Synthesis and Characterization of Novel Lipid Functionalized Poly(ε-caprolactone)s

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ABSTRACT: A series of novel lipid functionalized poly(ε-caprolactone)s (PCLs) were synthesized through ROP of ε-caprolactone in the presence of *threo*-9,10-dihydroxyoctadecanoic acid, synthesized from oleic acid. PCLs with different molecular weights were obtained by controlling the molar ratio of the initiator to the monomer. DSC and XRD analysis indicate that the crystallinity of PCLs decreased when compared to unfunctionalized PCL. The enzymatic degradation study shows that for samples with lower lipid derivatives content, a higher enzymatic degradation rate was observed because the lipase enzymes attack the ester bonds of the polymer; increased lipid content therefore inhibits the action of the lipase enzymes. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 119: 1848–1856, 2011

Key words: ring-opening polymerization; thermal properties; modification; biodegradable; initiators

INTRODUCTION

Biodegradable polymers belong to a class of important and desirable biomaterials because of their wide applications in the biomedical field; including tissue engineering, controlled drug delivery and gene therapy.[1–3] Aliphatic polyesters, such as polylactide (PLA), poly(ε-caprolactone) (PCL) and polyglycolide (PGA) are the most commonly used, because of their good biocompatibility, low immunogenicity and suitable mechanical properties.[4,5] The properties of these materials can be further improved by modification and functionalization, for example by incorporation of bioactive compounds such as lipids, vitamins, hormones, or peptides to the polymer chain.[6,7] In the family of biodegradable polymers, PCL appears to be most advantageous owing to its good thermal properties. PCL can be degraded hydrolytically or in the presence of microorganisms or lipase enzymes.[8,9] Three kinds of lipases were found to accelerate the degradation of PCL: *Rhizopus delemer* lipase,[10] *Rhizopus arrhizus* lipase,[11] and *Pseudomonas* lipase.[12,13] High crystalline PCL has been reported to totally degrade over 4 days in the presence of *Pseudomonas* lipase while the hydrolytic degradation takes several years.[12,13]

Fatty acids are natural body components and are considered biologically safe; therefore they are suitable candidates for the preparation of biodegradable polymers. Fatty acids may also be able to retain an encapsulated drug if used as drug carriers.[14–17] However; most fatty acids sourced from commercial oilseed crops are monofunctional and therefore cannot act in the polymerization reactions as monomers or initiators. Oleic acid is a C18 fatty acid, which contains a double bond between the 9th and 10th carbon atoms in the chain. The presence of the double bond in the alkyl chain provides opportunities to improve the chemical functionality of the fatty acid chain, for example by the formation of diols in the reaction of oleic acid with formic acid in the presence of hydrogen peroxide.[18]

In general, any polymer which is used for biomedical application should be evaluated on basis of a number of appropriate properties. For example polymer should be biocompatible when implanted in the target organs, should be completely eliminated from the implantation site after a predictable time, should have suitable physical properties for device fabrication (e.g., low melting temperature, solubility) and should be fluid enough after drug incorporation to

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allow easy injection. Fatty acid-based polymers possess many of the above properties to claim their utility as drug delivery carriers.[14–17] For biomedical applications, PCL as a nontoxic polymer has been demonstrated exhibiting good biocompatibility, low immunogenicity, and high permeability to drugs. However, PCL has a high degree of crystallinity which limited its application. Incorporation of lipid-derived compounds into PCL chains can disturb the crystallization ability of the PCL segments and decrease the melting point of the polymers, which could improve the physical properties for device fabrication and drug incorporation. Moreover, it is also expected to extend the biocompatibility of the functionalized polymer by including lipid-derived compounds in the molecular structure. Therefore, the objective of this study was the synthesis and characterization of polymers based on ε-caprolactone (ε-CL) and oleic acid derivatives for potential use as drug carriers. The first step of this work was the synthesis of the initiator (*threo*-9,10-dihydroxyoctadecanoic acid) from oleic acid and then polymerization with ε-CL in the presence of stannous octanoate as the catalyst.

The enzymatic degradation studies of the functionalized polymers were carried out in a phosphate buffer solution containing *Pseudomonas cepecia* lipase. The results were then compared with results obtained using a PCL homopolymer as the control. Physicochemical property changes are discussed using results obtained by proton nuclear magnetic resonance (NMR), gel permeation chromatography (GPC), differential scanning calorimetry (DSC), thermal degradation analysis (TGA), X-ray diffractometry (XRD), polarized light microscopy (PLM), and scanning electron microscopy (SEM).

EXPERIMENTAL

Materials

ε-Caprolactone (2-Oxepanone, >99.0%, Sigma-Aldrich, USA) was dried and distilled under reduced pressure before use. Formic acid (>96.0%, Sigma-Aldrich, USA) was dried under vacuum prior to being used. Stannous octanoate (stannous 2-ethylhexanoate, >95.0%, Sigma, USA), oleic acid (>90.0%, Sigma-Aldrich, USA), hydrogen peroxide solution (35 wt % in water, Fischer Chemicals, USA), dichloromethane (>99.0%, Fischer Chemicals, USA), and methanol (>99.0%, Fischer Chemicals, USA) were used as received. For the enzymatic degradation test, *Pseudomonas cepacia* lipase (powder, $\sim$ 50 U mg^{-1}) was purchased from Fluka, USA and used as received.

