Macromolecular Research, Vol. 13, No. 1, pp 73-79 (2005)

Preparation and Characterization of Electrospun Poly(L-lactic acid-*co*-succinic acid-*co*-1,4-butane diol) Fibrous Membranes

Hyoung-Joon Jin*, Mi-Ok Hwang, Jin San Yoon, Kwang Hee Lee, and In-Joo Chin

Department of Polymer Science and Engineering, Inha University, 253 Younghyun, Nam, Incheon 402-751, Korea

Mal-Nam Kim

Department of Biology, Sangmyung University, 7 Hongji, Jongno, Seoul 110-743, Korea

Received November 25, 2004; Revised January 17, 2005

Abstract: Poly(L-lactic acid-*co*-succinic acid-*co*-1,4-butane diol) (PLASB) was synthesized by direct condensation copolymerization of L-lactic acid (LA), succinic acid (SA), and 1,4-butanediol (BD) in the bulk using titanium(IV) butoxide as a catalyst. The weight-average molecular weight of PLASB was 2.1×10^5 when the contents of SA and BD were each 0.5 mol/100 mol of LA. Electrospinning was used to fabricate porous membranes from this newly synthesized bioabsorbable PLASB dissolved in mixed solvents of methylene chloride and dimethylformamide. Scanning electron microscopy (SEM) images indicated that the fiber diameters and nanostructured morphologies of the electrospun membranes depended on the processing parameters, such as the solvent ratio and the polymer concentration. By adjusting both the solvent mixture ratio and the polymer concentration, we could fabricate uniform nanofiber non-woven membranes. Cell proliferation on the electrospun porous PLASB membranes was evaluated using mouse fibroblast cells; we compare these results with those of the cell responses on bulk PLASB films.

Keywords: biodegradable copolymers, electrospinning, nanofibers, non-woven membranes, scaffolds, fibroblast cells.

Introduction

Poly(L-lactic acid) (PLLA) has received much interest in recent years because it is synthesized from renewable resources. PLLA is degraded by hydrolytic cleavage of the ester bonds to produce lactic acid and its oligomers, which can then be metabolized by many microorganisms.[1] PLLA is also resorbable in the human body and is nontoxic after biodegradation.[2] However, application of PLLA has been limited to biomedical areas such as surgical sutures,[3-6] fixation of fractured bones,[7,8] tissue engineering,[9,10] and drug delivery systems.[11-13] This limitation is due to a number of factors, such as the cost for high purity polymer and the difficulty in controlled synthesis of high molecular weight to optimize mechanical properties.

Cargill Dow Co.[14] began to produce PLLA on a commercial scale under the trade name of Nature Works™. This production has led to a significant reduction in the price of PLLA, not to mention supplies of polymer. The polymer production process combines agricultural processes with biological and chemical technologies. Cargill Dow Co. produces PLLA through ring opening polymerization of L-lactide, which is formed by catalytic depolymerization of low molecular weight PLLA prepolymer[14] synthesized by direct polycondensation polymerization of L-lactic acid (LA). This route is employed mainly because direct condensation polymerization of LA in the bulk state gives only low- to intermediate-molecular-weight polymers due to the low equilibrium constant of the condensation polymerization reaction.

A solvent with high boiling point such as dicyclohexylcarbodiimide or diphenyl ether, which is compatible with PLLA, is used for the removal of dissociated water by azeotropic distillation,[15] in order to shift the equilibrium state to the polymer side. PLLA with high molecular weight can be produced through this solution polymerization route. This route requires large reactor volume and facilities for evaporation and recovery of the solvent, which increases the production cost of PLLA.[14]

The major route to convert lactic acid to high-molecular-weight polymer is ring-opening polymerization of lactide.[16,17] We have identified an alternative synthesis route based on direct condensation copolymerization of LA together with succinic acid (SA) and 1,4-butane diol (BD) to produce high-molecular-weight biodegradable copolymers of poly(L-lactic acid-*co*-succinic acid-*co*-1,4-butane diol) (PLASB). Most

*e-mail: hjjin@inha.ac.kr
1598-5032/02/73-07

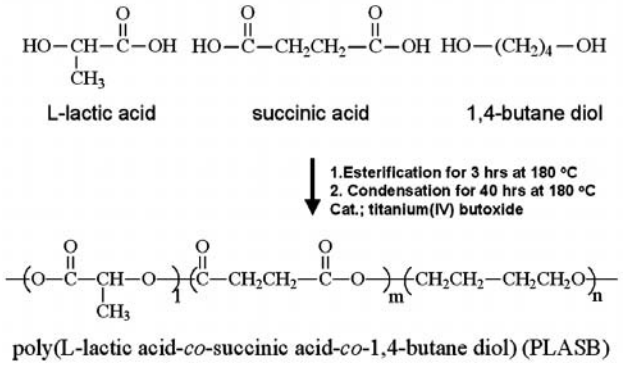
importantly, this process is potentially cost-effective. The effects of content of comonomers on the molecular weight of the copolymers were explored in this study. PLASB offers new possibilities to prepare degradable copolymers for biomedical applications by extending the range of polymer properties achievable.

