Exhibition of Soft and Tenacious Characteristics Based on Liquid Crystal Formation by Introduction of Cholesterol Groups on Biodegradable Lactide Copolymer

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Cholesterol side-functionalized poly(depsipeptide-*co*-DL-lactide) (PGD-DL-LA-(cholesterol)$_n$) and poly(depsipeptide) (PGD-(cholesterol)$_n$) were prepared as novel biodegradable liquid crystalline (LC) polymers. These polymer films exhibited different LC phases depending on the cholesterol unit content in the polymers. The thermodynamic stability of these LC phases was quite high, and PGD-(cholesterol)$_n$ film exhibited continuous LC phases up to 202 °C. The resulting cholesterol LC phases were indicated to act as physical cross-linking points to form noncovalent network structures among the polymer chains. Therefore, PGD-DL-LA-(cholesterol)$_n$ film exhibited a rubbery and stretchy nature at 37 °C due to physical cross-linking points based on cholesterol LC phase well-dispersed in the film. The cholesterol side-group effects leading to rubbery character and hydrolytic resistance reported herein are rather unique. The biodegradable LC material exhibiting a soft and tenacious nature is a promising candidate for a new class of implant biomaterials used with dynamic organs of the body such as the heart and blood vessels.

Introduction

Self-assembly strategies could bring novel capabilities to synthetic materials used in advanced technology. Liquid crystalline polymers have been used as dynamically functional materials for electro-optical displays, sensing, templates, and stimuli responsiveness.[1–5] Liquid crystalline polymers should also be useful in biorelated fields because the self-organizing structures of liquid crystals through noncovalent specific interactions are compatible with those in living systems.[6–11] Cholesterol containing liquid crystalline materials have especially attracted more and more attention and interest in the biomaterials field.[12–16] Abbott et al. recently reported a novel experimental system that permits study of the specific binding of proteins to receptors hosted at fluid-like biomimetic interfaces via a thermotropic liquid crystalline system.[13,14] Zhou at al. developed the polymer/liquid crystalline composite membrane and found the liquid crystalline component appeared to be beneficial in improving the blood compatibility.[15,16] On the other hand, Stupp et al. prepared the biodegradable polymer/liquid crystalline hybrid film consisting of cholesterol end functionalized oligo(L-lactic acid) (OLLA). The oligomers were found to be able to self-assemble to present ordered and periodic bulk structures to guide cell behavior.[12] Furthermore, a few research groups also reported the cholesterol end functionalized oligo- and polyesters as biodegradable materials.[17,18]

We have previously reported that the synthesis of biodegradable copolymers of depsipeptide and poly(L-lactide) (PLLA), poly(depsipeptide-*co*-L-lactide), having reactive side-chain groups, such as COOH, NH_2, and SH, by varying amino acid units of depsipeptide.[19–21] Moreover, it was found to be possible to endow many functionalities to these copolymers via chemical modification of various functional groups.

In this paper, we designed a novel liquid crystalline biodegradable film via self-assembly of cholesterol side-functionalized poly(depsipeptide-*co*-DL-lactide) copolymers in order to create an implantable biomaterial exhibiting a soft and tenacious nature. It was anticipated that the ability of side-cholesterol groups to form liquid crystalline phases could provide a driving force for the intermolecular self-assembly of the copolymers discussed here. In other words, the resulting cholesterol liquid crystalline phases should act as physical cross-linking points to form noncovalent network structures among the copolymer chains. Besides the self-assembling ability, cholesterol has high thermodynamic affinity for the cell membrane and ability to change the membrane's permeability and fluidity.[22,23] These characteristics make cholesterol an interesting component of bioactive biomaterials since it could have universal effects regardless of cell type and receptor map on the membrane. Thus, we propose that a biodegradable material exhibiting a soft and tenacious nature based on cholesterol liquid crystalline phases is a promising candidate for new class of tissue engineering cellular scaffold used with dynamic organs of the body such as the heart and blood vessels.

Herein, we discussed the synthesis and characterization of cholesterol side-functionalized poly(depsipeptide-*co*-DL-lactide) copolymers, preparation of copolymer films exhibiting liquid crystalline phases, their thermodynamic and mechanical properties, and their in vitro biodegradation profile.

Experimental Section

Materials. DL-Lactide (DL-LA) was purchased from PURAC and used without further purification. Cholesterol, poly(L-lactide) (P-L-LA) ($M_n = 3.0 \times 10^4$, $M_w/M_n = 1.5$), and poly(DL-lactide) (P-DL-LA) ($M_n = 3.2 \times 10^4$, $M_w/M_n = 1.6$) were purchased from Sigma Chemical Co. (St. Louis, Mo) and used without further purification. All other reagents were purchased from Wako Pure Chemical Co. (Tokyo) and used without further purification.

