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Morphology of Elastic Poly(L-lactide-*co*-ϵ-caprolactone) Copolymers and in Vitro and in Vivo Degradation Behavior of Their Scaffolds

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Very elastic PLCL [poly(L-lactide-*co*-ϵ-caprolactone), 50:50] copolymers were synthesized and extruded into porous tubular scaffolds (pore size 150 ± 50 μm, porosity 90%) for the application to tissue engineering. The copolymers were basically random and amorphous. However, two T_g's (glass transition temperatures) were observed in dynamic mechanical thermal analysis and also in differential scanning calorimetry thermograms. Furthermore, microdomains (about 17 nm in size) were indicated on the small-angle X-ray scattering profile and finally confirmed by transmission electron microscopy. Therefore, the PLCL copolymer was probably composed of a soft matrix of mainly ϵ-caprolactone moieties and hard domains containing more L-lactide units to exhibit a rubberlike elasticity in virtue of the physically cross-linked structure. The smooth muscle cells seeded scaffolds were implanted into nude mice subcutaneously for up to 15 weeks to monitor the in vivo degradation. In addition, they were degraded in vitro in phosphate buffer solution (pH 7.4) for up to 1 year to compare the results each other. All the scaffolds degraded slowly in vivo and in vitro even in the form of a highly porous thin membrane. However, the degradation rate was somewhat faster for in vivo than for in vitro. This should be explained by enzymes that might have played a certain role in the degradation in the body. In addition, the ϵ-caprolactone moieties degraded faster than the L-lactide units did in these PLCL scaffolds, although their hydrophilicities are in the opposite order. This behavior appeared more prominently in the in vivo case. This should result from that the amorphous regions composed of mainly ϵ-caprolactone units might have been first attacked by water because water can penetrate into the amorphous regions easier than the hard domains containing more L-lactides.

Introduction

Synthetic biodegradable polymers have been widely utilized in medical applications such as sutures, orthopedics, drug delivery systems, and recently scaffolds for tissue engineering.[1−5] Those include polyglycolide (PGA), polylactide (PLA), poly(ϵ-caprolactone) (PCL), and their copolymers. For scaffolds in tissue engineering, a proper mechanical stability, degradation rate, and cyto-compatibility to direct specific cell growth and differentiation are needed.[5] Recently, several studies have demonstrated that mechanical signals play a critical role in engineering functional smooth-muscle (SM) or SM-containing tissues (e.g., blood vessels). Mechanical stimulation increased collagen production and induced the phenotype of SMCs (smooth muscle cells) in engineered SM tissues to be more consistent with a contractile, differentiated phenotype.[6−10] To engineer any tissue under conditions of cyclic mechanical strain (mechano-active tissue engineering), it would be necessary to utilize soft but elastic scaffolds.[9−10] In our previous reports, we synthesized very elastic biodegradable poly(glycolide-*co*-ϵ-caprolactone) (50:50)[11] or PLCL [poly(L-lactide-*co*-ϵ-caprolactone), 50:50] scaffolds.[12] Those scaffolds were very flexible but rubberlike elastic to maintain a complete recovery even under cyclic loading in culture media for up to an initial 2 weeks. For efficient tissue engineering, the scaffolds should maintain their mechanical stability for the initial period but be degraded properly and replaced by new tissues. In addition, those PLCL scaffolds demonstrated excellent compatibility for SMCs when implanted in vivo.[13] Therefore, in the present work, the in vivo degradation behavior of PLCL scaffolds seeded with SMCs was investigated more in detail and compared with the degradation of those in vitro. In addition, the morphology of PLCL was analyzed to explain the rubberlike elasticity.

Experimental Section

Materials. L-Lactide (LA; Purac Biochem, The Netherlands) was purified by recrystallization from dried ethyl

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acetate and thoroughly dried for 24 h under a vacuum prior to use. ϵ-Caprolactone (CL; Aldrich) was dried over calcium hydride for 24 h at room temperature and then distilled under reduced pressure (ca. 0.3 mmHg) at 55 °C. Stannous octoate (Sigma) was purified by vacuum distillation at 175 °C (ca. 0.2 mmHg). 1,6-Hexanediol was distilled over calcium hydride too. Toluene was thoroughly dried by distillation over sodium and benzophenone. All other chemicals and solvents were of analytical grade and used without further purification.

Preparation of PLCL (50:50) and Its Scaffolds. The synthesis of PLCL and the preparation of tubular scaffolds were described in detail elsewhere.[12] Briefly, LA (100 mmol) and CL (100 mmol) were polymerized at 150 °C for 24 h in a 50-mL glass ampule containing 1,6-hexanediol (0.5 mmol) and stannous octoate (1 mmol). After reaction, the product was dissolved in chloroform and precipitated in methanol, filtered, and dried under a vacuum.