Synthesis of initiator *threo*-9, 10-dihydroxyoctadecanoic acid

The synthesis of *threo*-9,10-dihydroxyoctadecanoic acid was performed according to literature.[18,19] To a solution of oleic acid (10.0 g, 0.035 mol) and formic acid (29.5 mL, 0.035 mol), $\sim$ 3.4 mL of 34% hydrogen peroxide were added. After 5–10 min, the reaction became slightly exothermic. In the following 20–30 min, a homogeneous mixture was obtained. The temperature was maintained at 40°C with a cold water bath at the beginning, and with warm water at the end of the reaction. After about 3 h, the formic acid was removed by distillation under reduced pressure. The residue in the flask was heated for 1 h at 100°C with an excess of 3*N* aqueous sodium hydroxide, and the hot solution was cautiously poured into an excess of 3*N* hydrochloric acid with stirring. The oil phase was allowed to solidify and the aqueous layer was discarded. The solid was remelted in the steam bath by addition of hot water and stirred well to remove residual salts and water-soluble acids. When the oil solidified, the aqueous layer was discarded, and the solid was broken and dissolved in 350 mL of 95% ethanol by heating in the steam bath. After crystallization at 0°C for several hours, the product was collected on a filter paper and dried under vacuum. The yield of crude *threo*-9,10-dihydroxyoctadecanoic acid was 91.5% (9.15 g). After second and third recrystallizations from 200 mL of 95% ethanol, the product yield was 8.5 g or 85%. The reaction conditions of the dihydroxylation procedure is more likely to produce *threo*-9,10-dihydroxyoctadecanoic acid. Furthermore, determination of the melting temperature of the synthesized compound indicated the value at 92°C (DSC analysis, data not reported). Knothe et al.[19] found that the melting point for *threo*-9,10-dihydroxyoctadecanoic acid is 95°C and that of the *erythro*-9,10-dihydroxyoctadecanoic acid is 132°C. Given that our measured melting temperature is close to the literature value, we supposed that the *threo*-9,10-dihydroxyoctadecanoic acid was obtained. The structure of the product was confirmed by ^{1}H NMR spectra (CD_3OD-d_4, 400 MHz) δ (ppm): 0.91 (t, 3H, $-(CH_2)_6CH_2C\mathbf{H_3}$), 1.21–1.62 (m, 26H, $-C\mathbf{H_2}-$ from oleic acid derivatives chain), 2.28 (t, 2H, $-(CH_2)_6C\mathbf{H_2}COOH$, 3.39 (d, 2H, $-(CH_2)_7C\mathbf{H}(OH)-C\mathbf{H}(OH)(CH_2)_6-$). No signals derived from impurities were observed in the NMR spectra, indicating that a pure product was obtained.

Polymerization procedure

Functionalized PCLs with different molar ratios of the initiator, *threo*-9,10-dihydroxyoctadecanoic acid, to the monomer were prepared. The initiator/ε-caprolactone feed ratio for the functionalized PCLs

were: 1/25; 1/50; 1/75; 1/100 (mol/mol), referred to as OL/$(CL)_n$ 25; OL/$(CL)_n$ 50; OL/$(CL)_n$ 75; OL/$(CL)_n$ 100, respectively, where OL = *threo*-9,10-dihydroxyoctadecanoic acid and CL = ε-caprolactone. For each polymerization, monomers (ε-CL), initiator (*threo*-9,10-dihydroxyoctadecanoic acid) and catalyst $SnOct_2$ (0.1 mol %) were placed in 50-mL glass ampoules under a nitrogen atmosphere. The reaction vessel was then maintained at 100°C for 24 h in a temperature controlled oil bath. The cooled reaction product was dissolved in CH_2Cl_2, precipitated in cold methanol, and dried under vacuum for 72 h. For comparison, PCL was also synthesized utilizing the same reaction conditions for functionalized PCLs, but using *n*-BuOH as an initiator, with initiator/ε-caprolactone feed ratio of 1/100.

Enzymatic degradation

Polymer films for degradation studies were obtained by compression molding at 80°C, followed by rapid cooling to room temperature. The weight of the samples was ~ 100 mg, with a thickness of 0.5 mm. To carry out the enzymatic degradation study,[20] the samples was immersed in 10 mL of buffer solution (pH = 7.0) containing *Pseudomonas cepecia* lipase (0.2 mg mL^{-1}). The vials were placed in an oven at 37°C. The buffer/enzyme solution was changed every 1 day to restore original levels of enzymatic activity. After 1 day, the specimens were withdrawn from the buffer/enzyme solution and washed with distilled water. They were then vacuum-dried at room temperature for 1 week. A similar procedure was applied to specimens after 4 days of enzymatic degradation. All the samples were weighed and examined by SEM (Philips XL30 ESEM LaB_6, manufactured by FEI Company, Oregon, USA). Prior to SEM investigation, the surface of each of the films was coated with gold.[20]

Analysis and characterization

The polymerization products were characterized by means of ^1H and ^{13}C NMR (Varian 500 or 400 MHz, Varian, CA). The NMR spectra of the polymers were recorded in $CDCl_3$ or CD_3OD-d_4.

Number-average molecular weights (M_n) and polydispersity index (M_w/M_n) were estimated by GPC, which were performed on an Agilent GPC (Agilent series 1200, USA) equipped with RI detector. Chloroform ($CHCl_3$) was used as the mobile phase at a flow rate of 1.0 mL min^{-1}. A 10 μL amount of 0.4% (w/w) was injected for each analysis. Calibration was performed with polystyrene standards (Polysciences).