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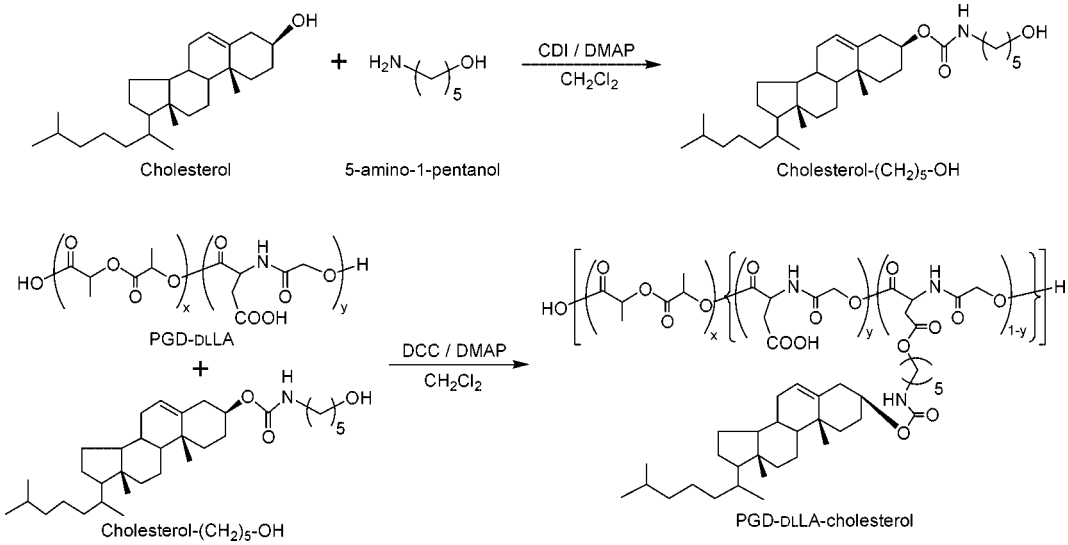
Scheme 1. Synthetic Route of the PGD-DL-LA-(cholesterol)$_{13}$ Conjugate



Measurements. IR spectra were recorded with a Perkin-Elmer 1600 series FTIR. ^{1}H NMR spectra were recorded on a JEOL GSX-400 using tetramethylsilane (TMS) as internal reference. Gel permeation chromatography (GPC) analysis was performed on a Tosoh GPC-8020 series system (column, TSK-GEL α-5000 × 2; eluent, *N*,*N*-dimethylformamide (DMF); detector, RI; standard, PEG). The thermal properties of the polymer films were measured by differential scanning calorimetry (DSC, Shimadzu DSC-60, TA-60WS) under nitrogen gas flow (40 cm^3/min). The temperature range was between −50 and 200 °C at a heating rate of 10 °C/min. The optical properties of polymer films were measured with an optical microscope (Olympus BHS-751P) under cross-polarization conditions at 25 °C. The dynamic contact angles of the air side of the polymer films against water were measured by the sessile drop method at 25 °C with the help of a CCD camera. The average values were calculated from measurements at 15 different points on the films excluding the maximum and minimum values. Tensile tests of polymer films (25 × 7.5 × 0.1 mm) were performed using AUTOGRAPH AGS-J series equipment (Shimadzu) with an elongation rate of 1 mm/min at 25 °C and 37 °C.

Synthesis of Poly[(Glc-Asp)-*r*-(DL-lactide)] Copolymer. Poly[(Glc-Asp)-*r*-(DL-lactide)] (PGD-DL-LA) copolymer was synthesized through bulk ring-opening copolymerization of DL-lactide (3.56 g, 24.8 mmol) and cyclo[Glc-Asp(OBzl)] (720 mg, 2.75 mmol) using tin 2-ethylhexanoate (11.1 mg, 27.5 μmol) as a catalyst according to the same method reported previously.[19] Moreover, poly(Glc-Asp) (PGD) homopolymer was synthesized through bulk ring-opening homopolymerization of cyclo[Glc-Asp(OBzl)] (2.50 g, 9.51 mmol) using tin 2-ethylhexanoate (3.9 mg, 9.51 μmol) as a catalyst. Deprotection reaction of the poly{[Glc-Asp(OBzl)]-*r*-(DL-lactide)} and poly[Glc-Asp(OBzl)] polymers was carried out by acid treatment with a mixture of trifluoromethane sulfonic acid, thioanisol, and trifluoroacetic acid. The purification of the reaction mixture was performed three times by a reprecipitation method using chloroform as a solvent and diethyl ether as a nonsolvent and dried under vacuum overnight to give PGD-DL-LA and PGD. The degree of polymerization of DL-LA and cyclo(Glc-Asp) and molecular composition of the obtained polymers were estimated from ^{1}H NMR measurement. The number averaged molecular weight (M_n) and the molecular weight distribution (M_w/M_n) of the polymers were estimated by GPC.