PLCL tubular scaffolds were prepared by an extrusion−particulate leaching technique.[12] PLCL solution in chloroform (1% w/v) was added by NaCl particles (100−200 μm, the ratio of salt to polymer was 90:10 by weight) and extruded to tubes (inner diameter 4 mm, outer diameter 6 mm, thickness 1 mm) by a custom-designed piston extrusion tool. The solvent was evaporated for 48 h at room temperature and then completely under vacuum for 24 h. The resulting PLCL/salt composite scaffolds were leached in distilled water with shaking for 3 days. The salt-free PLCL scaffolds were freeze-dried for 24 h and sterilized with ethylene oxide gas.

Measurements. ^{1}H NMR spectra were recorded at room temperature with a Varian Unity-300 (300 MHz) in $CDCl_3$. The IR spectroscopy was carried out with a 2000 FT-IR apparatus (Perkin-Elmer). Number-average (M_n) and weight-average (M_w) molecular weights were determined using a gel permeation chromatograph (Waters 410, Milford, MA) equipped with microstyragel columns calibrated with polystyrene standards. Chloroform was used as a mobile phase at a flow rate of 1.0 mL/min at 30 °C. The DSC (differential scanning calorimetry) study was conducted by TA5000/DSC2950 (TA Instrument, Inc.) with a heating rate of 10 °C/min. T_g's (glass transition temperatures) were taken from the midpoints of the transition zone. DMA (dynamic mechanical thermal analysis) was studied by Rheometrics Scientific IV in a single cantilever bending mode with rectangular samples (10 mm × 20 mm × 2 mm). Temperatures were scanned from −120 to 50 °C at a 5 °C/min heating rate and 1-Hz frequency. Small-angle X-ray diffraction measurements were carried out using a synchrotron radiation X-ray beam, beamline 4C1 at Pohang Accelerated Beam Center (Korea). The incident beam intensity, with a wavelength of 0.149 nm, was monitored using an ionization chamber to correct minor decreases in the primary beam intensity. The TEM (transmission electron microscopy) image was obtained using a JEOL1210 transmission electron microscope operated at 120 kV. The tensile properties of scaffolds were measured by Instron model 5567 with a 10-N load cell at a cross-head speed of 1 mm/min. Recovery after tension was calculated by the following formula: recovery (%) = recovered length/original length × 100. The pore diameter and porosity of PLCL scaffolds were measured from a scanning electron microscope (Hitachi, Tokyo) and a mercury intrusion porosimeter (Micromeritics, GA), respectively. For scanning electron microscopy (SEM) imaging, samples were coated with gold using a sputter coater (Eiko IB3, Tokyo) operated at 15 kV.

In Vitro Biodegradation Test. The tubular PLCL scaffolds were cut into pieces (length 5 cm, $n = 4$) and placed in closed bottles containing 50 mL of PBS (phosphate buffer solution, pH 7.4) on a shaker table set at 60 rpm at 37 °C for up to 1 year. The buffer solution was changed every week. After 1, 3, 5, 8, 15, 20, 40, and 52 weeks, the scaffolds were taken out, washed with distilled water, and dried in a vacuum. The specimens were weighed and analyzed by gel permeation chromatography (GPC) and NMR.

In Vivo Implantation Test. The cylindrical PLCL scaffolds (length 1 cm) were seeded with SMCs and implanted into the subcutaneous dorsum of 5-week-old male athymic mice (Japan SLC, Inc., two mice, two specimens per mouse). The procedure of cell isolation from rabbit aortas and culture was explained in detail elsewhere.[12,13] In brief, the descending aortas were incubated with collagenase/elastase enzymes and the resultant cell suspension was separated and cultured in a growth medium consisting of medium 199 (Gibco) supplemented with fetal bovine serum, L-glutamine, penicillin, and streptomycin. Each scaffold was inoculated by a cultured SMCs (passage number = 2) suspension in M199 (1.5×10^8 cells/mL), then incubated in the culture medium for 5 h at 37 °C, and subsequently transplanted into the mice. Implants were harvested after 1, 2, 5, 8, and 15 weeks to measure the dried weight, and the polymeric scaffolds were extracted by chloroform for 5 h and analyzed by GPC and NMR.

Results and Discussion

Characterization of PLCL. Random PLCL copolymers were synthesized by a ring opening polymerization of LA and CL with 1,6-hexanediol in the presence of stannous octoate (Figure 1). All the polymerizations were conducted in the bulk with continuous stirring. Under these conditions, high conversion in a homogeneous state is expected after a 24-h reaction time. The polymerizations were carried out at 150 °C with a constant molar feed ratio (f_{LA}/f_{CL}) of 5.0:5.0. At temperatures lower than 140 °C, the incorporation of CL units into polymer chains was incomplete because of the low reactivity of CL. The overall yield of the PLCL copolymers was 90%. The PLCL used for the in vitro degradation study had a M_n, M_w, and M_w/M_n of 167 000, 224 000, and 1.34, respectively, and the other one implanted had 186 000, 305 000, and 1.64, respectively (Table 1), although molecular weights obtained by GPC using polystyrene standards are generally overestimated approximately two times as reported.[14] The mole ratio of LA to CL in the copolymer (F_{LA}/F_{CL}) was 5.1:4.9 for both PLCLs. The structure of PLCL was analyzed by ^{1}H NMR (Figure 1). The methylene protons of the ϵ-caproyl unit beside the ester oxygen atom (Figure 1c′,c) and carbonyl group (Figure 2g′,g) appeared at δ 4.3−4.0 and δ 2.5−2.3, respectively, where c′ represents CH_2