A TA Q-100 modulated DSC (MDSC) system from TA Instruments (New Castle, USA.), equipped with a refrigerated cooling system was used to analyze the thermal transitions of the polymers. The sample was held at 20°C for 3 min to reach its equilibrium state and then was heated to 130°C at a rate of 20°C min^{-1} to erase its thermal history. To record the crystallization curve, the sample was cooled down to −80°C at a constant rate of 5°C min^{-1} and kept at this temperature for 3 min to allow for the completion of the crystallization. The sample was then heated to 130°C at a constant rate of 3°C min^{-1} to record the melting curve. All the procedures were performed in a dry nitrogen gas atmosphere.

The relative crystallinity of the polymers was calculated according to the following equation: $W_c = (\Delta H_f/\Delta H^\circ_f) \times 100$, where W_c is the relative crystallinity, ΔH_f is the heat fusion of the polymers and ΔH°_f is the heat of fusion of 100% crystalline PCL which has been reported to be 136 J g^{-1}.[21]

Thermal gravimetric analysis was carried out on a TGA Q50 (TA Instruments, New Castle, USA) following the ASTM D3850-94 standard. Approximately 20 mg of sample was loaded in the open platinum pan. The samples were heated from 25 to 600°C under dry nitrogen at constant heating rates of 10°C min^{-1}. All the samples were run in triplicate for thermal property measurements.

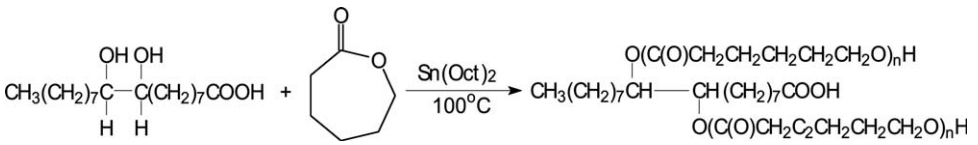
The crystallization structure of all the polymer samples was further studied by XRD. A Bruker AXS X-ray diffractometer (Madison, USA) equipped with a filtered Cu Kα radiation source (λ = 0.1542 nm) and a 2D detector was used to record the XRD patterns. The procedure was automated and controlled by the Bruker AXS's "GADDs V 4.1.08" software. The frames were processed using GADDS software and the resulting spectra were analyzed using Bruker AXS's "Topas V 2.1" software.

The microstructure was investigated using a Leica DMRX polarized light microscope (Leica Microsystems, Wetzlar, Germany) fitted with a Hamamatsu digital camera (C4742-95). The microscope/camera assembly was controlled by Impovision's "Openlab 4.02" software (Improvision, Coventry, UK). The sample was heated to 90°C and quenched to room temperature. Images were then obtained at a magnification of 500×.

RESULTS AND DISCUSSION

Polymer synthesis and characterization

Aliphatic polyesters, with high molecular weights, can be successfully synthesized by ring-opening polymerization of corresponding monomers.[22,23] Recently, ring-opening polymerization methods have been established for preparing linear, dendritic, hyperbranched, and multi-arm star-branched polymers.[24–27] According to these previous studies, ring-opening polymerization can be initiated by compounds



Scheme 1 Synthesis of the lipid functionalized PCLs with *threo*-9,10-dihydroxyoctadecanoic acid.

such as alcohols or amines[28] containing active hydrogen atoms. The ring-opening polymerization can proceed in the presence of carboxyl groups, but the carboxyl group does not initiate this process. The presence of carboxyl groups in the molecule can however accelerate the polymerization reaction when the temperature is very high.[29] In this study, oleic acid derivatives bearing two hydroxyl groups and a carboxyl group were engaged as initiators for the ring-opening polymerization of ε-CL. The polymerization reaction was carried out under anhydrous conditions—the monomer and initiator were dried before using to avoid traces of water. The reaction conditions (24 h and 100°C) were optimum to obtain the functionalized PCLs. A series of novel PCLs functionalized by oleic acid derivatives were synthesized (Scheme 1). As shown in the Table I, the molecular weights of OL/$(CL)_n$ could be controlled by adjusting the molar ratio of the initiator to the monomer. It is worth mentioning that the highest molecular weight was recorded by the OL/$(CL)_n$ sample with the least initiator content. Simultaneously with the increasing of molecular weight of the polymers, the value of the polydispersity index was also increased, what could be explained by a clear loss of the control when the high molecular weight PCLs were tried to be obtained.

The ^{1}H NMR spectrum of functionalized PCL [OL/$(CL)_n$ 50 as an example] is shown in Figure 1. From the ^{1}H NMR, we can easily verify that ring-opening polymerization of ε-caprolactone was successfully initiated by *threo*-9,10-dixydroxyoctadecanoic acid. The typical signals from PCL and oleic acid derivatives can be observed. Namely b (4,20 ppm, d, —C**H**—O, initiator chain), *f* (4.05 ppm, t, —C(O)$CH_2CH_2CH_2CH_2C\mathbf{H_2}$O—, ε-CL repeating unit), *f′* (3.64 ppm, t, —C(O)$CH_2CH_2CH_2CH_2C\mathbf{H_2}$OH, end group), *g* (2,35 ppm, t, —C$\mathbf{H_2}$COOH, initiator chain), *c* + *c′* (2.29 ppm, t, —C(O)C$\mathbf{H_2}CH_2CH_2CH_2CH_2$O—, ε-CL repeating unit), *i* + *i′* (1.75–1.79 ppm, m, —C$\mathbf{H_2}$—CH—CH—, initiator chain), *d* + *d′* (1.65 ppm, m, —C(O)$CH_2C\mathbf{H_2}CH_2C\mathbf{H_2}CH_2$O—, ε-CL repeating unit,), *e* + *e′* (1.33 ppm, t, —C(O)$CH_2CH_2C\mathbf{H_2}CH_2CH_2$O—, ε-CL repeating unit), *j* + *j′* (1.23–1.30 ppm, m, —C$\mathbf{H_2}$— unit of initiator chain) and *a* (0.86 ppm, t, C$\mathbf{H_3}(CH_2)_7$—, initiator chain).