Synthesis of a Cholesterol Derivative with a Primary Hydroxyl Group. A cholesterol derivative with an alkyl spacer and end primary hydroxyl group (cholesterol−C_5H_{10}−OH) was prepared through a coupling reaction between the hydroxyl group of the original cholesterol and the amino group of 5-amino-1-pentanol. Cholesterol (1.0 g, 2.59 mmol) and *N*,*N*′-carbonyldiimidazole (504 mg, 3.10 mmol) (CDI) were dissolved in anhydrous CH_2Cl_2 (10 mL) in a pear-shaped flask equipped with a magnetic stirring bar. This solution was cooled to 0 °C and stirred for 4 h. Then 5-amino-1-pentanol was added into the reaction flask. The mixture was further stirred at room temperature for 24 h. After the reaction, the reaction mixture was washed with pure water three times. The resulting organic phase was treated with anhydrous $MgSO_4$ and concentrated in vacuo to give white powder of cholesterol−C_5H_{10}−OH (78% yield). Mp: 145 °C. FTIR (KBr, cm^{-1}): 3390 (bd, −OH, ν_s); 2937, 2868 (−CH_2−, ν_s); 1695 (C═O, ν_s). ^{1}H NMR ($CDCl_3$, ppm): 0.68 (s, 3H, cholesterol C*H*$_3$), 2.19–2.42 (br, 2H, cholesterol C*H*$_2$), 5.37 (s, 1H, cholesterol C*H*), 3.18 (br, 2H, C*H*$_2$NH), 3.64 (br, 2H, C*H*$_2$OH).

Synthesis of PGDDLLA-(Cholesterol)$_n$ Conjugate. PGD-DL-LA-(cholesterol)$_n$ and PGD-(cholesterol)$_n$ conjugates were synthesized through a coupling reaction between the carboxyl side groups of the polymers and the hydroxyl end group of the cholesterol derivatives. In brief, *N*,*N*-dicyclohexyl carbodiimide (DCC) (74 mg, 0.36 mmol) was added to an ice-cooled solution of the PGD-DL-LA (500 mg, 0.30 mmol of COOH), cholesterol−C_5H_{10}−OH (180 mg, 0.36 mmol), and 4-(dimethylamino)pyridine (DMAP) (12 mg, 0.10 mmol) in anhydrous CH_2Cl_2 (5 mL). The reaction solution was stirred at 0 °C for 4 h and then at room temperature for 20 h. Precipitated dicyclohexylurea was removed by filtration, and the filtrate was pored into a *n*-hexane/ethanol mixture (8/2) to remove the excess cholesterol−C_5H_{10}−OH molecules and to give a white precipitate of PGD-DL-LA-(cholesterol)$_n$ conjugate (89% yield). PGD-(cholesterol)$_n$ conjugate was obtained by the same procedure described above (92% yield).

Preparation of the Polymer Film. The PGD-DL-LA-(cholesterol)$_n$ and the PGD-(cholesterol)$_n$ conjugate films were prepared by a solution cast method. A chloroform solution of the PGD-DL-LA-(cholesterol)$_n$ conjugate (4 wt %) was cast on a Teflon dish (50 mm diameter), and then the solvent was allowed to evaporate for 12 h at room temperature (25 °C). The obtained films were further dried under vacuum at 25 °C for 48 h. The thickness of the conjugate films is in the range of 100–110 μm. As the control film, PGD-DL-LA, P-DL-LA, and P-L-LA films were prepared using the same procedure described above.

Biodegradation Test of the Conjugate Film. Biodegradation behavior of the PGD-DL-LA-(cholesterol)$_n$ conjugate film was studied by weight loss and molecular weight reduction. The conjugate film (7 × 7 × 0.1 mm) was weighed after thorough drying (W_0) and incubated in 10 mL of 1/15 M KH_2PO_4−Na_2HPO_4 buffer (PBS; pH 7.0, I = 0.14) at 37 °C for 50 days. PBS was refreshed every 2 days to keep the constant pH values during hydrolysis test. After 1, 2, 4, 7, 14, 28, and 50 days, the conjugate film was washed with pure water and dried in vacuo, and then the film was weighed again (W_t). The weight loss was determined as follows: Weight loss (%) = $[(W_0 - W_t)/W_0)] \times 100$. The kinetics of ester bond hydrolysis (molecular weight reduction

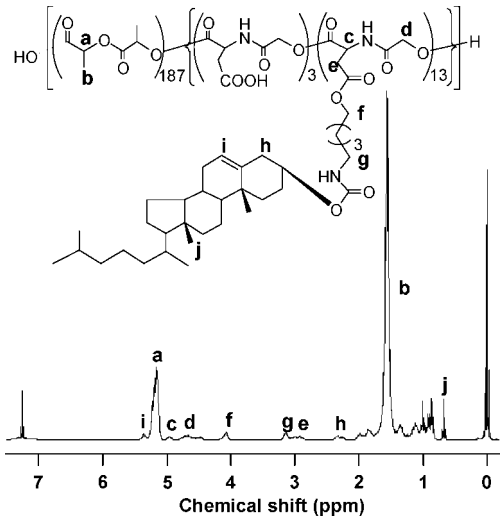


Figure 1. ^{1}H NMR spectrum of the PGD-DL-LA-(cholesterol)$_{13}$ conjugate ($CDCl_3$ solvent).

