s, 2H), 4.18 (d, 2H), 4.33 (d, 2H), 5.64 (s, 1H), 6.12 (s, 1H). Anal. Calcd for $C_{10}H_{14}O_5$: C, 56.07; H, 6.59. Found: C, 56.18; H, 6.52.

Scheme 1. Synthetic Pathway to AC (4a) and MAC (4b) Monomers[a]



[a] Conditions: (i) benzaldehyde, TsOH, THF, room temperature; (ii) acryloyl chloride (**2a**) or methacryloyl chloride (**2b**), Et_3N, CH_2Cl_2, 0 °C; (iii) HCl (1.0 M), methanol, room temperature; (iv) ethyl chloroformate, Et_3N, THF, 0 °C.

Ring-Opening Copolymerization. The polymerization was carried out in toluene at 110 °C using isopropanol as an initiator and $Sn(Oct)_2$ as a catalyst. The following is a typical example of synthesis of P(CL-*co*-AC) 8.9%. In a glovebox under a nitrogen atmosphere, to a stirred solution of ε-CL (1.539 g, 13.5 mmol) and AC (0.3 g, 1.5 mmol) in toluene (20 mL) was quickly added isopropanol stock solution (0.26 mL, 0.75 M) and $Sn(Oct)_2$ stock solution (1 mL, 0.1 M). 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 polymer P(CL-*co*-AC) 8.9% was isolated by precipitation in cold diethyl ether and dried in vacuo at room temperature.

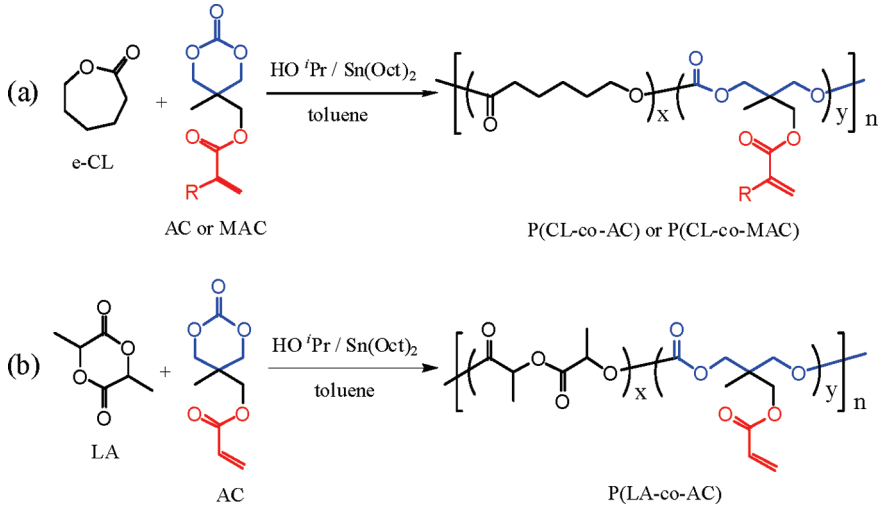
Characterization. ^{1}H NMR spectra were recorded on the Unity Inova 400 and NMRststem (Varian) operating at 400 and 300 MHz, respectively. $CDCl_3$ and DMSO-d_6 were used as solvents and the chemical shifts were calibrated against residual solvent signals. The molecular weight and polydispersity of 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. Contact angle was determined by POWEREACH Instrument (Micaren, JC2000C/X).

Post-Polymerization Modification by a Michael-Type Conjugate Addition Reactions. Michael-type conjugate addition reaction was carried out in DMF at room temperature under a nitrogen atmosphere. The following is a typical example on modification of P(CL-*co*-AC) 8.9%. P(CL-*co*-AC) 8.9%, a thiol-containing molecule (R−SH: 2-mercaptoethanol, 2-mercaptoethylamine hydrochloride, 3-mercaptopropanoic acid or L-cysteine), and pyridine were reacted in DMF at a mole ratio AC/R−SH/pyridine of 1/10/10 at room temperature (Scheme 3). The reaction was allowed to proceed for 2 to 3 d. The resulting functional polymers were isolated by precipitation from cold diethyl ether/ethanol and dried in vacuo at room temperature.

