A Chemoselective Approach to Grafting Biodegradable Polyesters

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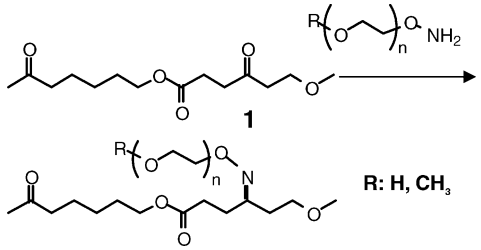
Aliphatic polyesters, such as poly(ϵ-caprolactone) (PCL), poly(L-lactide) (PLLA), and polyglycolide (PGA), are of great interest as biomaterials due to their excellent biodegradability, bioresorbability, and mechanical properties.[1,2] The potential applications of these polymers are further broadened when functional pendant groups are incorporated into the polymer backbones. Various chemical approaches have been developed to present amino,[3,4] carboxyl,[5,6] hydroxyl,[7−9] and sulfhydryl groups[10,11] onto such polyesters via ring-opening polymerization (ROP) of lactones or lactides with the appropriate functional monomer. While useful, these methods generally require a complex multistep synthesis of the protected monomer before polymerization and removal of the protective groups prior to further chemical manipulation, which can result in degradation of the polymer. The extent of copolymerization obtained by ROP of lactones and lactides can also be low due to large differences in monomer reactivity.[6,9,12−17]

Alternatively, the direct coupling of unprotected functional groups to the polymer backbone under mild reaction conditions would circumvent these shortcomings. In this communication, we report a new approach to graft unprotected functional groups onto the backbones of aliphatic polyesters. This approach is based on the selective reaction between a ketone-bearing aliphatic polyester and aminooxy-terminated functional groups. Our interest in using chemoselective coupling to graft functional groups onto aliphatic polyesters is motivated by several considerations: (1) The reactive partners are highly selective and therefore can tolerate the presence of diverse functional groups without using protection chemistries. (2) The reaction occurs rapidly and efficiently under mild reaction conditions, minimizing chemical degradation of the polymer. (3) The two partners react covalently to form a chemically stable linkage.

For this work, we grafted aminooxy-terminated poly(ethylene oxide) (PEO) onto the backbone of ketone-bearing PCL **1** as a demonstration of our approach (Scheme 1). Jérôme and co-workers first described the chemical synthesis of **1** in which the authors reported that reduction of the ketone to the corresponding alcohol gave a functional polyester in high yield.[18−20] Our attempts in the chemical reduction, however, invariably led to degradation of the polymer backbone. This result prompted us to consider an alternate route for functionalizing the ketone-bearing polyester of Jérôme using the chemoselective reaction of ketones with the aminooxy terminal groups of modified PEO. We chose PEO for our model reaction because its terminal OH group provides opportunity for further functionalization, while PEO itself is known to inhibit protein adsorption, reducing inflammatory response. The combination of these two properties can extend the spectrum of applications of biodegradable polyesters and enhance their performance in therapeutic devices.

Scheme 1. Synthetic Pathway for PCL-*graft*-PEO



Aminooxy-terminated PEOs[21] of lengths ranging from 3 to 45 ethylene glycol units were prepared following the reaction shown in Scheme 2.[22] The PCL backbone, a ketone-bearing PCL, was synthesized following methods published by Jérôme and co-workers,[18−20] except using tin 2-ethylhexanoate as a catalyst in ROP of ϵ-CL and 1,4,8-trioxaspiro[4,6]-9-undecanone to achieve a high molecular weight PCL derivative (>100 kg/mol).[23] The incorporation ratio of 1,4,8-trioxaspiro[4,6]-9-undecanone to ϵ-CL in the resulting polymer and weight-average molecular weight (M_w) of the polymer were well controlled from 6.7 to 31.2 mol % and from 30 to 230 kg/mol, respectively. Molecular weight was determined by size exclusion chromatography (SEC) based on polystyrene standards.