Recently, electrospinning has attracted a great deal of attention because it is a polymer processing method that can lead to the formation of non-woven membranes of nanofibers.[18-20] Great efforts have been made to study the effects of processing parameters on the structure and morphology of electrospun fibers.[21-26] For example, Zong *et al.*[25] investigated the electrospinning process and the morphological properties of electrospun polymeric nanofibers. They found that higher solution concentration favored the formation of uniform nanofibers with no bead-like features. Furthermore, electrospinning technology was found to be a suitable to process natural biopolymers[18,22,27-35] and synthetic biocompatible or bioabsorbable polymers[24,25,36-46] for biomedical applications.

In the present study, the effects of the processing parameters in electrospinning on the microstructure of biodegradable PLASB membranes was determined. The electrospun nanofibrous structure is known to be capable of supporting cell attachment and proliferation.[27,32,36-40,47,48] The mechanical properties of the PLASB electrospun membranes were also determined.

Experimental

Materials. L-lactic acid (LA, 85% aqueous solution, Tedia), 1,4-butane diol (BD, Aldrich) and succinic acid (SA, Aldrich) were used as received without further purification. Titanium(IV) butoxide (Aldrich) was used as received to catalyze the condensation polymerization reactions.[49-53] A typical procedure of copolymerization is shown in Scheme I. After LA, SA and BD were added into a flask, the mixture was heated in an oil bath to 120 °C with stirring under dried nitrogen for 2 h at about 300 torr. Equal amounts of SA and BD 0.1~1.0 mole each per 100 mole of LA, were used



Scheme I. Synthesis of poly(L-lactic acid-*co*-succinic acid-*co*-1,4-butane diol).

(Table I). Then, it was heated to 180 °C under nitrogen atmosphere for 3 h to initiate the esterification reaction. The reactor pressure was reduced step by step to 1 torr for 2 h, and then the reaction was continued for another 40 h for the condensation polymerization to proceed. The resulting copolymer was dissolved in chloroform, and then was precipitated twice in excess methanol. The product was dried in a vacuum oven at 60 °C until a constant weight was attained.

Electrospinning. To prepare electrospun fibers of PLASB, mixed solvents of methylene chloride (MC) (HPLC; Fisher Scientific) and dimethylformamide (DMF) (certified A.C.S.; Fisher Scientific) were used at mixture ratios and different concentrations. PLASB was dissolved at room temperature, and the concentrations and the MC/DMF ratio are listed in Tables II and III, respectively. Electrospinning was performed with a steel capillary tube mounted on an adjustable, electrically insulated stand as described elsewhere.[18,19,27] The inside diameter of the tips of the capillary tube was 1.5 mm. The capillary tube was maintained at a high electric potential, and it was connected to a syringe filled with 10 mL of the PLASB solution. A constant volume flow rate was maintained using a syringe pump, set to keep the solution at the tip of the tube without dripping. The electric potential, the flow rate, and the distance between the capillary tip and the collection screen were adjusted so that a stable jet was

Table I. Characterization of Poly(L-lactic acid/Succinic acid/Butane diol)(PLASB)

Sample Code	Molar Composition		M_w ($\times 10^4$)	T_m (°C)
	Feed LA/SA/BD	Copolymer LA/SA/BD		
PLLA	100/0/0	100/0/0	4.1	152.4
PLASB01	100/0.1/0.1	100/0.26/0.26	8.8	138.1
PLASB05	100/0.5/0.5	100/0.82/0.69	21.0	124.6
PLASB10	100/1.0/1.0	100/1.68/1.35	17.7	120.2

Table II. Electrospun Fiber Diameter vs. Solvent Ratio (Conc.=35 wt%)

MC:DMF[a] (v/v)	2 : 1	3 : 2	1 : 1	2 : 3
Diameter[b] (nm)	920 ± 70	810 ± 105	530 ± 40	625 ± 300

[a]MC; Methylene Chloride, DMF; Dimethylformamide.
[b]N=50, Average ± standard deviation.