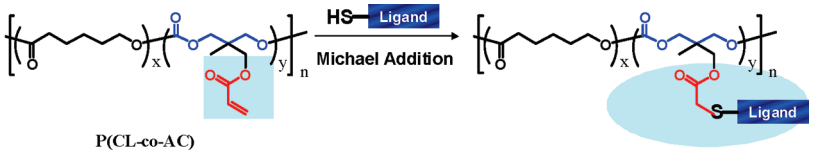
For modification with RGDC, P(CL-*co*-AC) 8.9%, RGDC and pyridine were reacted in DMF at a mole ratio AC/RGDC/pyridine of 1/1/10 at room temperature (Scheme 3). The reaction was allowed to proceed for 7 d. Then, 2-mercaptoethanol (5-fold relative to AC units) was added to react with the remaining AC groups. The reaction was continued for another 2 d (Scheme 3). The resulting RGD functionalized polymer was isolated by precipitation from cold ethanol and dried in vacuo at room temperature.

Preparation of Functional Copolymer Films and Contact Angle Measurements. Thin films were prepared by casting functionalized copolymer solutions in DMF (0.5 wt %) on microscope slides. The films on the slides were dried by placing in a desiccator for 18 h followed by vacuum-drying for 3 days to

Scheme 2. Ring-Opening Copolymerization of (M)AC with ε-CL and LA: (a) ε-CL and (M)AC; (b) LA and AC



Scheme 3. Modifications of P(CL-*co*-AC) 8.9% Copolymer with Thiol-Containing Molecules by Michael-Type Conjugate Addition



Functional copolymer	Ligand	AC/SH [a]	Conv. [b] (%)	Funct. [c] (%)
P(CL-co-AC) $(OH)_{8.9\%}$	OH	1/10	100	8.9
P(CL-co-AC) $(NH_2)_{8.9\%}$	NH_2	1/10	100	8.9
P(CL-co-AC) $(COOH)_{2.7\%}$	COOH	1/10	30	2.7
P(CL-co-AC) $(cys)_{8.9\%}$	NH_2, COOH	1/10	100	8.9
P(CL-co-AC) $(RGDC)_{5.2\%}(OH)_{3.7\%}$	**RGDC** (OH)	1/1/(5)	58 (42)	5.2 (3.7)

remove DMF thoroughly. The static contact angle of the films was measured using a POWEREACH instrument.

Cell Culture. The above-prepared functionalized copolymer coated microscope slides were placed in a 24-well tissue culture plate. The whole plate was sterilized by radiation prior to use. L929 fibroblasts were cultured in Dulbecco's modified Eagle medium (DMEM), containing 10% FBS at a density of 5×10^5 cell/well in a humidified 5% CO_2 atmosphere at 37 °C. The culture media was replaced each day. After 3 d culture, the media was removed, and the cells were rinsed two times with fresh media prior to the microscope observation. To visualize cell nuclei by fluorescence microscopy, the cells were washed three times with PBS, fixed with 4% paraformaldehyde, and stained with Hoechst 33258 (KeyGEN, China). The cells were observed under an inverted microscope (Nikon Eclipse 80i Microscope equipped with a DS camera cable).

Results and Discussion

Synthesis of AC and MAC Monomers. The two functional cyclic carbonate monomers, AC (**4a**) and MAC (**4b**), were synthesized in four steps (Scheme 1). The reaction of THME with benzaldehyde in THF at room temperature in the presence of catalytic amount of TsOH, followed by washing with pH 7.4 phosphate buffer, produced benzylidene-THME (**1**) with high yield. The (meth)acrylation of **1** was subsequently ensued by acid hydrolysis of benzylidene acetal groups, which afforded after column purification monoacryloyl THME (**3a**) and monomethacryloyl THME (**3b**). The structures of **3a** and **3b** were corroborated by ^{1}H NMR.