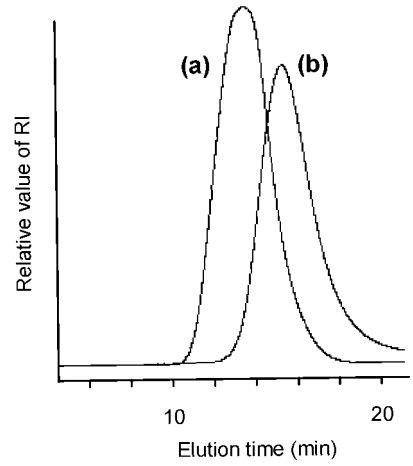


Figure 2. GPC curves of PGD-DL-LA-(cholesterol)$_{13}$ (a) and PGD-DL-LA polymers (b).

of the conjugate was studied in PBS at 37 °C. At predetermined time periods, the conjugate film was washed with pure water and dried in vacuo and the M_n of the conjugate after hydrolysis was determined by GPC measurement. Molecular weight reduction of the conjugate was calculated from the initial molecular weight (M_0) and the molecular weight after hydrolysis (M_t): molecular weight reduction (%) = $[(M_0 - M_t)/M_0)] \times 100$.

Results and Discussion

Synthesis and Characterization of PGD-DL-LA-(cholesterol)$_n$ Conjugate. Synthetic route of the PGD-DL-LA-(cholesterol)$_n$ copolymer is shown in Scheme 1. First, the cholesterol−C_5H_{10}−OH derivative was prepared by a coupling reaction of the hydroxyl group in cholesterol and the amino group of 5-amino-1-pentanol. The IR spectrum of the cholesterol−C_5H_{10}−OH derivative showed an absorption peak at 1695 cm^{-1}, which was assigned to C═O stretching of the urethane bond (Figure S1 in Supporting Information). The integral ratio of methyl peak of cholesterol (0.68 ppm) to methylene peak of 5-amino-1-pentanol (3.64 ppm) was almost 3:8 in the ^{1}H NMR spectrum of the synthesized cholesterol−C_5H_{10}−OH. These results proved that the cholesterol−C_5H_{10}−OH derivative was successfully synthesized. Next, the cholesterol−C_5H_{10}−OH molecules were reacted with PGD-DL-LA or PGD polymers though a coupling reaction between the end hydroxyl group of cholesterol−C_5H_{10}−OH and side carboxyl group of the PGD-DL-LA or PGD polymers. The degree of polymerization of DL-LA and cyclo(Glc-Asp) and molecular composition of the obtained polymers were estimated from the ^{1}H NMR measurement in chlorofolm-d_3. The number averaged molecular weight (M_n) and the molecular weight distribution (M_w/M_n) of the polymers were estimated by GPC. Figure 2 shows typical GPC curves of the synthesized polymers. All polymers gave a single peak in the GPC curve, indicating the absence of monomers and uncoupled cholesterol−C_5H_{10}−OH molecules. The characteristics of the obtained polymers are summarized in Table 1. Figure 1 shows a ^{1}H NMR spectrum of the resulting PGD-DL-LA-(cholesterol)$_n$ conjugate. The average number of introduced cholesterol groups per a PGD-DL-LA or PGD backbone was estimated from the integral ratios of peak d (4.72 ppm, −CH_2O− in PGD-DL-LA or PGD) and peak j (0.68 ppm, −$C(CH_3)$− in cholesterol) in ^{1}H NMR spectrum, and they were found to be 13 for PGD-DL-LA [PGD-DL-LA-(cholesterol)$_{13}$] and 11 for PGD [PGD-(cholesterol)$_{11}$], respectively. So, we calculated molar percentage of the cholesterol groups in the conjugate. The cholesterol unit contents were found to be 14 wt % for the PGD-DL-LA-(cholesterol)$_{13}$ and 55 wt % for the PGD-(cholesterol)$_{11}$, respectively. These cholesterol unit contents should be high enough to form liquid crystalline phase in the polymer films. These conjugates were well dissolved in various organic solvent such as chloroform, acetone, DMSO, DMF, THF, and so on. The solution cast films having a thickness ca. 100 μm were prepared from these conjugates and used in subsequent experiments on their physicochemical properties, self-assembling behavior, optical micrographic observation, and degradation behavior. Besides the conjugate films, the polymer films were also prepared from the PD-L-LA and PGD-DL-LA without side cholesterol groups as the control, and their characters were compared with those of the conjugate films.