By comparing the integration value of peak *a* and *f′*, we can conclude that both hydroxyl groups initiated the polymerization of ε-CL and that functionalized polymers have been obtained. The molecular weights $M_n^{(calc.)}$ of the obtained functionalized polymers were calculated from the equation M_n = (MW(CL))([CL]$_o$/[OL]$_o$) + MW(OL).[20,30] It was assumed, there was 100% conversion from ε-caprolactone to poly(ε-caprolactone) due to the chemical shifts changes in the ^{1}H NMR spectra between these two compounds. Also, the number average molecular weight M_n^{NMR} of functionalized PCLs were determined qualitatively from the peak intensities of the methylene proton at the hydroxyl terminus (peak *f′*, ^{1}H NMR spectra of OL/$(CL)_n$ 50 in the Fig. 1) and the main chain of methylene proton (peak f, ^{1}H NMR spectra of OL/$(CL)_n$ 50 in the Fig. 1). This calculation assumes a precise number of hydroxyl termination groups.

TABLE I
The Molecular Characterization of the PCL Homopolymer and Lipid Functionalized PCLs

Sample	OL/ε-CL (mol mol^{-1})	Yield (%)[a]	M_n^{GPC}		$M_n^{(calc.)b}$	M_n^{NMRc}
			M_n	M_w/M_n		
OL/$(CL)_n$ 25	1/25[d]	89	7050	1.33	3167	3210
OL/$(CL)_n$ 50	1/50[d]	98	12,800	1.42	6017	6070
OL/$(CL)_n$ 75	1/75[d]	81	18,500	1.50	8867	8840
OL/$(CL)_n$ 100	1/100[d]	87	25,000	1.75	11,720	11,900
PCL	1/100[e]	95	11,900	1.80	–	–

[a] Part insoluble in cold methanol.
[b] Obtained from the equation M_n = (MW(CL))([CL]o/[OL]o) + MW(OL).
[c] Number average molecular weight determined by ^{1}H NMR. Obtained from the equation $M_{n(NMR)} = (M_w(M))(DP_{(NMR)}) + M_w$(initiator); $DP_{(NMR)} = I_{pol}/I_{ter} + 1$, where M_w is the molecular weight of ε-caprolactone monomer or threo-9.10-dihydroxyoctadecanoic acid; I_{pol} and I_{ter} represent the integrals obtained by ^{1}H NMR from the polymer (4.05 ppm) [—CH_2O—] and hydroxyl end group (3.64 ppm) [—CH_2OH] peaks, respectively.
[d] Molar ratio of OL to ε-CL:1/25; 1/50; 1/75; 1/100; respectively.
[e] Molar ratio of initiator (n-BuOH) to ε-CL: 1/100.

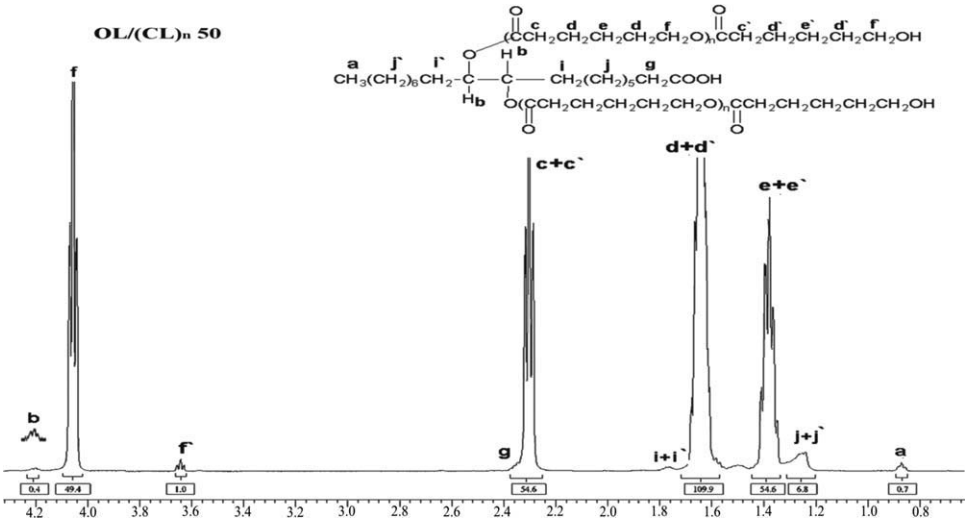


Figure 1 ^{1}H NMR spectra of the lipid functionalized PCL.

As shown in Table I, the M_n^{NMR} values were in agreement with the $M_n^{(calc.)}$ values. These results indicate that hydroxyl groups in the initiator molecule act as active sites of polymerization of ε-CL. Moreover, the polymerization yields estimated from the insoluble part in methanol were found to be comparatively high (81–98%). The above results clearly demonstrate that the *threo*-9,10-dihydroxyoctadecanoic acid effectively initiated ring-opening polymerization of ε-CL, leading to the formation of lipid functionalized PCLs.