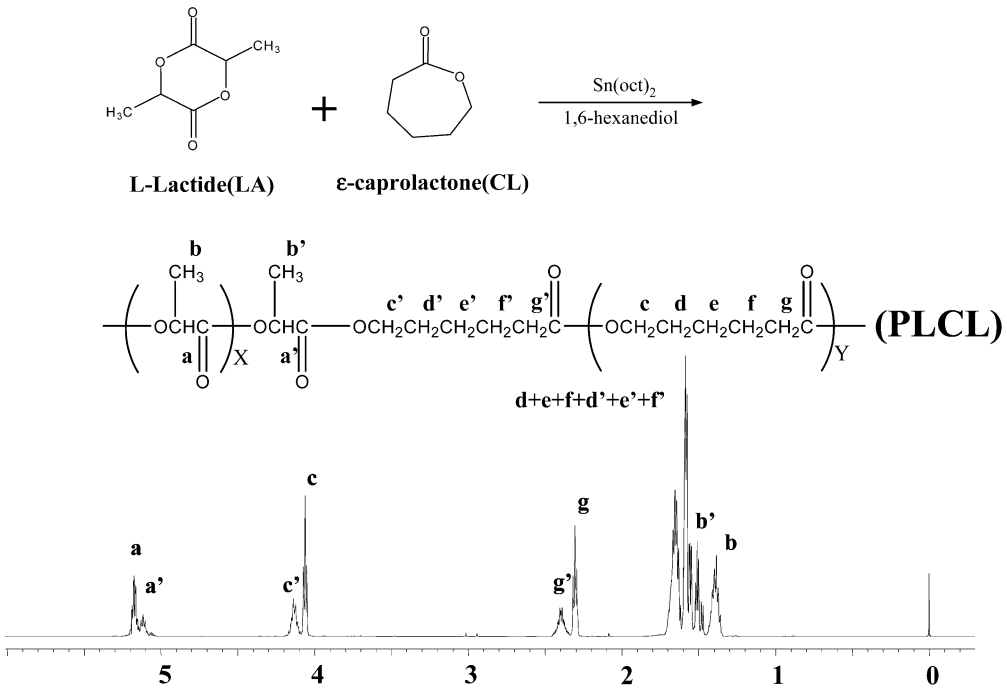

Figure 1. Preparation and ^{1}H NMR spectrum of PLCL (50:50) copolymer, polymerized by stannous octoate in melt at 150 °C for 24 h.

Table 1. Characterization of PLCL and Their Scaffolds Studied in Vitro and in Vivo Degradation (Porosity 90%, Pore Size 150 ± 50 μm)

	f_{LA}/f_{CL}[a]	F_{LA}/F_{CL}[b]	M_n[c] ($\times 10^3$)	M_w[c] ($\times 10^3$)	M_w/M_n	T_g[d]	tensile strength (MPa)	elongation (%)	recovery (%)
PLCL in vitro	5/5	5.1/4.9	167	224	1.34	−38; −11	0.80	203	98
PLCL in vivo	5/5	5.1/4.9	186	305	1.64	−37; −10	0.81	210	98

[a] Mole ratio in the feed. [b] Mole ratio in the copolymer. [c] Determined by GPC. [d] Determined by DMA.

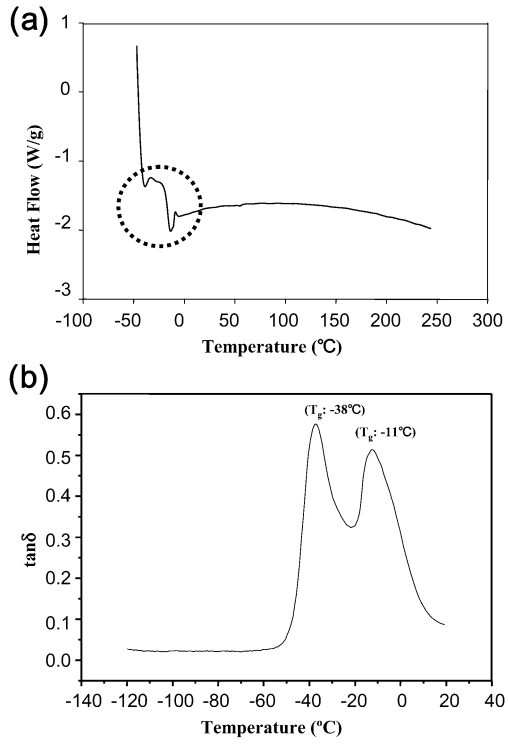

Figure 2. DSC (a) and DMA (b) thermograms of the PLCL (50:50) copolymer showing two glass transition temperatures.

coupled to the LA unit and c indicates CH_2 connected to the other CL unit.[15] However, the IR spectra exhibited only one ester carbonyl absorption band at 1756 cm^{-1} (data were not shown here).