Next, cyclization was performed in the presence of ethyl chloroformate in dilute anhydrous THF solution at 0 °C via dropwise addition of triethylamine, similar to preparation of acid-labile cyclic carbonate monomer.[52] The resulting cyclic carbonate monomers, AC and MAC, were purified by recrystallization from anhydrous diethyl ether. Notably, moderate overall yields of ca. 40% were obtained for both monomers. The ^{1}H NMR spectra ($CDCl_3$) of AC and MAC as well as the signal assignments are shown in Figure 1. Taking AC monomer as an example, the resonances at δ 1.14 (s), 4.17 and 4.33 (dd), 4.19 (s), and 5.91−6.45 were attributable to the methyl protons, methylene protons next to the carbonate, methylene protons neighboring to the ester, and acryloyl protons, respectively (Figure 1A). Importantly, the integral ratio of signals at δ 1.14 (methyl protons) and 5.91−6.45 (acryloyl protons) was close to the theoretical value (1:1), which combined with results of elemental

Table 1. Synthesis of (Meth)acryloyl Functionalized Biodegradable Copolymers by Ring-Opening Copolymerization[a]

			monomer convn,[c] %			$M_n \times 10^{-3}$			
entry	copolymer	f,[b] %	(M)AC	CL or LA	F,[d] %	theor[e]	^{1}H NMR[f]	GPC[g]	PDI GPC[g]
1	P(CL-*co*-AC) 8.9%	10	88.7	98.8	8.9	9.5	9.7	12.2	1.41
2	P(LA-*co*-AC) 6.1%	10	51.2	87.5	6.1	9.9	9.9	14.0	1.26
3	P(CL-*co*-MAC) 7.6%	10	87.5	99.2	7.6	9.6	11.2	18.5	1.27
4	P(LA-*co*-MAC) 8.6%	10	73.1	85.2	8.6	10.1	10.2	16.3	1.40
5	P(CL-*co*-AC) 16%	20	90.0	100	16	10.2	11.5	19.3	1.44
6	P(CL-*co*-AC) 33.3%	40	75.1	100	33.3	10.3	10.3	14.1	1.54
7	P(CL-*co*-MAC) 32.5%	40	70.7	99.8	32.5	10.3	10.2	13.4	1.35

[a] The copolymerization was carried out in toluene at 110 °C using isopropanol as an initiator and $Sn(Oct)_2$ as the catalyst at a total monomer-to-initiator ratio of 80/1. [b] Molar fraction of (M)AC monomer in feed. [c] Determined by ^{1}H NMR. [d] Molar fraction of (M)AC units in the resulting copolymer determined by ^{1}H NMR. [e] Theoretical molecular weights calculated according to the following equation: $M_{n(theor)} = MW_{(M)AC} \times 80 \times f \times convn_{(M)AC} + MW_{CLorLA} \times 80 \times (1 - f) \times convn_{CLorLA}$. [f] Estimated by ^{1}H NMR end-group analysis. [g] Determined by GPC (eluent, THF; flow rate, 1.0 mL/min; standards, polystyrene).

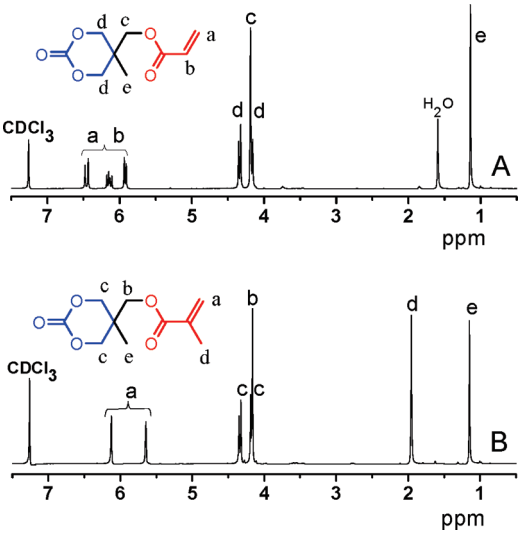


Figure 1. ^{1}H NMR spectra (400 MHz, $CDCl_3$) of AC (A) and MAC (B) monomers.

analysis confirmed successful synthesis of AC monomer. In a similar way, ^{1}H NMR (Figure 1B) and elemental analysis also pointed to successful synthesis of MAC monomer.

Synthesis of Biodegradable Copolymers Containing Acryloyl or Methacryloyl Functional Groups. The aim of this study was to provide a versatile approach to functionalizing biodegradable polymers such as PCL and PLA. Hence, copolymerization behaviors of (M)AC with ε-CL or LA were investigated. The copolymerization was performed in toluene at 110 °C using isopropanol as an initiator and $Sn(Oct)_2$ as a catalyst (Scheme 2). The monomer to initiator mole ratio was set at 80/1 and (M)AC monomer in feed was 10 mol %. The results showed that copolymerization of (M)AC with both ε-CL and LA went smoothly affording copolymers with controlled molecular weights and moderate polydispersities (PDI = 1.26−1.54) (Table 1).