Table III. Electrospun Fiber Diameter vs. Solution Concentration (MC:DMF=1:1 v/v)

Solution Conc. (wt%)	15	20	30	35
Diameter[a] (nm)	160 ± 30	390 ± 200	500 ± 110	530 ± 40

[a]N=50, Average ± standard deviation.

obtained. Once a stable jet was produced, either dry or wet fibers were collected on the screen by varying the distance between the capillary tip and the collection screen.

Characterization. ^{1}H NMR analysis was performed on a Bruker DPX250 FT-NMR (Bruker Instuments, Billerica, MA). Molecular weights of copolymers were measured by gel permeation chromatography (GPC) analysis performed with tetrahydrofuran as eluent (eluent rate of 1 mL/min) on a Waters 410 (RI detector and column (porosity: 10 μm, Styragel HR 1, HR 2, HR 4, linear)). Calibration was performed using the polystyrene standard. Thermal properties of the copolymers were determined by a differential scanning calorimetry (DSC) (Perkin Elmer DSC 7, Norwalk, CT). DSC thermograms were obtained by scanning from 30 to 180 °C at the heating rate of 20 °C/min. Mechanical properties of electrospun membranes were measured using an Instron tensile tester (Instron 4,200, Canton, MA) at ambient condition with a crosshead speed of 20 mm/min. The membranes measuring 0.5 mm in thickness were cut into 8 × 40 mm rectangular shapes for tensile test. Gauge length was set at 20 mm and a load cell of 100 kg_f was used. All samples were stored in vacuum at room temperature before test. Each test was performed 5 times. The tensile stress and strain were graphed and the average tensile strength as well as the tensile modulus and standard deviation determined.

Cell Studies. L929 mouse fibroblast cells (KCLB-10001) were supplied by the Korea Cell Line Bank (KCLB). The cells were cultured in 50 mL cell culture flasks containing Dulbeccos Modified Eagles Medium (DMEM; Gibco). The cell culture was maintained in a gas-jacket incubator equilibrated with 5% CO_2 at 37 °C. When the cells had grown to confluence, the cells were digested by 1 mL of 0.05% trypsin for 1-2 min, then 3 mL of culture medium was added to stop digestion, and the culture medium was aspirated to cause cell dispersion after counting the cells. The electrospun PLASB membranes and the bulk PLASB films cast in chloroform were cut into small disks (10 mm in diameter, 0.2 mm in thickness) with the aid of a cork borer and located into a 24 well cell culture plate. All the disks were sterilized by ultraviolet light for 2 h. 1 mL of cell suspension was evenly placed on the samples. The cell-seeded disks were maintained at 37 °C under 5% CO_2 condition. Subsequently, the culture medium was removed and then the samples were rinsed with 0.01 M phosphate-buffered saline (PBS; pH 7.2) to remove any of the residual culture medium and unattached cells. After the attached cells on the disks were digested by trypsin, the cell attachment efficiency was determined by counting the number of cells remaining in the well.[54] Cell attachment and proliferation was measured by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) (Sigma, St. Louis, MO) staining. Seeded electrospun membranes and bulk films were incubated in MTT solution (0.5 mg/mL, 37 °C/5% CO_2) for 2 h. The intense red colored formazan derivatives formed was dissolved and the absorbance at 590 nm was measured with a microplate spectrophotometer (Spectra Max 250, Molecular Devices, Inc, Sunnyvale, CA). The reference wavelength was 690 nm. Cell number was correlated to optical density (OD).

SEM was used to determine cell morphology seeded on the electrospun membranes and the bulk films. Following harvest, seeded samples were immediately rinsed in 0.2 M sodium cacodylate buffer, fixed in Karnovsky fixative (2.5% glutaraldehyde in 0.1 M sodium cacodylate) overnight at 4 °C. Fixed samples were dehydrated through exposure to a gradient of alcohol followed by Freon (1,1,2-trichlorotrifluoroethane, Aldrich, Milwaukee, USA) and allowed to air dry in a fume hood. Specimens were sputter coated with Au using a Polaron SC502 Sputter Coater (Fison Instruments), and examined using a filed emission scanning electron microscope (FESEM, S-4300, Hitachi, Japan) at 15 kV. Fiber and pore sizes of the membrane were measured from the electronic SEM images using Corel computer software. A total of 100 samples were averaged for each image.