Notice that the number-average molecular weights calculated by ^{1}H NMR is lower than the molecular weights obtained by GPC. Overestimation of the M_n obtained by GPC for PCL is common because polystyrene standards are used in the construction of the calibration curve. Solvent selected for the GPC measurement will also slightly affect the M_n value. Ba′ez et al.[31] found that the $M_n^{(calc.)}/M_n^{(GPC)}$ ratio for PCL was between 0.36 and 0.60, where $M_n^{(calc.)}$ values are similar to those derived from ^{1}H NMR spectra. Kricheldorf and Eggerstedt[32] also found that the M_n values of PCL determined from GPC measurements are larger than the values obtained from ^{1}H NMR spectra analyses by about 15–25%. In our study, the ratios of $M_n^{(calc.)}$ and $M_n^{(GPC)}$ ranged from 0.45 to 0.48. The average value 0.47 was applied as the pertinent factor to convert the $M_n^{(GPC)}$ values to the actual values in this study.

Thermal degradation of PCL and functionalized PCLs were studied by DTGA and the results are shown in Figure 2.

The DTGA curves display one main degradation mechanism with the inflection points at 298°C for OL/(CL)$_n$ 25, at 305°C for OL/(CL)$_n$ 50, at 312°C for OL/(CL)$_n$ 75, at 320°C for OL/(CL)$_n$ 100 and at 322°C for PCL. These results suggest that the thermal stability of polymers depends strongly on the molecular weights, resulting from the different molar ratio of substrates (OL to ε-CL). It is difficult to separate the dependence solely on molecular weight or structural influences due to the incorporation of lipid content, however. A single step degradation was observed for PCL and functionalized polymers in this study, although a two-step degradation mechanism has been proposed by Persenaire et al.[33] Employing a similar procedure to study thermal degradation as was used in this work, a one-stage degradation mechanism of the thermal degradation of PCL was also found by Su et al.[34] To further enquire into the discrepancy between their results and reported two-stage processes, they performed kinetic analysis of multiple degradation curves and found a two-stage degradation mechanism by applying Friedman plots to the data. The results shown in this study therefore do not preclude the possibility of a two-stage degradation mechanism.

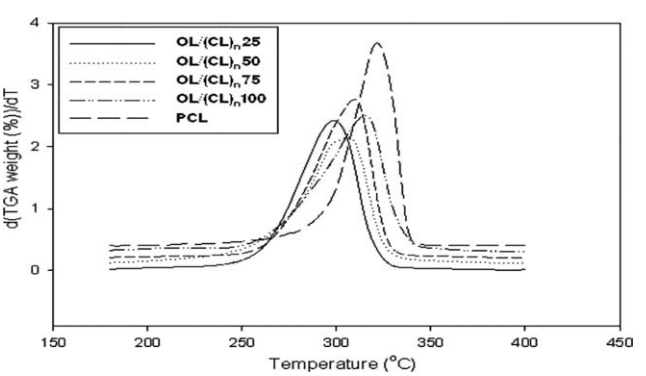


Figure 2 DTGA curves of thermal degradation of the PCL homopolymer and the lipid functionalized PCLs.

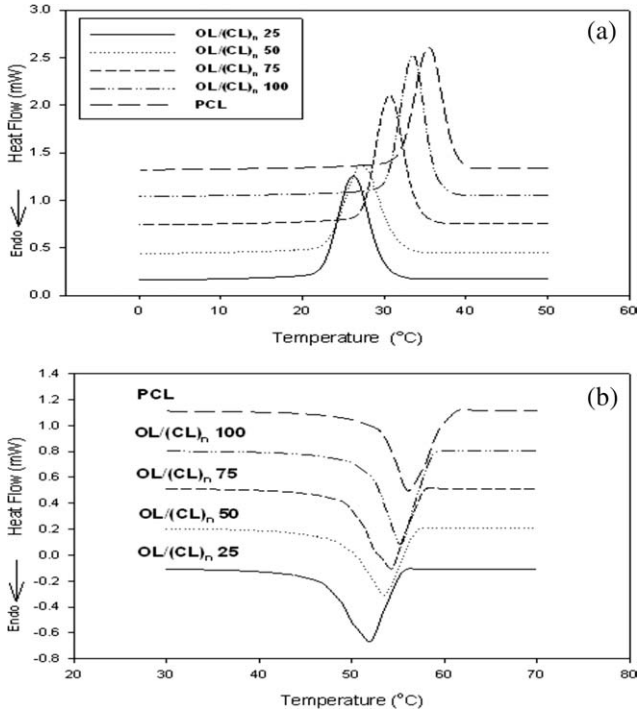


Figure 3 DSC thermograms of the PCL homopolymer and the lipid functionalized PCLs: (a) cooling at the constant rate 5°C min^{-1}; (b) second scan of heating at a constant rate of 3°C min^{-1}.

The thermal properties of the polymers were also investigated by MDSC. The results are shown in Figure 3(a,b).