Liquid Crystalline Phases in Conjugate Films. Thermodynamic properties of the polymer films were characterized by DSC measurements, as listed in Table 2. T_g value of the PGD-DL-LA film was lower than that of P-DL-LA film owing to random copolymerization with depsipeptide unit. Interestingly, a meaningful drop in T_g value was also observed when a certain number of cholesterol groups were conjugated to the PGD-DL-LA film. In other words, the T_g value of the PGD-DL-LA-(cholesterol)$_{13}$ film were almost 11 °C lower than that of the PGD-DL-LA film. The result indicates that the side cholesterol groups introduced to the PGD-DL-LA backbone act effectively as internal plasticizer. Figure 3 shows the DSC curves of the conjugate and the polymer films. In both PGD-DL-LA and P-DL-LA films, T_m was not detected, indicating the absence of crystalline phases in these polymer films. On the other hand, obvious endothermic peak were observed at 117 °C in PGD-DL-LA-(cholesterol)$_{13}$ film, indicating the presence of crystalline phases in the film. Considering the T_m value of general PLA (about 150 °C), it is very difficult to suppose this endothermic peak to be PLA crystalline phases. Accordingly, the crystalline phases should correspond to self-assembled crystalline phases composed of side cholesterol molecules in PGD-DL-LA-(cholesterol)$_{13}$ conjugates. In order to analyze the self-assembled morphology of the cholesterol molecules, polarized optical microscopic observation of the PGD-DL-LA-(cholesterol)$_{13}$ films was performed, as shown in Figure 4. Both PGD-DL-LA and P-DL-LA films showed dark images, indicating isotropic character (panels C and D of Figure 4). On the other hand, we

Table 1. Characteristics of the Synthesized Polymers

polymer	$X^{a,b}$ (%)	$M_n{}^c \times 10-4$ (M_w/M_n)^c	av no. of cholesterol units^b	cholesterol content^b (wt %)
PGD-DL-LA-(cholesterol)$_{13}$	7.7	3.6 (3.2)	13	14
PGD-DL-LA	8.7	3.1 (1.8)	0	0
P-DL-LA	0	3.2 (1.6)	0	0
PGD-(cholesterol)$_{11}$	100	0.77 (1.7)	11	55

[a] *X* means the depsipeptide unit content in copolymer. [b] Estimated by ^{1}H NMR spectroscopy ($CDCl_3$ solvent). [c] Estimated by GPC (eluent, DMF; standard, PEG).

Table 2. Results in DSC Measurement and Dynamic Water Contact Angles[b] of PGD-DL-LA-(cholesterol)$_{13}$, PGD-DL-LA, P-DL-LA, and PGD-(cholesterol)$_{11}$ Films

sample film	$T_g{}^a$ (°C)	$T_m{}^a$ (°C)	θ_d (deg)	θ_{adv} (deg)	θ_{rec} (deg)	hysteresis (deg)
PGD-DL-LA-(cholesterol)$_{13}$	12.0	117	70.9 ± 1.9	85.6	56.2	29.4
PGD-DL-LA	32.5	N.D.[c]	62.3 ± 2.2	75.0	50.1	24.9
P-DL-LA	41.2	N.D.[c]	64.9 ± 3.1	78.1	51.8	26.3
PGD-(cholesterol)$_{11}$	63.2	202				

[a] Determined by DSC. The sample was heated at 10 °C/min. [b] Dynamic contact angles were measured at 25 °C. θ_d = dynamic contact angle; θ_{adv} = advancing contact angle; θ_{rec} = receding contact angle; hysteresis = $\theta_{adv} - \theta_{rec}$. [c] Not detected.

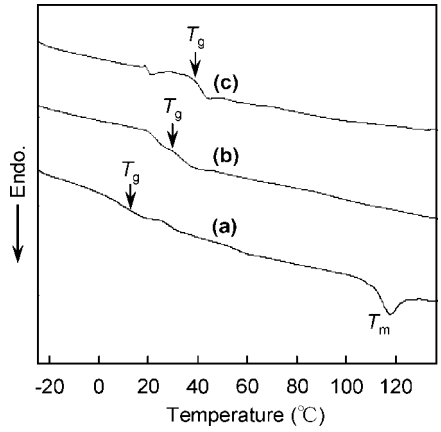


Figure 3. DSC curves of PGD-DL-LA-(cholesterol)$_{13}$ (a), PGD-DL-LA (b), and P-DL-LA (c) films.