Chemoselective coupling was performed by simply mixing the ketone-bearing PCL and aminooxy-terminated PEO in chloroform at various stoichiometries and temperatures. After a predetermined time, the product was recovered by reprecipitation in cold methanol, which efficiently removed any unreacted PEO. Figure 1 shows the ^{1}H NMR spectra of ketone-bearing PCL (M_w: 30 kg/mol; M_w/M_n: 1.3; ketone: 30 mol %)[23] before and after reaction with monoaminooxy-terminated hexaethylene glycol (MW: 297 g/mol).[22] In this case, the stoichiometry of aminooxy groups to ketone was 3:1, and the coupling was carried out at 25 °C for 3 days. PCL-*graft*-PEO comb copolymer with 43.9 wt % PEO was obtained. The peaks of ϵ-methylene protons of ketone-bearing ϵ-CL units and methylene protons adjacent to the aminooxy terminus of PEO shift from 4.35 to 4.25 ppm and 3.82 to 4.15 ppm, respectively. However, since the peaks of ϵ-methylene protons of the PCL chain end ϵ-CL units (4.1−4.3 ppm) overlap with those peaks, the

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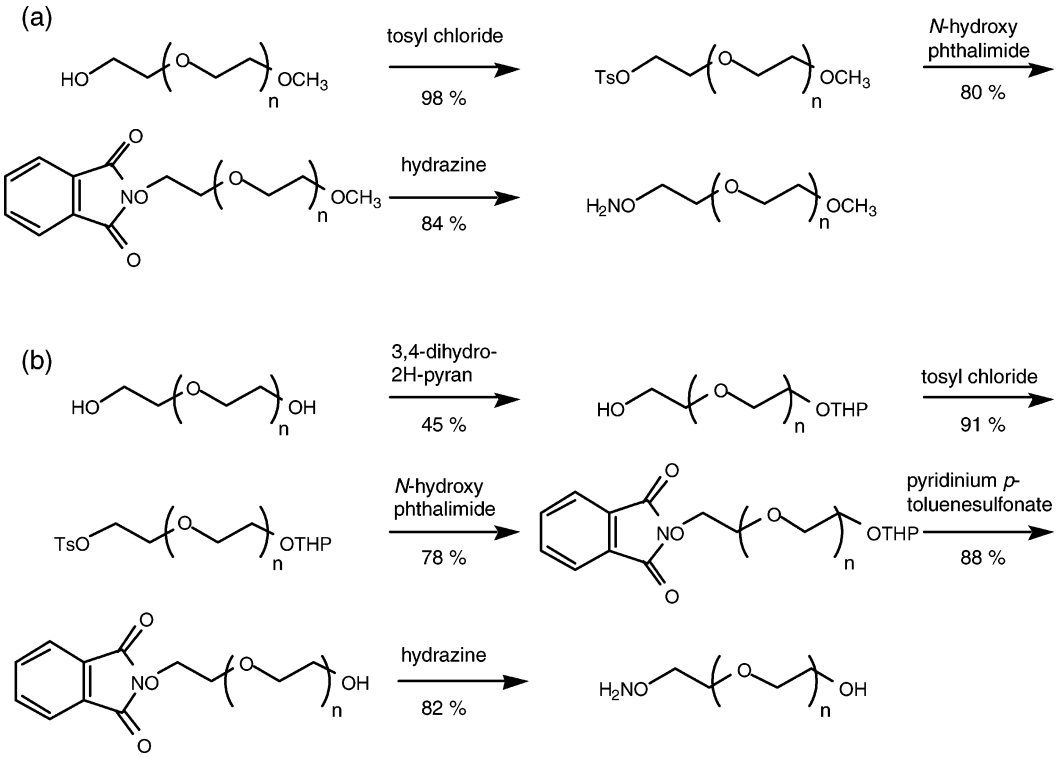
Scheme 2. Synthetic Pathways for (a) Aminooxy-Terminated Methoxy-PEO and (b) Monoaminooxy-Terminated Hexaethylene Glycol