Results and Discussion

Synthesis of Biodegradable Poly(L-lactic acid-*co*-succinic acid-*co*-1,4-butane diol) (PLASB). Figure 1 shows a ^{1}H-NMR spectrum of PLASB synthesized in this study. The peaks centered at 5.14 ppm and those at 1.5 ppm correspond to the methine and methyl protons of LA units, respectively. The methylene protons of SA units exhibit their peaks at 2.70 ppm, and those of BD units appear at 1.5 and 4.15 ppm.[55-57] The composition of PLASB was determined from the intensity of the peaks at 5.14, 4.15, and 2.70 ppm corresponding to LA, BD and SA units, respectively. Table I summarizes the copolymer composition and weight-average molecular weight (M_w) measured by GPC. A direct condensation polymerization of LA yielded PLLA with M_w as low as 2.3 × 10^4. In sharp contrast, M_w of PLASB increased to 2.1 × 10^5,

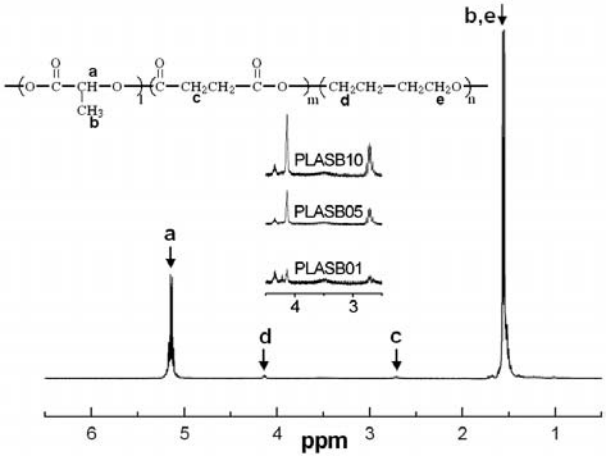


Figure 1. ^{1}H-NMR spectra of poly(L-lactic acid-*co*-succinic acid-*co*-1,4-butane diol).

as the contents of SA and BD in the copolymerization medium rose from 0.1 to 0.5 mole per 100 mole of LA. However, further increase of the contents of SA and BD to 1.0 mole per 100 mole of LA reduced M_w of PLASB down to 1.8×10^5.

Condensation polymerization of SA/BD with an alkoxide catalyst produces much higher molecular weight polymer than that of LA with the same catalyst, indicating that the equilibrium of the former reaction is much more forward to the polymer side compared to the latter reaction. Thus, the content of SA and BD rise is believed to increase M_w of PLASB due to the shift of the equilibrium.

Thermal properties of PLASB were measured by DSC. PLLA homoploymer exhibits melting peak (T_m) at 152.4 °C (Table I). T_m of PLASB copolymer was depressed to 120.2 °C. This result implies that the successive LA units in the PLASB copolymer were short due to random incorporation of SA and BD units into PLASB so that the crystallization of the segments composed of the successive LA units was greatly suppressed. It should be noted that the T_m of the PLASB copolymer is mainly due to the successive LA units.

Electrospun Porous Membrane. In order to fabricate porous membranes for cell seeding by electrospinning of PLASB, the solvent mixture of MC and DMF was used. MC is a good solvent to dissolve aliphatic polyesters. However, due to its low boiling point (39.8 °C) and high volatility, the tip of spinneret capillary is easily clogged while the polymer solutions are electrospun in this solution. Thus, DMF (b.p. 153 °C) was added in various ratios to increase the boiling point of the solution, as shown in Table II. The solution of two solvent mixture was suitable to form a stable drop at the end of the capillary for electrospinning. The distance between the tip and the collector was 20 cm and the flow rate of the fluid was 0.01 mL/min. As the potential difference between the capillary tip and the aluminum plate counter electrode was gradually increased to 28 kV (E=1.4 kV/cm), the drop at the end of the capillary tip was elongated from a hemispherical shape into a cone shape. Figure 2 shows the details of the morphology of the electrospun fibers examined by SEM. The individual electrospun fibers appeared to be randomly distributed in the non-woven membrane. The concentration of the polymer solution was fixed at 35 wt% and the solvent ratios of the MC/DMF mixture 2/1, 3/2, 1/1 and 2/3 vol% in the order of decreasing vapor pressure of the solvent mixture. As the DMF content increased up to 1/1 vol% in the MC/DMF mixture, the fibers became smaller and more uniform, while 2/3 vol% of MC/DMF, the fiber became larger and the size distribution was much broader than others as shown in Figure 2 and Table II. Then, we fixed the solvent ratio at 1/1 vol% ratio for electrospinnig and varied the concentration of the polymer solution. The concentration is known to be one of the most critical parameters controlling the fiber morphology.[25] The SEM images of the membranes spun from the PLASB solutions of different viscosities are shown in Figure 3. Drastic morphological changes were found when the concentration of the polymer solution was changed. It was impossible to collect continuous fibers at the concentrations below 15 wt%, from which only the beads, not the fibers were produced. A composite of large beads and fibers was generated by electrospinning the PLASB solution at 15 wt% (Figure 3(a)). The membrane morphology is seen to change gradually to the uniform fiber-structure (Figure 3(b), Figure 3(c) and Figure 3(d)). As the solution became more concentrated, more uniform fibers were formed. On the other hand, at concentrations higher than 35 wt%, the high viscosity of the solution prevented the PLASB solution from electrospinning, as the droplet at the end of capillary was dried out before the constant jet could be formed. In addition, the