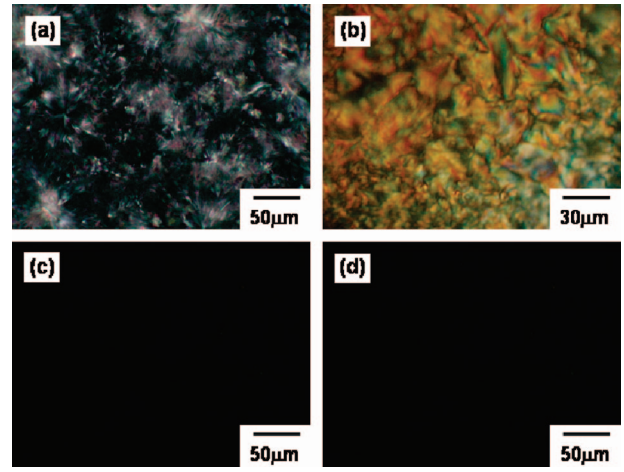


Figure 4. Polarized optical microscopic images of PGD-DL-LA-(cholesterol)$_{13}$ (a), PGD-(cholesterol)$_{11}$ (b), PGD-DL-LA (c), and P-DL-LA (d) films at 25 °C.

dispersed birefringent texture was observed for PGD-DL-LA-(cholesterol)$_{13}$ film under cross polarization at 25 °C (Figure 4A). When the temperature was raised up to 130 °C (above T_m observed in DSC curve), the birefringent texture disappeared immediately. These observations clearly prove self-assembling behavior of the side cholesterol groups in PGD-DL-LA-(cholesterol)$_{13}$ conjugate and fabrication of thermotropic liquid crystalline phases in this system. The size of these liquid crystalline phases ranged from 10 to 40 μm, indicating the intermolecular self-assembly behavior. To the best our knowledge, this is the first report of fabrication of thermotropic liquid crystalline phases via self-assembly of cholesterol side-functionalized synthetic biodegradable polymer.

Interestingly, continuous liquid crystalline phases were observed for PGD-(cholesterol)$_{11}$ film at 25 °C (Figure 4B), although the cholesterol unit content in PGD-(cholesterol)$_{11}$ conjugate is 55 wt %. This result clearly supports the formation of hierarchical organized liquid crystalline phases composed of PGD backbone and side cholesterol groups. Moreover, the liquid crystalline phases were found to be quite stable, and PGD-(cholesterol)$_{11}$ film exhibited the obvious birefringent texture up to 202 °C. This significantly higher thermodynamic stability is rather unique. Consequently, the continuous liquid crystalline phases consisting of biodegradable PGD-(cholesterol)$_{11}$ conjugate are a very important factor for construction of cell layers onto material surface as tissue engineering scaffold.

Investigating the interaction between water and any material to be used as a biological implant is very important. So, we evaluated the dynamic water contact angles of the polymer film surfaces and also confirmed the influence of liquid crystalline phases on the surface wettability. The surface contact angles of the polymer films are listed in Table 2. We certainly confirmed that all polymer films used in this study had flat surfaces without porous structure in the bulk phase (data not shown). The values of the water contact angles of PGD-DL-LA-(cholesterol)$_{13}$, PGD-DL-LA, and P-DL-LA films were found to be 70.9°, 62.3°, and 64.7°, respectively. On comparison between PGD-DL-LA and P-DL-LA films, it was revealed that the introduction of depsipeptide units into P-DL-LA hardly affected the surface contact angles. Moreover, the PGD-DL-LA-(cholesterol)$_{13}$ film was found to exhibit significant higher contact angle than PGD-DL-LA film. These results strongly support that this difference in contact angle should be mainly attributed to the liquid crystalline phases self-assembled at around the film surface. Thus, PGD-DL-LA-(cholesterol)$_{13}$ and PGD-(cholesterol)$_{11}$ films having surface liquid crystalline phases consisting of cholesterol molecules are expected to show effective cellular attachment.

Tensile Property of Liquid Crystalline Polymer Films. Tensile properties of dry polymer films were evaluated from their stress−strain curves, as shown in Figure 5 and Table 3. PGD-DL-LA and P-DL-LA films exhibited similar tensile properties at 25 °C. These films and P-L-LA film (Figure S3, Supporting Information) showed a distinct yield point in an early

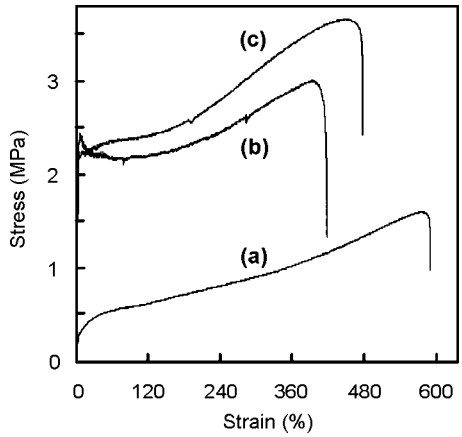


Figure 5. Stress−strain curves of PGD-DL-LA-(cholesterol)$_{13}$ (a), PGD-DL-LA (b), and P-DL-LA (c) films at 25 °C.