Morphology of PLCL (50:50). As we reported in the previous study,[12] the PLCL (50:50) is a basically soft copolymer but exhibits a completely rubberlike elasticity indicating the presence of physical cross-linking such as in the case of styrene-butadiene-styrene block copolymer. For such a case, the hard domain would be composed of LA moieties. It is well-known that the copolymerization rate of LA is much faster than that of CL,[16−19] although the homopolymerization rate of CL is faster than LA so that LA monomers might have polymerized first to form blocks working as hard domains and the CL units reacted later to form a soft matrix. The synthesis of PLCL and other lactone copolymers often including their degradation behavior has been widely studied in recent years.[16−27] Pennings et al. reported the elastic property of PLCL copolymers first but did not describe the morphology in detail.[25]

The DSC analysis of PLCL copolymer was shown in Figure 2a. There was no crystalline melting peak, and, therefore, it is amorphous, which was expected for a random copolymer. But, its T_g curve was not simple, which indicates maybe two T_g's in the 0 to −40 °C region. However, the DMA profile revealed clearly two T_g's, one at −38 °C and the other at −11 °C, as seen on Figure 2b. This indicates very strongly a phase-separated structure of this PLCL. Because the T_g of PCL and PLA homopolymers were reported as −60 and +50 °C,[26] respectively, the T_g at −38 °C would represent a phase composed of mainly CL units and the T_g at −11 °C indicates the other phase containing more LA moiety.

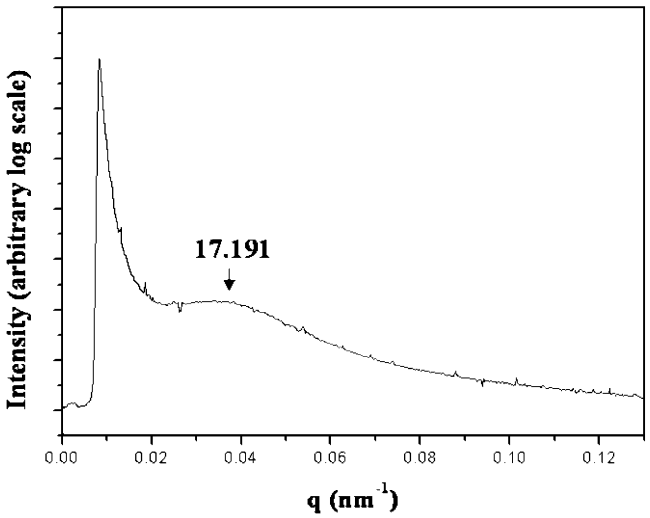

Figure 3. SAXS profile of the PLCL copolymer indicating an ordered structure 17.191 nm in size.

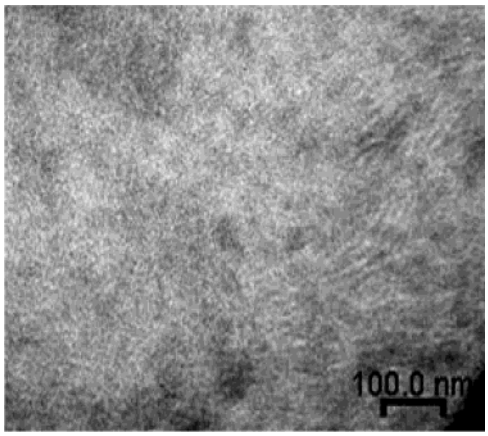

Figure 4. TEM image of the PLCL copolymer; stained by OsO_4, the image was revealed by the density difference of the CL and LA moieties. The domain size was 16.8 nm.

The small-angle X-ray scattering (SAXS) profile in Figure 3 showed a typical amorphous character too, but a wide shoulder was observed to indicate a weakly ordered structure and the size corresponded to 17.2 nm. To investigate the morphology in more detail, thin cast films (thickness ca. 50 nm) stained with OsO_4 were subjected to TEM with a specially accelerated X-ray beam. Under this condition, each CL or LA moiety revealed an image produced by the difference in density. As seen in Figure 4, the TEM image confirmed the presence of domains whose diameters were about 16.8 nm, a similar value to that of the SAXS data. These hard domains should be responsible for the high elasticity of PLCL by acting as physical cross-linkings, as described above.

Table 2. Analysis of Scaffold Materials Degraded in Vitro (in PBS at 37 °C, pH 7.4, $n = 4$) and in Vivo (Implanted Subdermally in Two Nude Mice, Two Specimens per Mouse)