Importantly, ^{1}H NMR displayed clearly resonances at δ 5.6−6.4 attributable to intact acryloyl protons (Figure 2, parts A and B) and at δ 5.6−6.1 assignable to intact methacryloyl protons (Figure 2C) for copolymers of AC and MAC, respectively. The composition of the copolymers could be determined by comparing integrals of signals of (meth)acryloyl protons with those of PCL methylene protons at δ 2.30 or PLA methine proton at δ 5.16. Notably, P(CL-*co*-AC) chains contained 8.9 mol % of AC units, close to the feed of 10 mol % (Table 1, entry 1). This copolymer is accordingly denoted as P(CL-*co*-AC) 8.9%. The copolymerization of AC with LA yielded copolymers with 6.1 mol % AC (Table 1, entry 2). This is in contrast to acryloyl

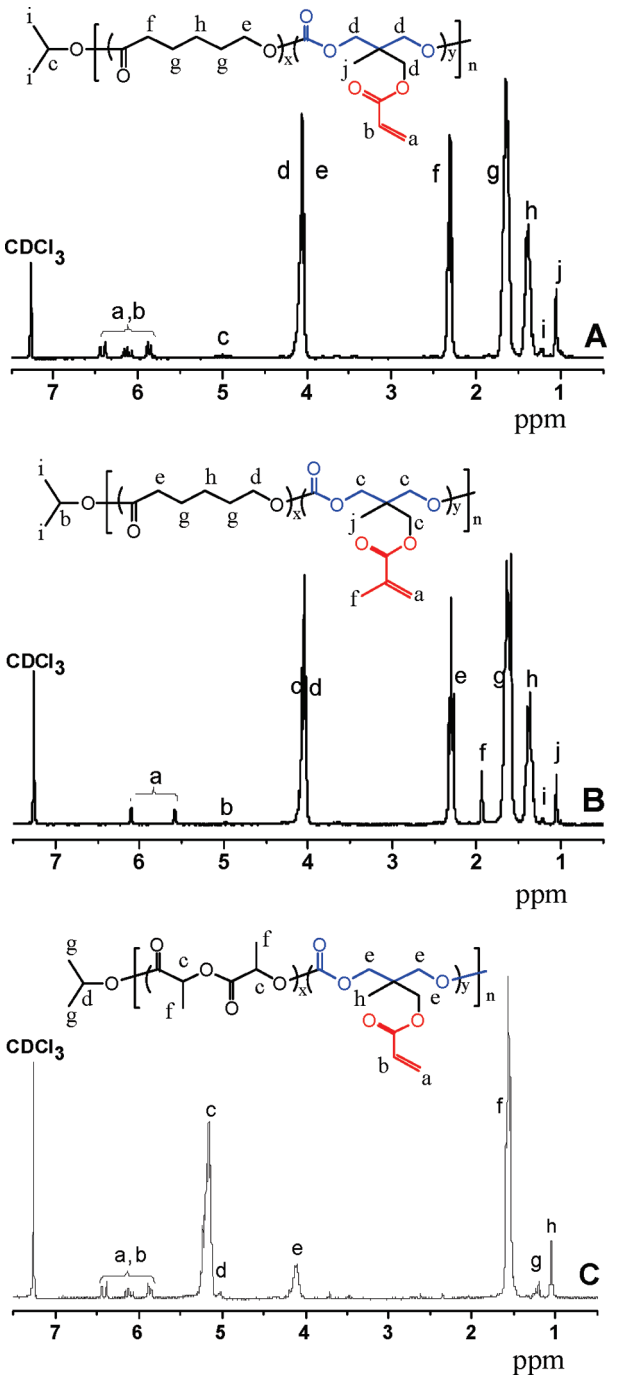


Figure 2. ^{1}H NMR spectra (300 MHz, $CDCl_3$) of (M)AC copolymers: (A) P(CL-*co*-AC); (B) P(CL-*co*-MAC); (C) P(LA-*co*-AC).