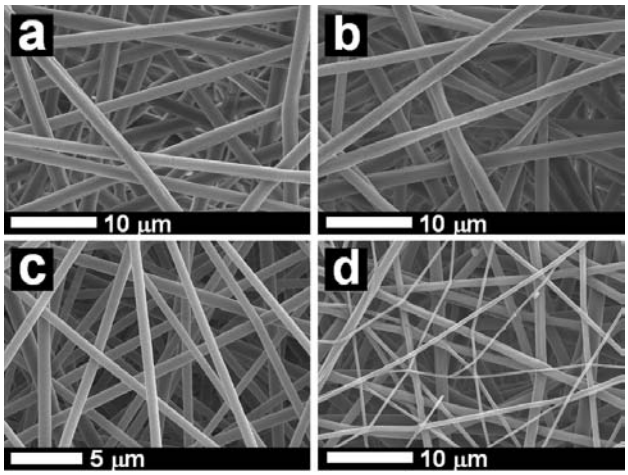


Figure 2. SEM images of electrospun PLASB fibers from different solvent mixture compositions (MC/DMF v/v); (a) 2:1, (b) 3:2, (c) 1:1, and (d) 2:3 (fixed solution concentration; 35 wt%).

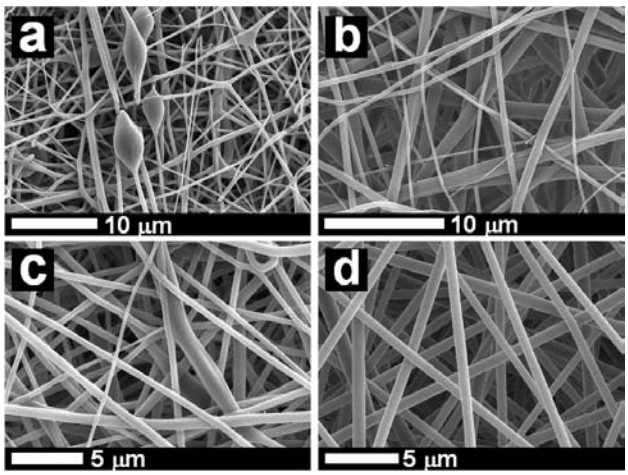


Figure 3. SEM images of electrospun PLASB fibers from different solution concentrations; (a) 15, (b) 20, (c) 30, and (d) 35 wt% (fixed solvent mixture compositions; MC/DMF 1:1).

diameter of the fibers became bigger as the concentration of the PLASB solution increased, as shown in Table III. Figure 3 and Table III also shows that the size distribution of the fiber was broad except for the fibers spun from 35 wt% solution. At lower concentrations, electrospun fibers are harder to dry before they reach the collector. The wet fiber often would result in the undulating morphology as seen in Figure 2(b) and Figure 3(c). In contrast, at higher concentrations, the electrospun fibers are mostly dried by the time they are collected. To obtain a uniform fiber-structure, higher polymer concentration is preferred within the processing limit.

Mechanical properties, such as tensile modulus, ultimate tensile stress, and ultimate strain, were evaluated as shown in Table IV. Since the fibers were randomly oriented within the nanofibrous structures, mechanical isotropy is expected in the X-Y plane of the specimen. The tensile modulus of electrospun membranes decreased as the concentration of the solution increased from 20 to 35 wt%, but the tensile strength and elongation increased up to 45 ± 4 MPa and 45.4 ± 12.7%, respectively. We decided to use the membrane electrospun from the 35 wt% polymer solution for the cell seeding experiment because it was least brittle and had the most uniform fiber size.