coupling was characterized by monitoring the chemical shifts for the protons corresponding to the two methylene groups adjacent to the ketone before (2.7−2.9 ppm) and after (2.5−2.7 ppm) chemoselective coupling. The coupling reaction was further confirmed by ^{13}C NMR (Supporting Information). The peak of the ketone carbon at 206 ppm decreases while two peaks at 155 ppm emerge, which can be assigned to the oxime carbon, split due to the two geometric isomers of the C=N−O group. Fourier transfer infrared spectroscopy (FT-IR) also revealed the formation of oxime by the coupling reaction (Supporting Information). The ketone stretching peak at 1716 cm^{-1} shifted to 1640 cm^{-1}, consistent with the stretching of the C=N bond. Ketone groups in the PCL backbone react exclusively with the oxyamine even in the presence of unprotected hydroxyl groups, allowing the preparation of functionalizable PEO-grafted PCL.

The coupling efficiency was investigated in various ONH_2:C=O molar ratios from 1 to 25 at 25 or 40 °C. The polymer concentration in chloroform was kept at 5.0 wt %. As expected, the coupling efficiency is affected by both the stoichiometry and temperature of the reaction, as shown in Figure 2. The coupling rate increased with increase in the reaction temperature. With excess of aminooxy-terminated PEO, the coupling was completed in a day. The results obtained show that the coupling proceeds efficiently even at room temperature.

SEC results suggest that no degradation of the polymer backbone occurs during the coupling reaction. The values of M_w and polydispersity index (M_w/M_n) of the polymer fraction monitored by refractive index before (233 kg/mol and 1.9) and after (237 kg/mol and 1.8) the coupling were nearly identical (Supporting Information). This conclusion is further supported by the ^1H NMR spectrum of a 30 kg/mol PCL-*g*-PEO prepared with $Al(O^iPr)_3$.[11−13,23] The peak ratio of the chain end methyl group at 1.24 ppm and other protons of the polymer backbone did not change during the coupling reaction.

Thermal stability of the comb copolymer was investigated by ^1H NMR and thermal gravimetric analysis (TGA). Heat treatment in air at 100 °C for 10 min resulted in no changes to the ^1H NMR spectrum. TGA also revealed higher thermal stability of PCL-*g*-PEO than the pregrafted polymer (Supporting Information), showing 0.6% weight loss at 200 °C, and 5% at 290 °C, compared to 5% at 260 °C for the parent polyester.

Several methods to tether PEO side chains onto biodegradable polyesters have been published.[9,24−27] However, the molecular weights of the obtained graft copolymers were usually low.[9,24,25,27] Because of severe reaction conditions, chain cleavage of the backbone might be expected.[26] In addition, such synthetic strategies typically exclude the possibility for further modification of the free PEO end. By using this chemoselective technique, PCL-*g*-PEO biodegradable comb copolymers with controlled PEO content can be prepared. In addition, the molecular weights of both side chains and backbone are simultaneously well controlled with suitable combinations of PCL backbone and aminooxy-terminated PEO. PCL-*g*-PEO copolymers containing up to 70 wt % PEO have been synthesized. When the PEO content exceeds 50 wt %, however, the resulting material becomes soluble in both water and methanol, and thus separation of excess PEO is difficult.

In conclusion, PEO side chains have been grafted to ketone-bearing polyesters through a chemoselective reaction, yielding biodegradable amphiphilic comb polyesters of well-defined chemical structure under mild synthetic conditions. The chemoselective approach presented here illustrates a general route to chemically introducing functional pendant groups onto ketone-bearing polyesters obtained by ROP. The selectivity for