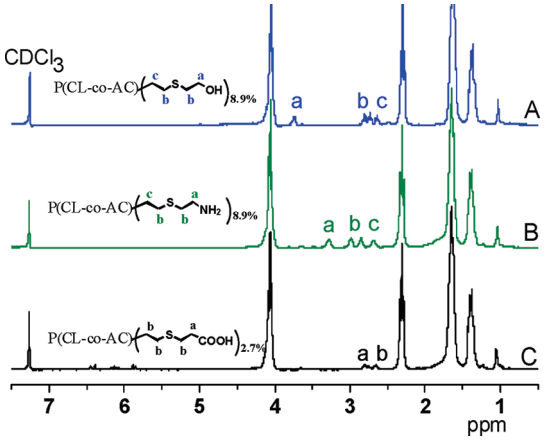


Figure 3. ^{1}H NMR spectra (300 MHz, $CDCl_3$) of P(CL-*co*-AC) 8.9% copolymer modified with 2-mercaptoethanol (A), 2-mercaptoethylamine (B), and 3-mercaptopropanoic acid (C).

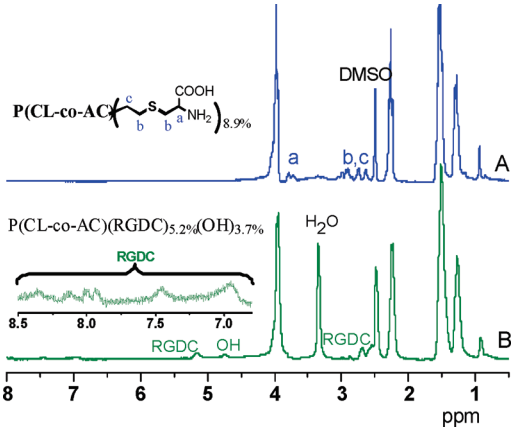


Figure 4. ^{1} H NMR spectra (300 MHz, DMSO-d_6) of P(CL-*co*-AC) 8.9% copolymer modified with L-cysteine (A) and RGDC peptide (B).

ε-CL monomer reported by Jerome and co-workers,[40,49] wherein copolymerization with LA is generally not feasible. The capability of copolymerization with LA renders these functional cyclic carbonate monomers highly attractive for diverse biomedical applications. Similarly, copolymerization of MAC with ε-CL and LA afforded P(CL-*co*-MAC) containing 7.6 mol % MAC (Table 1, entry 3) and P(LA-*co*-MAC) containing 8.6 mol % MAC (Table 1, entry 4), respectively. It should be noted that at higher feed ratios of 20 and 40 mol % AC monomer, P(CL-*co*-AC) copolymers with 16 mol % and 33.3 mol % AC units were obtained (Table 1, entries 5 and 6). The copolymerization of MAC and LA at a high feed ratio of 40 mol % MAC monomer afforded P(LA-*co*-MAC) copolymer with 32.5 mol % MAC (Table 1, entry 7). ^{1}H NMR end-group analysis revealed that all copolymers had molecular weights close to the theoretical values (Table 1). The M_n data determined by GPC were in general higher than those determined by ^{1}H NMR (Table 1), which is most likely due to use of polystyrene standards for molecular weight calibration in our GPC measurements. It is evident, therefore, that both (meth)acryloyl functionality and molecular weights of these functional copolymers could be readily controlled.

Modification of Copolymers by Michael-Type Conjugate Addition. To investigate whether acryloyl functional groups are amenable to derivatization by Michael-type conjugate addition, P(CL-*co*-AC) 8.9% was reacted under a nitrogen atmosphere with varying thiol-containing molecules such as 2-mercaptoethanol, cysteamine, and 3-mercaptopropanoic acid. The reaction was performed in DMF at room temperature for 2 to 3 d at an AC/R−SH/pyridine mole ratio of 1/10/10 (Scheme 3). Remarkably, ^{1}H NMR revealed complete disappearance of peaks assignable to acryloyl groups and occurrence of new signals corresponding to 2-mercaptoethanol and cysteamine moieties, respectively (Figure 3, parts A and B), indicating 100% functionalization with 2-mercaptoethanol and cysteamine (denoted as P(CL-*co*-AC)$(OH)_{8.9\%}$ and P(CL-*co*-AC)$(NH_2)_{8.9\%}$, respectively). The treatment of P(CL-*co*-AC) 8.9% with 3-mercaptopropanoic acid, however, yielded only 30% conversion of acryloyl groups, to give P(CL-*co*-AC)$(COOH)_{2.7\%}$ (Figure 3C). This is in line with a report by Hubbell and co-workers that neighboring carboxylic groups greatly decrease the reactivity of thiol molecules toward Michael-type conjugate addition modification.[53] It should be noted that the GPC curves of all modified copolymers remain unimodal with similar PDI to the parent copolymer, indicating minimal degradation during modification.