Cell Studies. One of the main objectives in the field of biomaterials is to fabricate matrices that can mimic the structure and biological function of the extracellular matrix (ECM).[38,58,59] ECM is composed of proteoglycans and fibrous collagen. Ideally, the dimensions of the building blocks of a tissue-engineered scaffold should be on the similar scale as those of natural ECM.[60,61] The scaffold should also have mechanically supportive properties for tissue regeneration while at the same time guiding cell differentiation and function. To achieve this goal, natural and synthetic polymers have been electrospun to produce nanofibrous membranes. A tissue engineering scaffold material must also support cellular attachment and growth. To evaluate cellular behavior on the electrospun PLASB porous membranes, L929 mouse fibroblast cells (KCLB-10001) were seeded and cultivated on the membranes. Cells were also cultivated on the PLASB cast films for comparison. The samples were prepared in discs with the thickness of 0.2 mm and the diameter of 0.5 cm. On day 1 approximately 30% more cells (6.2×10^4 cells/mL) were attached to PLASB bulk film when compared with the PLASB porous membrane (4.2×10^4 cells/mL). In both bulk film and porous membrane, the cell numbers were slightly increased on day 3 when compared with day 1, which suggests the cell growth had occurred (Figure 4). After 7 days, the cell numbers for both cases were significantly increased and the numbers were similar. The difference in cell density at day 1 between the bulk film and porous membrane may be due to the different initial cell attachment caused by the different surface morphology. In case of porous membrane, when the cell was seeded initially, some cells could be lost through the pores of membrane. Cell density was also determined by the MTT analysis (Figure 5) for the porous membrane and the bulk film. Fibroblast cells grown on bulk films (for both 7 and 14 days) showed higher MTT values compared with cells grown on porous membranes. Fibroblast cell attachment and cultivation was confirmed by SEM. SEM analysis in Figure 6 shows that after 7 days of cultivation, most parts of the bulk films and porous membranes were densely populated with fibroblast cells and the surfaces were covered by a cell sheet. While the cells on bulk films remained on the surface (Figure 6(a)), some cells migrated underneath the fibers on porous membranes (Figure 6(b)). After 14 days, the cells migrated into the fibrous membranes and covered fibers as shown in Figure 7. In view of the cell proliferation assessment, the electrospun nanofibrous membrane structure was found to show favorable cell growth as a scaffold. This novel biodegradable scaffold has potential applications for tissue engineering based upon its unique architecture, degradable composition and overall biocompatibility.

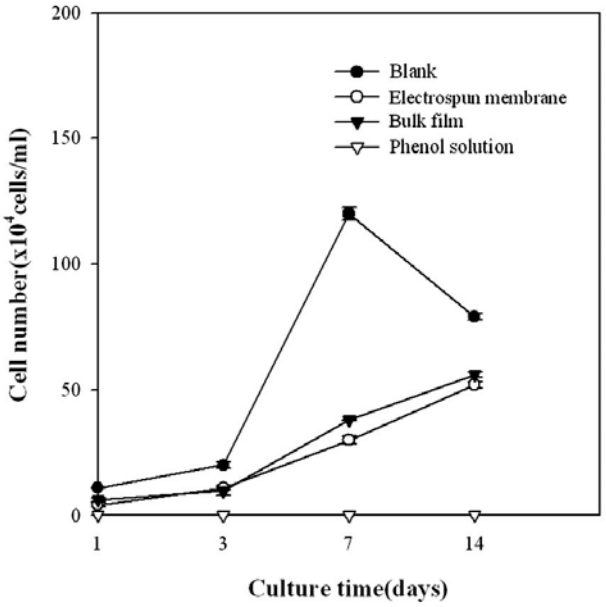


Figure 4. The number of fibroblast grown on PLASB electrospun membranes and bulk films. All experiments were performed in triplicate and the error bars indicate the standard deviation. (Blank; positive control, Phenol solution; negative control).

Table IV. Tensile Properties of Electrospun Fibers

Concentration (wt%)	Modulus[a] (MPa)	Strength[a] (MPa)	Elongation[a] (%)
20	302 ± 38	22 ± 5	17.0 ± 3.3
30	222 ± 35	34 ± 3	20.4 ± 6.5
35	156 ± 42	45 ± 4	45.4 ± 12.7

[a]*N*=5, Average ± standard deviation.

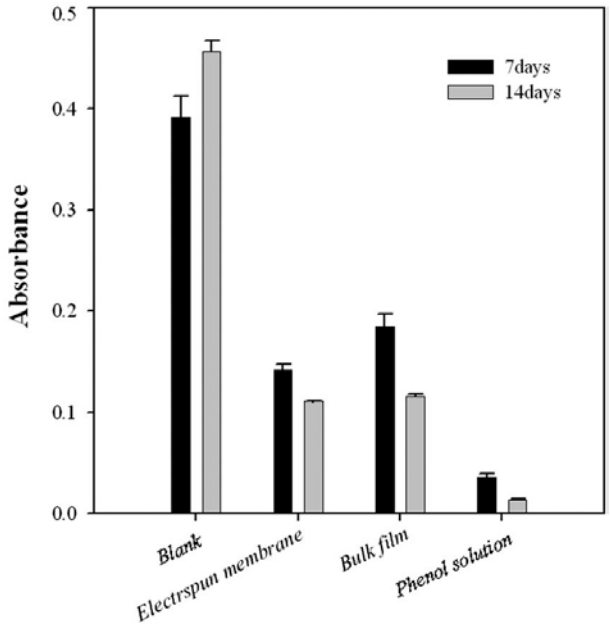


Figure 5. MTT results of fibroblast seeded on the PLASB electrospun membrane and the PLASB bulk film.