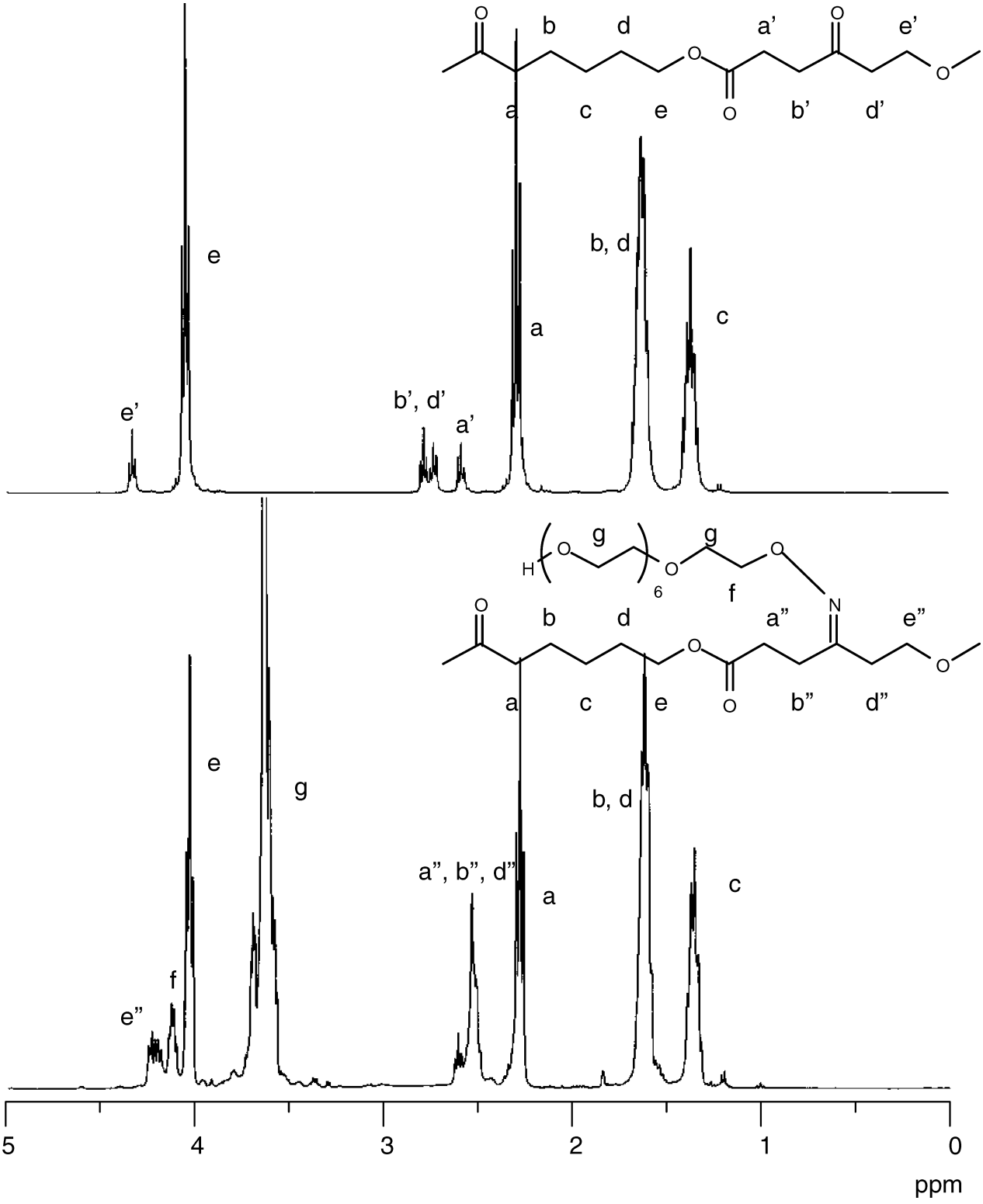


Figure 1. ^{1}H NMR spectra of PCL backbone (upper) and PCL backbone reacted with monoaminooxy-terminated hexaethylene glycol (bottom).

the coupling between ketones and aminooxy groups, even in the presence of other potentially reactive groups such as hydroxyls, is highly useful for the syntheses of biodegradable combs with bioinert side chains that can be further functionalized with cell-signaling peptides for a desired biological response.[28−30]

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Supporting Information Available: SEC traces, ^{13}C NMR spectra, FT-IR spectra, and TGA traces of parent PCL and PCL-*g*-PEO. This material is available free of charge via the Internet at http://pubs.acs.org.