The melting temperature (T_m), enthalpy of fusion (ΔH_f), crystallization temperature (T_c), and crystallization enthalpy (ΔH_c) of all the polymers were determined and listed in Table II. For unfunctionalized PCL, $T_m = 56$°C, $\Delta H_f = 90$ J g^{-1}, $T_c = 37$°C, and $\Delta H_c = 91$ J g^{-1}. Whereas for lipid functionalized PCL having similar molecular weight (i.e., OL/$(CL)_n$ 50), $T_m = 53$°C, $\Delta H_f = 68$ J g^{-1}, $T_c = 27$°C, and $\Delta H_c = 65$ J g^{-1}, which are much lower than the unfunctionalized PCL. These results indicate that the incorporation of lipid content influences the structure of the polymers. Moreover, the T_m values of the functionalized polymers were between 51 and 55°C, and the T_c values were in the range of 26–34°C, both decreased with the increasing of lipid content. This suggests that lipid-functionalized polymer with more lipid content, crystallized at relatively lower temperature due to more branches and shorter chain lengths. Additionally, the relative crystallinity (W_c) for the functionalized polymers decreased from 53 to 49% with the increasing lipid content in the macromolecule (see Table II). Other than the obvious dependence of enthalpy of fusion on the molecular weight of the crystallizing strands, this also suggests that the final degree of crystallinity of the functionalized polymers is lower than their homopolymer counterpart. It has been reported that end-capped PCL with phosphorylcholine groups demonstrated lower crystallization capabilities than PCL.[35] Teomim and Domb also found that nonlinear fatty acid terminated polyanhydrides based on ricinoleic acid possessed lower values of melting temperature and heat capacity than unmodified poly(sebacic acid).[14]

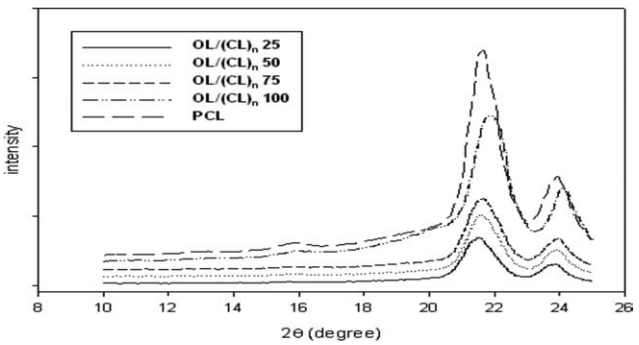


Figure 4 X-ray diffraction spectra of the PCL homopolymer and the lipid functionalized PCLs.

The glass transition temperature values for all samples cluster around −63°C, implying that the oleic acid derivatives had little influence on the glass-transition temperature of the functionalized PCLs.

The crystallization structure of PCL homopolymer and lipid functionalized PCLs was studied by XRD.

As shown in Figure 4, PCL homopolymer exhibited two peaks at $2\theta = 21.5° \pm 0.1°$ and $23.8° \pm 0.1°$, which correspond to (110) and (200) plane of PCL.[36] In the case of functionalized PCLs with lipid derivatives, the two peaks were situated at $2\theta = 21.6° \pm 0.1°$ and $23.9° \pm 0.1°$. These changes are within the error associated with the measurement, suggesting that there were no real significant changes to the packing of the crystals. The relative intensity of the XRD peaks clearly and unambiguously demonstrates

TABLE II
Thermal Properties of the PCL Homopolymer and Lipid Functionalized PCLs

Sample	T_c (°C)	ΔH_c (J g^{-1})	T_m (°C)	ΔH_f (J g^{-1})	W_c (%)
OL/$(CL)_n$ 25	26	64	51	66	49
OL/$(CL)_n$ 50	27	65	53	68	50
OL/$(CL)_n$ 75	30	74	54	70	52
OL/$(CL)_n$ 100	34	76	55	72	53
PCL	37	91	56	90	66

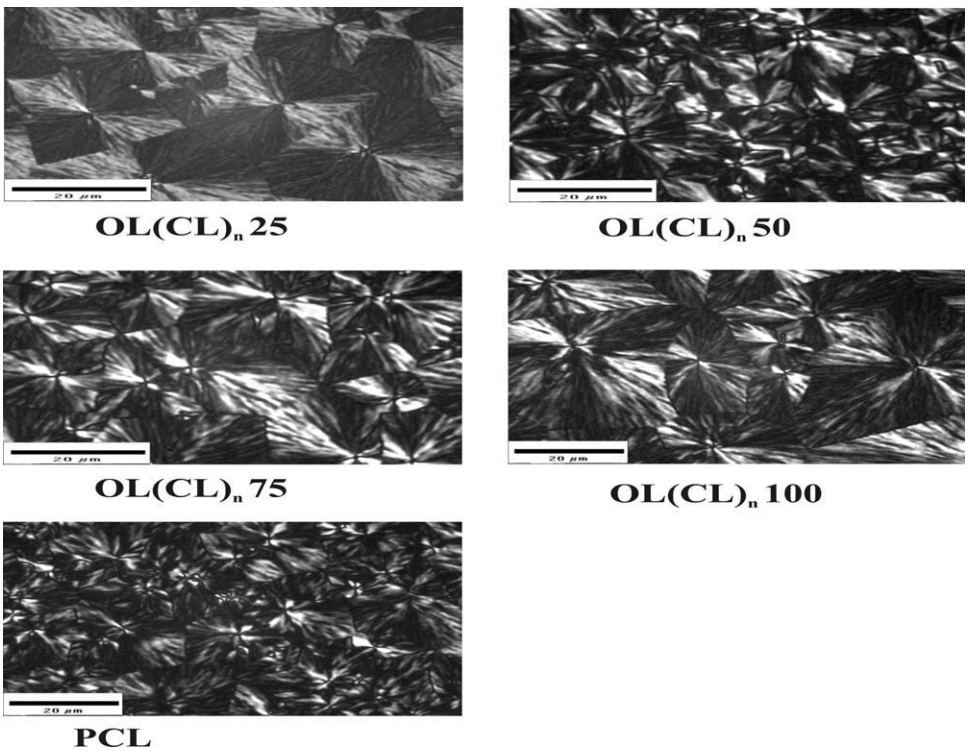


Figure 5 The polarized light microscope images of the PCL homopolymer and the lipid functionalized PCLs.