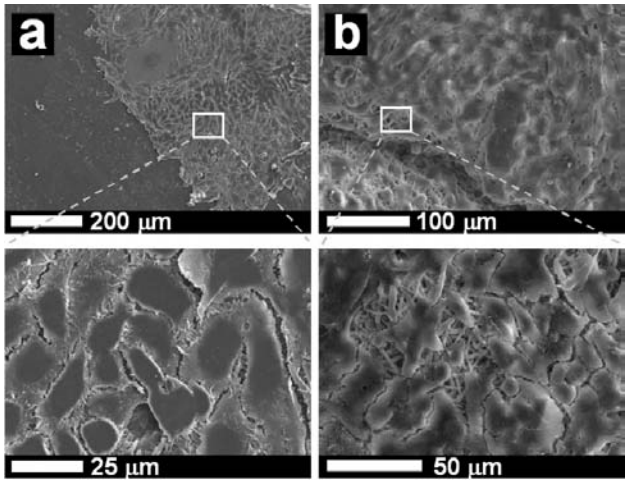


Figure 6. SEM images of cell proliferation on (a) PLASB film and (b) electrospun PLASB membrane after 7 days.

Conclusions

A direct condensation polymerization of L-lactic acid (LA), succinic acid (SA) and 1,4-butane diol (BD) with titanium (IV) butoxide produced the high-molecular weight poly(L-lactic aicd-*co*-succinic acid-*co*-1,4-butane diol) (PLASB). M_w of PLASB was 2.1×10^5 g/mol when the content of SA and BD in the copolymerization medium was 0.5 mole per 100 mole of LA, and M_w of PLLA was 2.3×10^4 g/mol in the absence of SA and BD.

Fine fibrous membranes with fiber diameter of 530 ± 40 nm were formed from the PLASB solution (35 wt%) in MC/DMF (1/1 vol%) mixed solvents by electrospinning. To evaluate cell behavior on the electrospun PLASB porous membranes, L929 mouse fibroblast cells (KCLB-10001) were seeded and cultivated on the porous membranes and compared with the bulk films. Even though initial cell attachment on the bulk film was better than on porous membranes, cell proliferation on both was similar after 14 days of incubation. Furthermore, while most of the cells on bulk films remained on the surface, some cells migrated underneath the fibers on porous membranes, which indicates the electrospun nanofibrous structure has favorable cell growth characteristics as a scaffold.

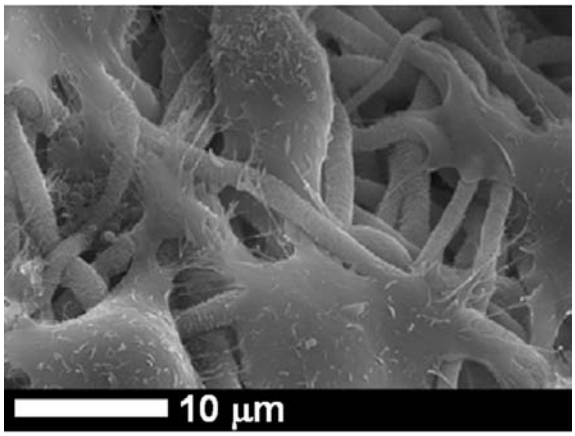


Figure 7. SEM images of cell migration into the membrane after 14 days.

Acknowldgements. This work was supported by grant No. R01-2002-000-00146-0 from the interdisciplinary research program of the KOSEF.