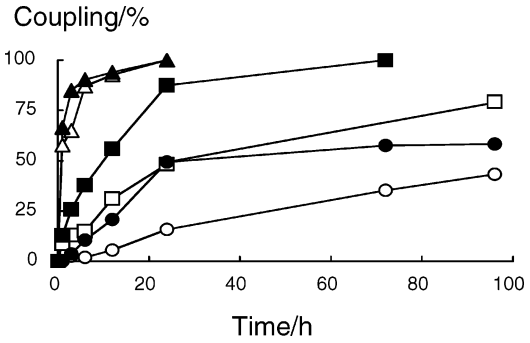

Figure 2. Effect of stoichiometry and temperature on aminooxy-terminated PEO (MW: 750) coupling as a function of time at 25 °C (open symbols) and 40 °C (filled symbols). Stoichiometry: aminooxy group/ketone = 1 (circles), 3 (squares), and 25 (triangles).

References and Notes

(1) Chasin, M.; Langer, R. *Biodegradable Polymers as Drug Delivery Systems*; Marcel Dekker: New York, 1990.
(2) Shalaby, S. W.; Burg, K. J. L. *Absorbable and Biodegradable Polymers*; CRC Press: Boca Raton, FL, 2004.
(3) Barrera, D. A.; Zylstra, E.; Lansbury, P. T.; Langer, R. *J. Am. Chem. Soc.* **1993**, *115*, 11010.
(4) Barrera, D. A.; Zylstra, E.; Lansbury, P. T.; Langer, R. *Macromolecules* **1995**, *28*, 425.
(5) Vert, M.; Lenz, R. W. *Polym. Prepr.* (*Am. Chem. Soc., Div. Polym. Chem.*) **1979**, *20*, 608.
(6) Kimura, Y.; Shirotani, K.; Yamane, H.; Kitao, T. *Macromolecules* **1988**, *21*, 3338.
(7) Chiellini, E.; Solaro, R. *Macrimol. Symp.* **1992**, *54/55*, 483.
(8) Stassin, F.; Halleux, O.; Dubois, P.; Detrembleur, C.; Lecomte, P.; Jérôme, R. *Macromol. Symp.* **2000**, *153*, 27.
(9) Parrish, B.; Emrick, T. *Macromolecules* **2004**, *37*, 5863.
(10) Ni, Q.; Yu, L. P. *J. Am. Chem. Soc.* **1998**, *120*, 1645.
(11) Carrot, G.; Hilborn, J. G.; Trollsas, M.; Hedrick, J. L. *Macromolecules* **1999**, *32*, 5264.
(12) Ouchi, T.; Fujino, A. *Makromol. Chem.* **1989**, *190*, 1523.
(13) Kimura, Y.; Shirotani, K.; Yamane, H.; Kitao, T. *Polymer* **1993**, *34*, 1741.
(14) Vert, M.; Lenz, R. W. *ACS. Polym. Prepr.* **1979**, *20*, 608.
(15) Cammas, M. S.; Guerin, P. *Macromol. Symp.* **2000**, *153*, 167.
(16) Bizzarri, R.; Chiellini, F.; Solaro, R.; Chiellini, E.; Cammas, M. S.; Guerin, P. *Macromolecules* **2002**, *35*, 1215.
(17) Zhou, Q. X.; Kohn, J. *Macromolecules* **1990**, *23*, 3399.
(18) Tian, D.; Dubois, P.; Grandfils, C.; Jérôme, R. *Macromolecules* **1997**, *30*, 406.
(19) Tian, D.; Dubois, P.; Jérôme, R. *Macromolecules* **1997**, *30*, 1947.
(20) Tian, D.; Dubois, P.; Jérôme, R. *Macromolecules* **1997**, *30*, 2575.
(21) Aminooxy-terminated PEO is preferred over commercially available amine terminated PEO. While the coupling of amine to ketone is known, the imine product is highly unstable and susceptible to hydrolysis. Furthermore, the reaction requires the use of base as a catalyst, which can lead to degradation of the polyester backbone. Using aminooxy-terminated PEO circumvents these shortcomings by forming a chemically stable oxime bond with the ketone without the use of a base.