	composition (LA/CL mol %)[a]	M_n[b] ($\times 10^3$)	M_w[b] ($\times 10^3$)	M_n/M_w	mass[c] (%)
control	51.4:48.6	115(100%)	215(100%)	1.82	100
in vitro 1w	51.2:48.8	109(95%)	180(84%)	1.86	99.7
3w	51.0:49.0	96(84%)	165(76%)	1.97	99.5
5w	50.9:49.1	63(55%)	107(68%)	1.95	98.6
8w	50.6:49.4	48(42%)	87(57%)	1.96	98.4
15w	54.5:45.5	41(36%)	80(36%)	2.01	94.3
20w	61.3:39.2	34(30%)	78(36%)	2.01	91.3
40w	68.4:32.6	21(18%)	54(25%)	2.01	78.4
50w	79.3:20.7	5(4%)	8(4%)	1.47	30.1
control	51.0:49.0	186(100%)	305(100%)	1.30	100
in vivo 1w	52.8:47.2	188(101%)	282(92%)	1.50	98.7
3w	54.3:45.7	100(54%)	233(76%)	2.32	96.3
5w	54.1:45.9	90(48%)	210(68%)	2.35	92.1
8w	55.9:44.1	75(40%)	174(57%)	2.43	89.3
15w	58.4:41.6	43(23%)	122(39%)	2.54	81.3

[a] Measured by ^{1}H NMR. [b] Measured by GPC. [c] Mass remained as percent.

Degradation. There have been several reports on the in vitro or in vivo degradation already in the literature. Those works were studied usually in the form of films or sheets but none in the scaffolds shape. Porous scaffolds have a much larger surface area and an interconnected structure so that the degradation would be accelerated compared to films or sheets.[17,18,20,21,27] In the present work, tubular porous PLCL scaffolds were prepared by an extrusion−particulate leaching technique. Their mechanical property and compatibility to SMCs were already reported in our previous study.[12,13] The typical pictures of the scaffolds are shown in Figure 5A−C. They exhibited a homogeneously interconnected porous structure. The average pore size and porosity of the scaffolds were about 150 ± 50 μm and 90%, as determined by SEM and a mercury porosimeter, respectively. The PLCL scaffolds indicated a tensile strength of 0.80 MPa and an elongation of more than 200% so that they were soft and flexible. Nevertheless, they showed a recovery of 98% after 200% elongation (Table 1).

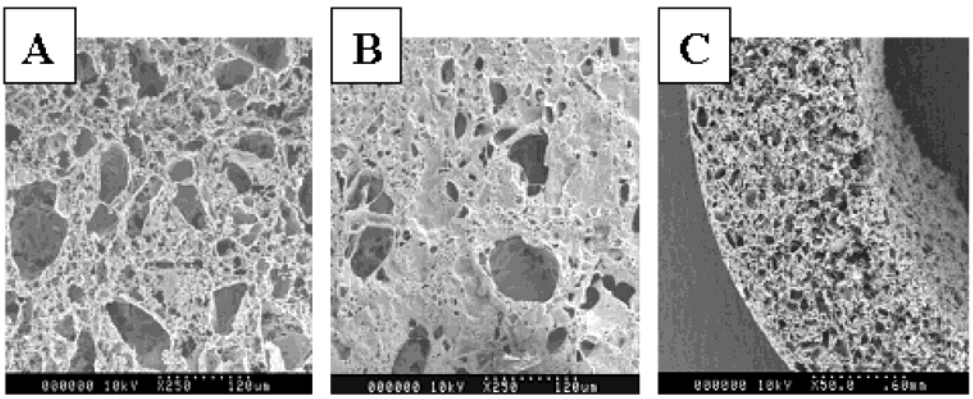

Figure 5. SEM micrographs of tubular PLCL scaffolds (inner diameter 4 mm, outer diameter 6 mm, thickness 1 mm) manufactured by an extrusion−salt particulate leaching method, pore size 150 ± 50 μm, porosity 90%; (A) lumen surface, (B) outer surface, and (C) cross section.