Table 3. Results in Tensile Test for PGDDLLA-(cholesterol)$_{13}$, PGDDLLA, and PDLLA films at 25 °C

sample film	Young's modulus (MPa)	tensile strength (MPa)	strain at break (%)
PGD-DL-LA-(cholesterol)$_{13}$	0.86	1.56	591
PGD-DL-LA	360	2.96	418
P-DL-LA	432	3.61	478
P-L-LA	1445	31.5	4.15

stage of the elongation process (within 20%), indicating that plastic deformation was derived over the yield point by further elongation. However, a yield point was not observed in the stress−strain curve for PGD-DL-LA-(cholesterol)$_{13}$ film, and the film showed the much lower Young's modulus (0.86 MPa) than those of PGD-DL-LA (360 MPa), P-DL-LA (432 MPa), and P-L-LA (1445 MPa) films. Moreover PGD-DL-LA-(cholesterol)$_{13}$ film showed the most highest elongation at break (591%) among the three polymer film used in this study. Consequently, PGD-DL-LA-(cholesterol)$_{13}$ film was found to exhibit not only a soft but also an almost tenacious nature at 25 °C. These results strongly support that the intermolecular self-assembled liquid crystalline phase is very operative for their soft and rubbery nature because the cholesterol liquid crystalline phases well-dispersed in the film act as internal plasticizer and physical cross-linking points to construct noncovalent network structures among PGD-DL-LA chains. When the P-DL-LA film is stretched continuously, the interchain overlapping and entangling of P-DL-LA molecules gradually come loose and then the film is broken. On the contrary, the physical cross-linking points based on cholesterol liquid crystalline phase in PGD-DL-LA-(cholesterol)$_{13}$ film can endow a rubbery and stretchy nature to the film. It should be noted that PGD-DL-LA-(cholesterol)$_{13}$ film exhibited relatively low Young's modulus and quite high elongation at break (710%) at 37 °C (Figure S2 in Supporting Information). The tensile property of PGD-DL-LA-(cholesterol)$_{13}$ film at 37 °C is very important for an ideal cellular scaffold used with dynamic organs of the body such as the heart and blood vessels and for a drug encapsulating film to cover a stent.

Biodegradation Behavior of Liquid Crystalline Polymer Films. Since PGD-DL-LA-(cholesterol)$_{13}$ and PGD-DL-LA polymers actually had the same molecular weight of PGD-DL-LA segment, it was expected that both polymer films would show a similar degradation profile. However, PGD-DL-LA film dissolved in PBS at 37 °C within 1 day. Therefore, biodegradation behavior of the PGD-DL-LA-(cholesterol)$_{13}$ film was investigated by the in vitro hydrolysis test. Parts A and B of

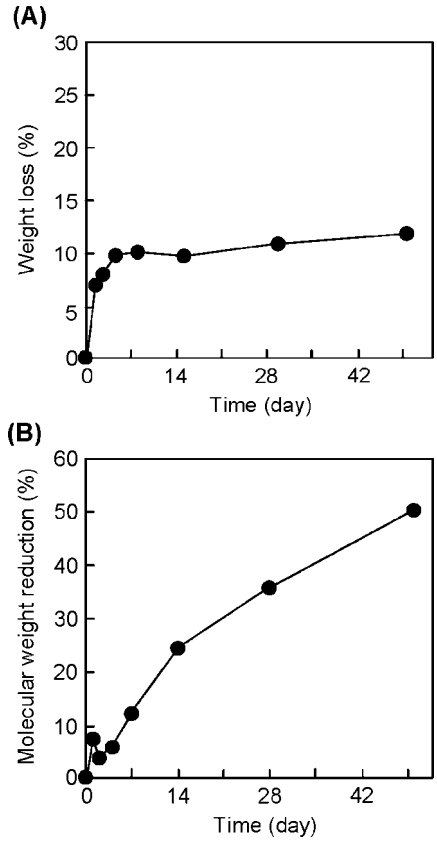


Figure 6. (A) Weight loss and (B) molecular weight reduction of PGD-DL-LA-(cholesterol)$_{13}$ film in PBS at 37 °C in vitro.

Figure 6 show the time course curves of the weight loss and molecular weight reduction of the conjugate film, respectively. PGD-DL-LA film completely dissolved in PBS within 24 h, although almost 10% of the PGD-DL-LA-(cholesterol)$_{13}$ film eroded away by 50 day through hydrolysis of the polymer chain. The difference in elusion profile between PGD-DL-LA-(cholesterol)$_{13}$ and PGD-DL-LA films may be related to the increase in hydrophobicity by introduction of cholesterol groups on the PGD-DL-LA molecule. However, the significant difference in elusion profile among polymer films should be attributed to the cholesterol liquid crystalline phases well-dispersed near the surface of the PGD-DL-LA-(cholesterol)$_{13}$ film. In other words, the physical cross-linking points consisting of cholesterol liquid crystalline phases should prevent elusion of the degradation products.

Notably, PGD-DL-LA-(cholesterol)$_{13}$ film kept its soft and stretchy nature even after 50 days, and the film did not fracture in the least when bent and twisted. The successful maintenance in an original mechanical property even after degradation for PGD-DL-LA-(cholesterol)$_{13}$ film is a great advantage for use as implant biomaterials contacting with soft tissues.