Michael-type conjugate addition is appealing for postpolymerization modification because its reaction condition is extremely mild and different bioactive molecules including peptides and proteins may readily be immobilized through their cysteine moieties.[54−56] In the following, modification of P(CL-*co*-AC) 8.9% with cysteine (an amino acid) or RGDC peptide was undertaken (Scheme 3). Interestingly, 100% functionalization was observed for modification with cysteine under the same reaction conditions as described above to afford P(CL-*co*-AC)$(cys)_{8.9\%}$, as shown by ^{1}H NMR (Figure 4A). The modification of P(LA-*co*-AC) 6.1% with cysteine also resulted in quantitative functionalization, yielding P(LA-*co*-AC)$(cys)_{6.1\%}$. The reaction with RGDC peptide was slightly different, in which AC/RGDC/pyridine mole ratio was set at 1/1/10 and after 7 d reaction 2-mercaptoethanol (5-fold relative to AC units) was added to consume the remaining AC groups. Markedly, ^{1}H NMR showed that RGDC has successfully been conjugated to the copolymer with 58% RGDC functionalization (Figure 4B). The remaining AC units were completely derivatized with 2-mercaptoethanol, yielding P(CL-*co*-AC)$(RGDC)_{5.2\%}(OH)_{3.7\%}$.

Therefore, combination of ROP and postpolymerization modification by Michael-type conjugate addition offers a direct and versatile access to functional biodegradable materials with vastly different functionalities including hydroxyl, amino, carboxyl, amino acid, and peptides.

Films of Functional Biodegradable Copolymers. Thin films of functionalized copolymers were prepared on microscope slides using 0.5 wt % copolymer solution in DMF. The static contact angle measurements demonstrated that P(CL-*co*-AC) 8.9% modified with 2-mercaptoethanol, cysteamine, cysteine and RGDC, except 3-mercaptopropanoic acid, all displayed increased hydrophilicity as compared to the parent copolymer (Figure 5). The negligible change of hydrophilicity of P(CL-*co*-AC) 8.9% modified with 3-mercaptopropanoic acid is most likely due to its low functionalization. In a similar way, increased hydrophilicity was also observed for cysteine-modified P(LA-*co*-AC) 6.1% copolymer (Figure 5), though decrease of contact angle was to a less extent than cysteine-modified P(CL-*co*-AC) 8.9%.

Initial cell culture studies were performed on the thin films of functional copolymers using L929 fibroblasts. Figure 6 shows images of cells after 3 d of culture. The cell nuclei were stained with Hoechst 33258 (blue). It is interesting to note that films of RGD modified P(CL-*co*-AC) supported better cell adhesion and growth as compared to those of the parent copolymer. Importantly, the morphology of the cells as well as their nuclei on RGD modified copolymer films appeared

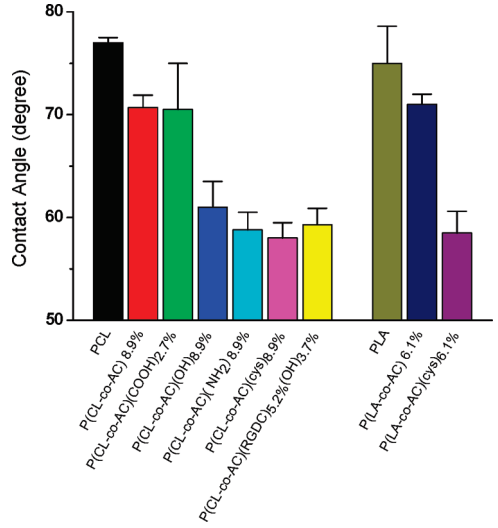


Figure 5. Contact angle measurements of functional copolymer films. Films of PCL (M_n = 12.4K) and PLA (M_n = 12.4K) were used as controls.