(22) Representative synthetic procedures: 3 g of methoxy-PEO (MW: 750, 4.0 mmol) was reacted with 1.14 g of *p*-toluenesulfonyl chloride (tosyl chloride) (6.0 mmol) and 0.84 mL of triethylamine (6.0 mmol) in dichloromethane for 5 h at 0 °C. The product was purified by filtration followed by reprecipitation in diethyl ether. ^{1}H NMR (400 MHz in $CDCl_3$), δ (ppm): 2.32 (s, 3H, Ar−CH_3), 3.35 (s, 3H, CH_3), 3.5−3.8 (m, 62H, CH_2CH_2O), 4.14 (t, 2H, CH_2OSO_2Ar), 7.12 (d, 2H, Ar), 7.76 (d, 2H, Ar). Tosylated PEO derivative (2.0 g, 2.2 mmol) was reacted with *N*-hydroxyphthalimide (0.54 g, 3.3 mmol) and triethylamine (0.46 mL, 3.3 mmol) in THF at 65 °C for 24 h. The product was purified by the same methods as described above. ^{1}H NMR (400 MHz in $CDCl_3$), δ (ppm): 3.35 (s, 3H, CH_3), 3.5−3.8 (m, 62H, CH_2CH_2O), 4.36 (t, 2H, CH_2ON), 7.71 (d, 2H, Ar), 7.83 (d, 2H, Ar). The obtained PEO derivative (2.0 g, 2.2 mmol) was then treated with hydrazine (0.11 mL, 3.3 mmol) in dichloromethane at 25 °C for 8 h. The product was collected by reprecipitation in diethyl ether. ^{1}H NMR (400 MHz in $CDCl_3$), δ (ppm): 3.35 (s, 3H, CH_3), 3.5−3.8 (m, 62H, CH_2CH_2O), 3.82 (t, 2H, CH_2ON). When the molecular weight of methoxy-PEO was less than 750, its derivative was purified by column chromatography. In the case of monoaminooxy-terminated hexaethylene glycol, PEO (MW: 282) was at first reacted with 3,4-dihydro-2*H*-pyran, and monoprotected PEO was isolated by column chromatography. The protecting group was removed by adding pyridinium *p*-toluenesulfonate after tosylation of the hydroxyl group (Scheme 2b).
(23) Lower molecular weight (<100 kg/mol) PCL derivatives were prepared with aluminum isopropoxide.[11−13] For higher molecular weight PCL derivatives, typical synthetic procedures were as follows. The ring-opening copolymerization of ϵ-CL (9.3 g, 81.6 mmol) and 1,4,8-trioxaspiro[4,6]-9-undecanone (1.0 g, 5.8 mmol) was carried out with tin octoate (40 mg, 100 μmol) at 110 °C in 5 mL of toluene for 24 h. After polymerization, an excess of 1 N HCl was added to terminate polymerization, and the copolymer was collected by reprecipitation in methanol. The M_w and M_w/M_n of the resulting polymer were determined to be 233 kg/mol and 1.9, respectively, by size exclusion chromatography. Finally, PCL incorporating 6.6 mol % of ketone group was obtained after deacetalization with triphenylcarbenium tetrafluoroborate.
(24) Ikeda, I.; Horie, N.; Suzuki, K. *J. Appl. Polym. Sci.* **1994**, *54*, 1123.
(25) Cho, K. Y.; Kim, C. H.; Lee, J. W.; Park, J. K. *Macromol. Rapid Commun.* **1999**, *20*, 598.
(26) Ponsart, S.; Coudane, J.; McGrath, J.; Vert, M. *J. Bioact. Compat. Polym.* **2002**, *17*, 417.
(27) Mayes, A. M.; Irvine, D. J.; Griffith, L. G. *Mater. Res. Soc. Symp.* **1998**, *530*, 73.
(28) Irvine, D. J.; Mayes, A. M.; Griffith, L. G. *Biomacromolecules* **2001**, *2*, 85.
(29) Irvine, D. J.; Ruzette, A. V. G.; Mayes, A. M.; Griffith, L. G. *Biomacromolecules* **2001**, *2*, 545.
(30) Koo, L. Y.; Irvine, D. J.; Mayes, A. M.; Lauffenburger, D. A.; Griffith, L. G. *J. Cell Sci.* **2002**, *115*, 1423.

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