a reduction in the crystallinity of the polymers as a function of increasing lipid content. This is due to the increasingly difficult process of accommodating polymers with more branches effectively into crystals. There is therefore a significant entropic barrier to nucleation of the branched polymers with the lipid derivatives. Therefore, a significant portion of the chains of the functionalized polymers reside in the amorphous phase. The XRD data provides convincing support for the conclusions of the MDSC results discussed previously.

The crystal morphology of both the PCL homopolymer and the lipid functionalized PCL was investigated by PLM.

All the polymers displayed a typical spherulitic morphology, as shown in Figure 5. The size of spherulites increased with increasing lipid content. This is not surprising, since the branched chains of the functionalized polymers presents an entropic barrier to nucleation; there are thus fewer centers of growth of the crystals. This results in the formation of relatively few, well-formed and large crystals, as opposed to many smaller crystals in the homopolymer. Within the crystallizing system the branched polymer chains are required to diffuse over to a growing crystal surface in the required conformation, and these results in the formation of fewer, larger crystals and spherulites.

Based on MDSC, XRD, and PLM results, it can be concluded that the functionalization of PCL with oleic acid derivative lowers the overall crystallinity of the polymer.

Degradation studies

The enzymatic degradation of PCL polymers have been investigated by several groups.[12,13,37–39] *Pseudomonas cepecia* is one of the enzymes which have significant effect on the degradation of PCL.[40] It belongs to the esterase family and is capable of cleaving the ester bond on hydrophobic substrates. In our study, enzymatic degradation of PCL and its functionalized polymers was examined. The weight losses of OL/$(CL)_n$ 25, 50, 75, 100, and PCL after 1, 2, 3, and 4 days of enzymatic degradation are shown in Figure 6.

The weight losses increased significantly after 4 days of enzymatic degradation for all functionalized PCLs. For unfunctionalized PCL, the weight loss value reached 97 wt % after 4 days of enzymatic degradation, indicating that its degradation was almost complete. These results are in good agreement with the results reported by Gana et al.[12,13]

The surface morphologies of OL/$(CL)_n$ 25, 50, 75, 100 and PCL before and after 1- and 4-day enzymatic degradation are shown in Figure 7(A–C), respectively.

Before degradation all samples had a smooth surface [Fig. 7(A)]. After enzymatic degradation for 1 day [Fig. 7(B)], samples OL/$(CL)_n$ 25, OL/$(CL)_n$ 50,

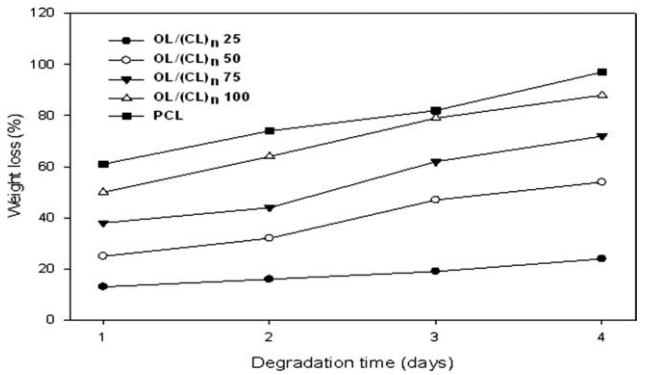


Figure 6 The weight loss of the lipid functionalized PCLs: OL/(CL)$_n$ 25, OL/(CL)$_n$ 50, OL/(CL)$_n$ 75, OL/(CL)$_n$ 100, and the PCL homopolymer during the course of enzymatic degradation.

OL/(CL)$_n$ 75, and OL/(CL)$_n$ 100 demonstrated varying amounts of degradation. This might be due to the presence of the lipid molecule in the macromolecule which influences polymer surface properties. In the case of unfunctionalized PCL [Fig. 7(B)], numerous holes were formed after 1 day of enzymatic degradation. After 4 days of enzymatic degradation all samples were more eroded [Fig. 7(C)].

From the degradation experiment, the difference in the degradation rate strongly depends on the presence of enzyme in the degradation system and the content of oleic acid derivatives in the polymer molecule. PCL shows the highest weight loss during enzymatic degradation as the *Pseudomonas cepecia* lipase has a specific activity on PCL segment degradation. For samples OL/(CL)$_n$ 75 and OL/(CL)$_n$ 100 with lower lipid derivatives content, a faster enzymatic degradation rate can be observed because of the effect of lipase catalysis. For samples OL/(CL)$_n$ 25 and OL/(CL)$_n$ 50 with higher lipid derivatives content, the degradation rate is relatively lower (13–25 wt % after 1 day of degradation). Li et al. observed that the weight loss of block copolymers prepared from ε-CL and poly(ethylene glycol) reached 25 wt % after 1 day of enzymatic degradation and 65 wt % after 2 days.[20] In this study the degradation rate for all samples is distinguishably lower, which might be due to the incorporation of fatty acid terminals to PCL.