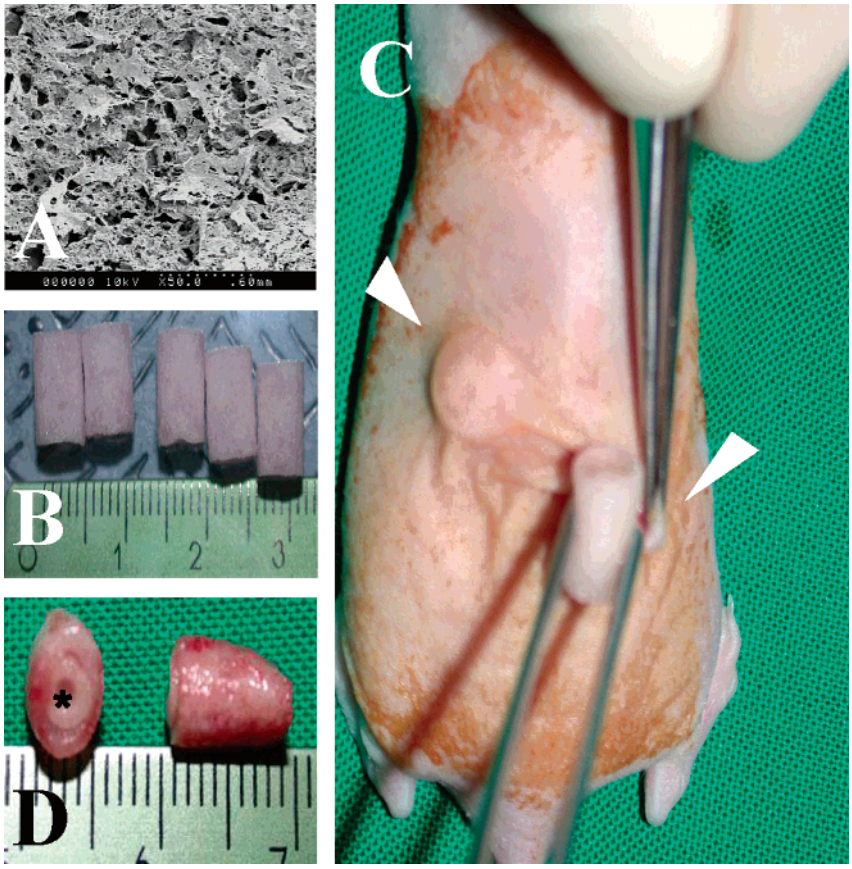

Figure 6. Implantation of PLCL scaffolds seeded with SMCs; (A) surface of SMCs-seeded scaffolds, (B) scaffolds before implantation, (C) scaffolds implanted subdermally into a nude mouse as indicated by the white arrows, implanted to two mice, two specimens per mouse, and (D) explanted scaffolds after 8 weeks (asterisk indicates silicon tube stent).

The PLCL scaffolds (length 1 cm) were seeded with SMCs (Figure 6A,B) and subsequently implanted into the subcutaneous space of nude mice (Figure 6C) for 1, 2, 5, 8, and 15 weeks to monitor the in vivo degradation. The explanted specimens (Figure 6D) were extracted by chloroform, and the polymeric residue was weighed and analyzed by NMR and GPC. In addition, the in vivo results were compared to the in vitro study.

All the data were summarized in Table 2. The PLCL scaffolds degraded very slowly even in the form of a highly porous thin membrane, which was expected from their hydrophobic characters. In vivo, the M_n and M_w decreased gradually to 23 and 39% of the initial value, respectively, after 15 weeks, as seen in Table 2 and Figure 7. At the same time, the polydispersity (M_w/M_n) increased gradually, indicating an active chain scission of the polymer. The mass decrease was even smaller to indicate an about 20% loss after 15 weeks. In vitro in PBS (pH 7.4), both the M_n and M_w decreased gradually to 36% after 15 weeks and finally to 4% after 1 year. In addition, the mass loss was only about 6% after 15 weeks but 70% after 50 weeks. It was noticeable to find that the degradation in vivo was somewhat faster than that in vitro, especially in the case of mass loss (Table 2 and Figure 8). Furthermore, the polymer used for in vivo study had a higher M_n and M_w than the one for in vitro work. This should be explained by the enzymes possibly having played a certain role in the degradation in the body. It is well accepted that aliphatic polyesters such as PGA and PLA degrade by nonenzymatic hydrolytic cleavage of ester bonds, and, therefore, the degradation rate is PGA > PLA > PCL according to their hydrophilicites. However, in the case of PCL, which is most hydrophobic and degrades most slowly, there have been reports that show a contribution of enzymes.[26,27]

The other interesting result was that the CL fractions of the specimens were gradually decreased with the degradation time. As shown in Table 2 and Figure 9, the CL % decreased from 49.0 to 41.6% in vivo for 15 weeks, while it diminished to 45.5% in vitro for 15 weeks and to 20.7% after 1 year. Therefore, the CL moieties degraded faster than LA units did in these PLCL scaffolds, although their hydrophilicities were in the opposite order. This behavior appeared more prominently in the in vivo case, as seen in Figure 9. This should be explained by the amorphous regions composed of mainly CL units possibly having been first attacked by water because water can penetrate into the amorphous regions easier than the hard domains containing more LAs.

The PLCL scaffolds exhibited a complete elastic recovery under cyclic mechanical stress,[12] good biocompatibility to SMCs,[12,13] and proper biodegradability studied here and, therefore, may be very useful for mechano-active tissue engineering and tissue culture under cyclic mechanical stimulation. The report on vascular tissue engineering will be followed soon elsewhere.[28]