Conclusions

We have designed a series of novel liquid crystalline biodegradable polymer films via self-assembly of cholesterol side-functionalized poly(depsipeptide-*co*-DL-lactide) copolymers in order to create an implant biomaterial exhibiting soft and tenacious nature. These polymer films showed different birefringent textures depending on the cholesterol unit content in the polymers. Interestingly, the thermodynamic stability of these liquid crystalline phases was quite high, and PGD-(cholesterol)$_{11}$ film especially exhibited continuous liquid crystalline phase

up to 202 °C. The physical cross-linking points based on cholesterol liquid crystalline phase in PGD-DL-LA-(cholesterol)$_{13}$ film can endow the rubbery and stretchy nature to the film. The cholesterol side-group effects leading to the rubbery character and hydrolytic resistance reported herein are rather unique. Herein, we propose that the soft and tenacious biodegradable material having cholesterol liquid crystalline phases should be a candidate for tissue engineering of a cellular scaffold used with dynamic organs of the body such as the heart and blood vessels. A future report will detail the results of in vitro protein adsorption, cell attachment, and cell proliferation behaviors using various kinds of cells on the polymer film as well as the three-dimensional matrices to evaluate a potential as cellar scaffold.

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Supporting Information Available. IR spectrum of cholesterol−C_5H_{10}−OH, stress−strain curves of PGD-DL-LA-(cholesterol)$_{13}$ film at 37 °C, and stress−strain curves of P-L-LA film at 25 °C. This material is available free of charge via the internet at http://pubs.acs.org.

References and Notes

(1) Demus, D.; Goodby, J. W.; Gray, G. W.; Spiess, H.-W. *Handbook of Liquid Crystals*; Wiley-VCH, Weinheim, 1998.
(2) *Opin. Solid State Mater. Sci.* **2002**, *6*, 513–587 (Section of Liquid Crystals).
(3) Kato, T. *Science* **2002**, *295*, 2414–2418.
(4) Haaren, J.; Broer, D. J. *Chem. Ind.* **1998**, 1017–1021.
(5) Goodby, J. W.; Mehl, G. H.; Saez, I. M.; Tuffin, R. P. *Chem. Commun.* **1998**, 2057–2070.
(6) Skoulios, A.; Guillon, D. *Mol. Cryst. Liq. Cryst.* **1988**, *165*, 317–332.
(7) Rädler, J. O.; Koltover, I.; Salditt, T.; Safinya, C. R. *Science* **1997**, *275*, 810–814.
(8) Koltover, I.; Salditt, T.; Rädler, J. O.; Safinya, C. R. *Science* **1998**, *281*, 78–81.
(9) Denisov, I. G.; Grinkova, Y. V.; Lazarides, A. A.; Sligar, S. G. *J. Am. Chem. Soc.* **2004**, *126*, 3477–3487.
(10) Ringsdorf, B.; Schlarb, J.; Venzmer, J. *Angew. Chem., Int. Ed.* **1988**, *27*, 113–158.
(11) Kunitake, T. *Angew. Chem., Int. Ed.* **1992**, *31*, 709–726.
(12) Hwang, J. J.; Iyer, S. N.; Li, L. S.; Claussen, R.; Harrington, D. A.; Stupp, S. I. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9662–9667.
(13) Luk, Y. Y.; Tingey, M. L.; Hall, D. J.; Israel, B. A.; Murphy, C. J.; Bertics, P. J.; Abbott, N. L. *Langmuir* **2003**, *19*, 1671–1680.
(14) Brake, J. M.; Abbott, N. L. *Langmuir* **2002**, *18*, 6101–6109.
(15) Zhou, C. R.; Yi, Z. J. *Biomaterials* **1999**, *20*, 2093–2099.
(16) Li, L. H.; Tu, M.; Mou, S. S.; Zhou, C. R. *Biomaterials* **2001**, *22*, 2595–2599.
(17) Zhuo, R.-X.; Cheng, S.-X.; Zou, T. *Colloid Polym. Sci.* **2005**, *283*, 1091–1099.
(18) Wan, T.; Zou, T.; Cheng, S.-X.; Zhuo, R.-X. *Biomacromolecules* **2005**, *6*, 524–529.
(19) Ouchi, T.; Shiratani, M.; Jinno, M.; Hirao, M.; Ohya, Y. *Makromol. Chem. Rapid Commun.* **1993**, *14*, 825–831.
(20) Ouchi, T.; Nozaki, T.; Ishikawa, A.; Fujimoto, I.; Ohya, Y. *J. Polym. Sci. Part A: Polym. Chem.* **1997**, *35*, 377–383.
(21) Ohya, Y.; Matsunami, H.; Yamabe, E.; Oucgi, T. *J. Biomater. Sci., Polym. Ed.* **2004**, *15*, 111–123.
(22) Yeagle, P. L. *Biochim. Biophys. Acta* **1985**, *822*, 267–287.
(23) Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, J. D. *Molecular Biology of the Cell*, 2nd ed.; Garland Publishing Inc.: New York, 1989.

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