Although the molecular weights of the lipid-functionalized polymers were lower with increasing lipid content, previous work on various molecular weights of unfunctionalized PCL suggest strongly that the differences in degradation observed are not a function of molecular weight, but of the lipid inclusion. Gan et al. studied degradation of PCL with different molecular weights.[12] They found that degradation rates of PCL with different molecular weights were similar, because of the involvement of carbonate linkage in the degradation process. Other researchers also proved that for cholesteryl moiety functionalized PCLs with different molecular weights, only cholesteryl content plays a critical role in the degradation rate, while the effect of molecular weight is negligible.[41] Therefore, the different biodegradation rates of these polymers must be attributed

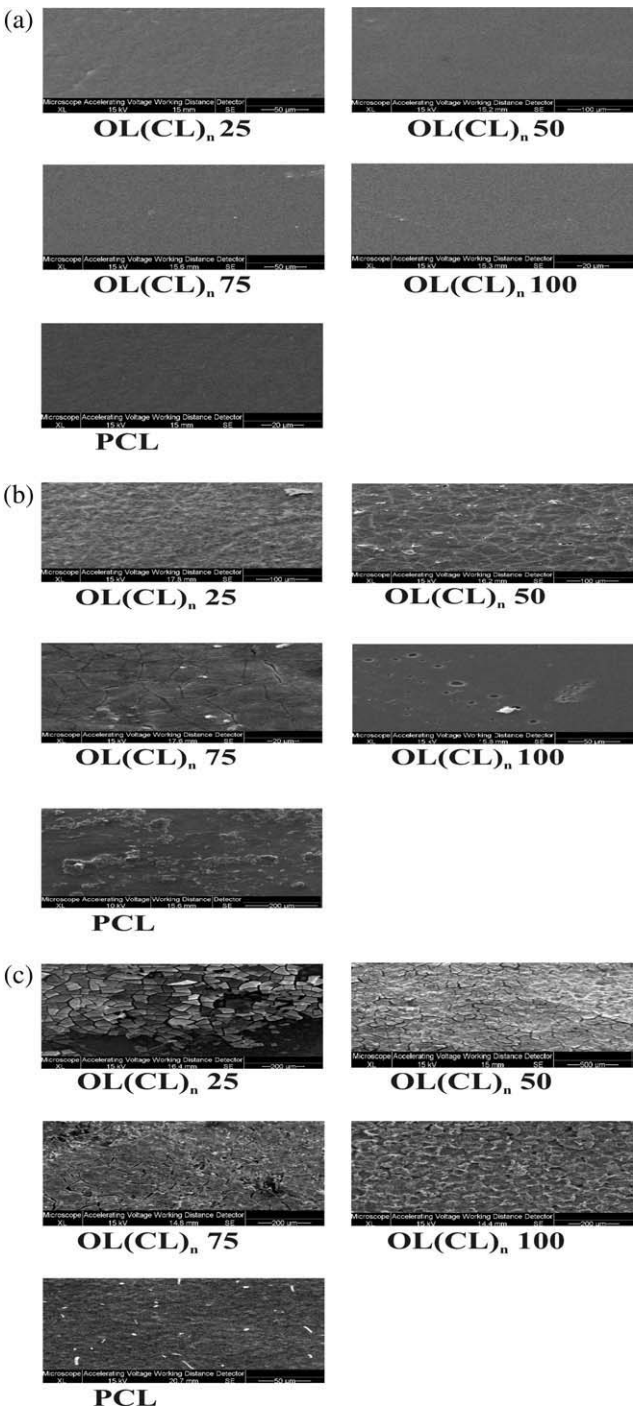


Figure 7 SEM images of the lipid functionalized PCLs: OL/(CL)$_n$ 25, OL/(CL)$_n$ 50, OL/(CL)$_n$ 75, OL/(CL)$_n$ 100, and the PCL homopolymer, **A**-before degradation, **B**-after enzymatic degradation for 1 day, **C**-after enzymatic degradation for 4 days.

to the different lipids content in the polymer macromolecule. Our degradation results are in accordance with previous studies.[12,36,41,42]

The conventional micelle drug delivery systems based on amphiphilic linear copolymers suffer from low encapsulation efficiency and fast release rate, which limit their applications in drug delivery.[43] Recently, it has been reported that amphiphilic copolymers with branched structures exhibited improved property as drug carrier.[44] In this study, the incorporation of lipid fatty acid molecule to PCL chains provided special branched structure of the polymer, which should be able to improve property as drug carrier. Nevertheless, the fatty acid molecule is also expected to increase hydrophobicity, which might limit its application in biomedical field, However, this problem could be mitigated by incorporation of some hydrophilic functionality to the molecule of functionalized PCLs. This work is still ongoing in our laboratory.

CONCLUSIONS

Novel biodegradable polyesters functionalized by fatty acid derivatives moieties were successfully synthesized by the ring-opening polymerization of ε-CL initiated by oleic acid derivatives bearing two hydroxyl groups. Polymers with different molecular weights were obtained by adjusting the molar ratio of the initiator to monomer. Because of the incorporation of fatty acid molecules resulted in branched polymer, functionalized polymers exhibited lower melting temperature and less crystallinity relative to unfunctionalized PCL. The *Pseudomonas cepecia* lipase significantly degraded all samples. The degradation rate was affected by the content of oleic acid derivatives in the polymer molecule. Higher oleic acid derivatives content resulted in slower degradation rates.

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