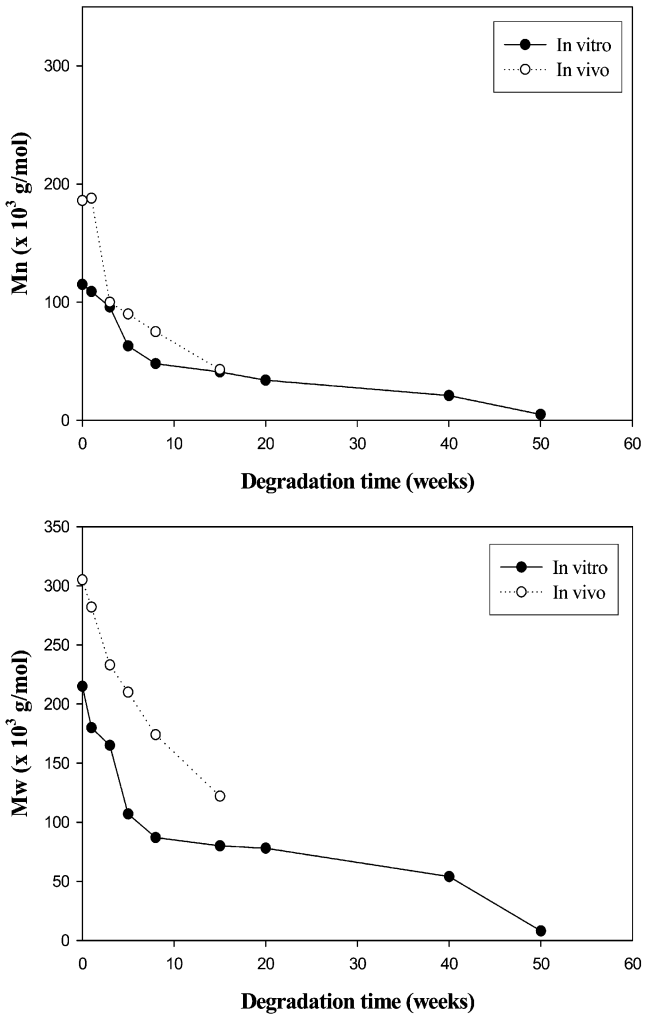

Figure 7. Change of the number-average (M_n, top) and weight-average (M_w, bottom) molecular weights of PLCL scaffolds on degradation time in vitro (filled circles, in PBS at 37 °C, $n = 4$) and in vivo (open circles, implanted subdermally into nude mice).

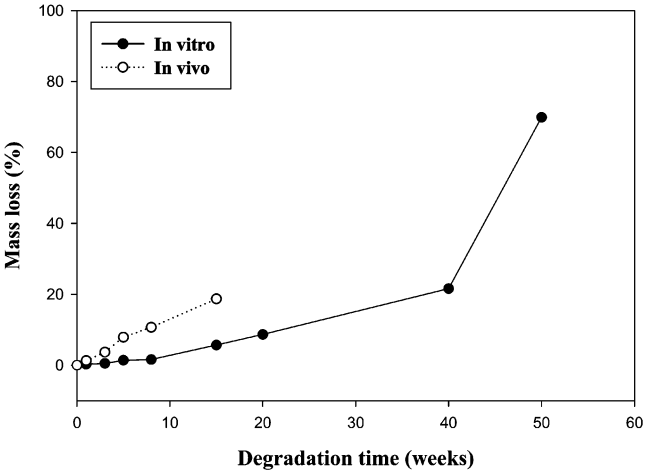

Figure 8. Mass loss fraction (weight %) of PLCL scaffolds on degradation time in vitro (filled circles) and in vivo (open circles).

Conclusions

Very elastic PLCL (50:50) copolymers were synthesized and extruded into porous tubular scaffolds (pore size 150 ± 50 μm, porosity 90%) for the application to tissue engineering. The copolymers were basically random and amorphous.

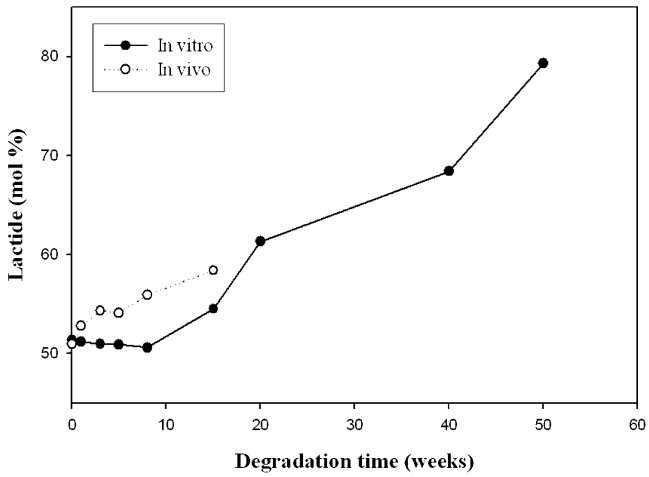

Figure 9. Change of LA contents in PLCL scaffolds on degradation time in vitro (filled circles) and in vivo (open circles).

However, two T_g's were observed in DMA and also in DSC thermograms. Furthermore, microdomains (about 17 nm in size) were indicated on the SAXS profile and, finally, confirmed by TEM. Therefore, the PLCL copolymer was probably composed of a soft matrix of mainly CL moieties and hard domains containing more LA units to exhibit a rubberlike elasticity in virtue of a physically cross-linked structure.

The SMCs-seeded scaffolds were implanted into nude mice subcutaneously for up to 15 weeks to monitor the in vivo degradation. In addition, they were degraded in vitro for up to 1 year to compare the results each other. All the scaffolds degraded slowly in vivo and in vitro even in the form of a highly porous thin membrane. However, the degradation rate was somewhat faster for in vivo than for in vitro. This should be explained by enzymes possibly having played a certain role in the degradation in the body. In addition, the CL moieties degraded faster than the LA units did in these PLCL scaffolds, although their hydrophilicities were in the opposite order. This behavior appeared more prominently in the in vivo case. This should result from the amorphous regions composed of mainly CL units possibly having been first attacked by water because water can penetrate into the amorphous regions easier than the hard domains containing more LA.