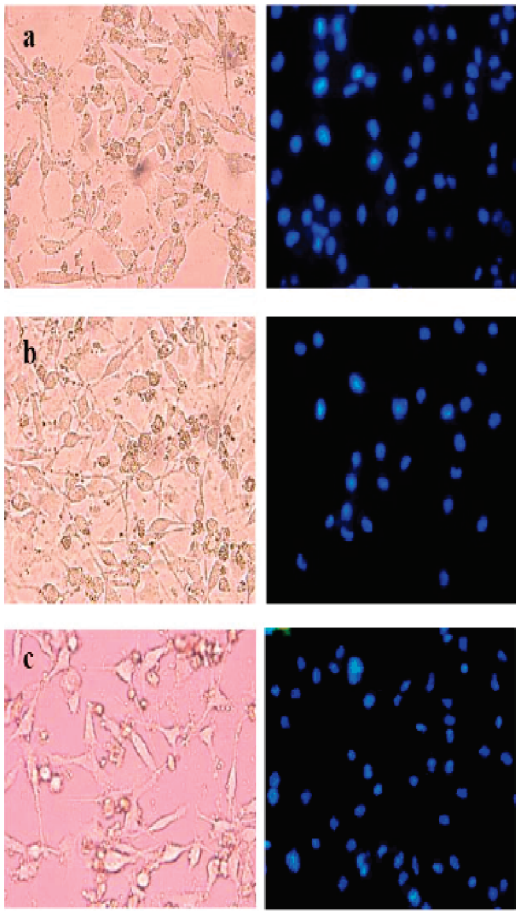


Figure 6. Images (×400) of L929 cells (left panel) and their nuclei stained with Hoechst 33258 (blue, right panel) after 3 day culture on film of P(CL-*co*-AC)$(RGDC)_{5.2\%}(OH)_{3.7\%}$ (a), film of P(CL-*co*-AC) 8.9% (b), and tissue culture plastic (c).

to be typical of fibroblasts in native tissues. Furthermore, unlike films of P(CL-*co*-AC), unhealthy round-shaped cells were practically absent for films of P(CL-*co*-AC)-$(RGDC)_{5.2\%}(OH)_{3.7\%}$. The cell nuclei counting results showed that RGD modified copolymer films had a comparable number of living cells to tissue culture plastic, while a significantly smaller number of living cells (ca. 63% relative to tissue culture plastic) were observed on P(CL-*co*-AC) films. These results indicated that activities of RGDC peptides maintained over the mild Michael-type conjugate addition reactions. RGD peptides have been widely applied to stimulate cell adhesion (e.g., in tissue engineering applications); however, proper immobilization of RGD peptides on biodegradable materials remains a huge challenge.[57] These (meth)acryloyl cyclic carbonate monomers provide a new entry to anchor peptides and perhaps proteins on synthetic biodegradable polymers for diverse biomedical applications including medical devices, tissue engineering, and drug delivery systems.[17,20,58,59]

Conclusions

We have demonstrated that combination of ring-opening polymerization and Michael-type conjugate addition chemistry represents an elegant and remarkably versatile approach to functionalizing biodegradable copolymers including PCL and PLA. It offers several interesting features: (1) the acryloyl cyclic carbonate monomers are readily prepared with good overall yields; (2) these acryloyl cyclic carbonate monomers are able to copolymerize with both ε-CL and LA, yielding copolymers with controlled acryloyl functional groups and molecular weights; (3) the acryloyl groups in the copolymers can be conveniently modified via Michael-type conjugate addition chemistry to different functionalities including hydroxyl, amine, carboxyl, amino acid, and peptides under extremely mild conditions, which does not involve any metal catalyst, protection−deprotection steps, and potentially toxic byproduct; (4) functionalization with many thiol-containing molecules including 2-mercaptoethanol, cysteamine, and cysteine is quantitative; and (5) RGDC peptides are readily anchored onto the biodegradable materials, resulting in enhanced cell adhesion and growth. We are convinced that combination of ring-opening polymerization and Michael-type conjugate addition has a great potential in developing multifunctional bioactive biomaterials for diverse biomedical applications including medical devices, tissue engineering, regenerative medicine, and drug delivery systems.

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