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References and Notes

(1) Hayashi, T. *Prog. Polym. Sci.* **1994**, *19*, 663.
(2) John C. M.; Arthur J. T. *Biomaterials* **2000**, *21*, 2335.
(3) Langer, R. *Nature* **1998**, *392*, 5.
(4) Tomihata, K.; Suzuki, M.; Oka, T.; Ikada, Y. *Polym. Degrad. Stab.* **1998**, *59*, 13.
(5) Patrick, C. W., Jr.; Mikos, A. G.; McIntire, L. V. *Frontiers in Tissue Engineering*; Pergamon: New York, 1998.
(6) Birukov, K. G.; Shirinsky, V. P.; Stepanova, O. V.; Tkachuk, V. A.; Hahn, A. W. A.; Resink, T. J.; Smirnov, V. N. *Mol. Cell. Biochem.* **1995**, *144*, 131.
(7) Reusch, P.; Wagdy, H.; Reusch, R.; Wilson, E.; Ives, H. E. *Circ. Res.* **1996**, *79*, 1046.
(8) Niklason, L. E.; Gao, J.; Abbott, W. M.; Hirschi, K. K.; Houser, S.; Marini, R.; Langer, R. *Science* **1999**, *284*, 489.

(9) Kim, B. S.; Nikolovski, J.; Bonadio, J.; Mooney, D. J. *Nat. Biotechnol.* **1999**, *17*, 979.
(10) Kim, B. S.; Mooney, D. J. *J. Biomech. Eng.* **2000**, *122*, 210.
(11) Lee, S. H.; Kim, B. S.; Kim, S. H.; Choi, S. W.; Jeong, S. I.; Kwon, I. K.; Kang, S. W.; Nikolovski, J.; Mooney, D. J.; Han, Y. K.; Kim, Y. H. *J. Biomed. Mater. Res.* **2003**, *66A*, 29.
(12) Jeong, S. I.; Kim, S. H.; Kim, Y. H.; Jung, Y.; Kwon, J. H.; Kim, B. S.; Lee, Y. M. *J. Biomat. Sci., Polym. Ed.* **2004**, accepted for publication.
(13) Jeong, S. I.; Kim, B. S.; Kang, S. W.; Kwon, J. H.; Jung, Y.; Lee, Y. M.; Kim, S. H.; Kim, Y. H. *Biomaterials* **2004**, accepted for publication.
(14) Save, M.; Schappacher, M.; Soum, A. *Macromol. Chem. Phys.* **2002**, *203*, 889.
(15) Pack, J. W.; Kim, S. H.; Cho, I. W.; Park, S. Y.; Kim, Y. H. *J. Polym. Sci., Polym. Chem. Ed.* **2002**, *40A*, 554.
(16) Feng, X. D.; Song, C. X.; Chen, H. Y. *J. Polym. Sci., Polym. Lett. Ed.* **1983**, 593.
(17) Qian, H.; Bei, J.; Wang, S. *Polym. Degrad. Stab.* **2000**, *68*, 423.
(18) Matsui, T.; Nakamura, T.; Tsuda, T. *J. Jpn. Soc. Biomater.* **1993**, *11*, 330.
(19) Duda, A.; Biela, T.; Libiszowski, J.; Penczek, S.; Dubois, P.; Mecerreyes, D.; Jerome, R. *Polym. Degrad. Stab.* **1998**, *59*, 215.
(20) Gan, Z.; Yu, D.; Zhong, Z.; Liang, Q.; Jing, X. *Polymer* **1999**, *40*, 2859.
(21) Pitt, C. G.; Gratzl, M. M.; Kimmel, G. L.; Surles, J.; Schindler, A. *Biomaterials* **1981**, *2*, 215.
(22) Grijpma, D. W.; Pennings, A. (Co)polymers of L-lactide, 2. *J. Macromol., Chem. Phys.* **1994**, *195*, 1649.
(23) Cui, Y.; Tang, X.; Huang, X.; Chen, Y. *Biomacromolecules* **2003**, *4*, 1491.
(24) Dong, C. M.; Qiu, K. Y.; Gu, Z. W.; Feng, X. D. *Macromolecules* **2001**, *34*, 4691.
(25) Grijpma, D. W.; Zondervan, G. J.; Pennings, A. J. *Polym. Bull.* **1991**, *25*, 327.
(26) Ye, W. P.; Du, F. S.; Jin, W. H.; Yang, J. Y.; Xu, Y. *Funtional. Polym.* **1997**, *32*, 161.
(27) Liu, L.; Li, S.; Garreau, H.; Vert, M. *Biomacromolecules* **2000**, *1*, 350.
(28) Jeong, S. I.; Kwon, J. H.; Lim, J. I.; Cho, S. W.; Jung, Y. M.; Sung, W. J.; Kim, B. S.; Lee, Y. M.; Kim, S. H.; Kim, Y. H. *Biomaterials* **2004**, accepted for publication.

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