References

(1) R. Auras, B. Harte, and S. Selke, *Macromolecular Bioscience*, **4**, 835 (2004).
(2) H. Tsuji and Y. Ikada, *Polymer*, **36**, 2709 (1995).
(3) J. P. Nuutinen, C. Clerc, T. Virta, and P. Tormala, *J. Biomat. Sci.-Polym. E*, **13**, 1325 (2002).
(4) P. Makela, T. Pohjonen, P. Tormala, T. Waris, and N. Ashammakhi, *Biomaterials*, **23**, 2587 (2002).
(5) X. Yuan, A. F. T. Mak, and K. Yao, *J. Appl. Polym. Sci.*, **85**, 936 (2002).
(6) Y. Ikada and H. Tsuji, *Macromol. Rapid Commun.*, **21**, 117 (2000).
(7) S. H. Hyon, F. Z. Jin, K. Jamshidi, S. Tsutsumi, and T. Kanamoto, *Macromol. Symp.*, **197**, 355 (2003).
(8) H. Winet and J. Bao, *J. Biomed. Mater. Res.*, **40**, 567 (1998).
(9) R. Langer, *Acc. Chem. Res.*, **33**, 94 (2000).
(10) L. C. Lu, S. J. Peter, M. D. Lyman, H. L. Lai, S. M. Leite, J. A. Tamada, J. P. Vacanti, R. Langer, and A. G. Mikos, *Biomaterials*, **21**, 1595 (2000).

(11) R. Bhardwaj and J. Blanchard, *Int. J. Pharm.*, **170**, 109 (1998).
(12) L. Calandrelli, G. De Rosa, M. E. Errico, M. I. La Rotonda, P. Laurienzo, M. Malinconico, A. Oliva, and F. Quaglia, *J. Biomed. Mater. Res.*, **62**, 244 (2002).
(13) T. W. Chung, Y. Y. Huang, and Y. Z. Liu, *Int. J. Pharm.*, **212**, 161 (2001).
(14) E. T. H. Vink, K. R. Rabago, D. A. Glassner, and P. R. Gruber, *Polym. Degrad. Stab.*, **80**, 403 (2003).
(15) R. Miyoshi, N. Hashimoto, K. Koyanagi, Y. Sumihiro, and T. Sakai, *Int. Polym. Proc.*, **11**, 320 (1996).
(16) A. C. Albertsson and I. K. Varma, *Biomacromolecules*, **4**, 1466 (2003).
(17) J. V. Seppälä, A. O. Helminen, and H. Korhonen, *Macromolecular Bioscience*, **4**, 208 (2004).
(18) H.-J. Jin, S. V. Fridrikh, G. C. Rutledge, and D. L. Kaplan, *Biomacromolecules*, **3**, 1233 (2002).
(19) Y. M. Shin, M. M. Hohman, M. P. Brenner, and G. C. Rutledge, *Polymer*, **42**, 9955 (2001).
(20) A. L. Yarin, S. Koombhongse, and D. H. Reneker, *J. Appl. Phys.*, **90**, 4836 (2001).
(21) S. V. Fridrikh, J. H. Yu, M. P. Brenner, and G. C. Rutledge, *Physical Review Letters*, **90**, 144502 (2003).
(22) S. Zarkoob, R. K. Eby, D. H. Reneker, S. D. Hudson, D. Ertley, and W. W. Adams, *Polymer*, **45**, 3973 (2004).
(23) H. Fong, I. Chun, and D. H. Reneker, *Polymer*, **40**, 4585 (1999).
(24) X. H. Zong, S. F. Ran, D. F. Fang, B. S. Hsiao, and B. Chu, *Polymer*, **44**, 4959 (2003).
(25) X. H. Zong, K. Kim, D. Fang, S. Ran, B. S. Hsiao, and B. Chu, *Polymer*, **43**, 4403 (2002).
(26) S.-H. Lee, J.-W. Yoon, and M. H. Suh, *Macromol. Res.*, **10**, 282 (2002).
(27) H.-J. Jin, J. Chen, V. Karageorgiou, G. H. Altman, and D. L. Kaplan, *Biomaterials*, **25**, 1039 (2004).
(28) S. Sukigara, M. Gandhi, J. Ayutsede, M. Micklus, and F. Ko, *Polymer*, **45**, 3701 (2004).
(29) S. Sukigara, M. Gandhi, J. Ayutsede, M. Micklus, and F. Ko, *Polymer*, **44**, 5721 (2003).
(30) J. A. Matthews, G. E. Wnek, D. G. Simpson, and G. L. Bowlin, *Biomacromolecules*, **3**, 232 (2002).
(31) J. A. Matthews, E. D. Boland, G. E. Wnek, D. G. Simpson, and G. L. Bowlin. *J. Bioact. Compat. Polym.*, **18**, 125 (2003).
(32) E. E. Boland, J. A. Matthews, K. J. Pawlowski, D. G. Simpson, G. E. Wnek, and G. L. Bowlin, *Frontiers in Bioscience*, **9**, 1422 (2004).
(33) L. Huang, R. P. Apkarian, and E. L. Chaikof, *Scanning*, **23**, 372 (2001).
(34) L. Huang, K. Nagapudi, R. P. Apkarian, and E. L. Chaikof, *J. Biomat. Sci.-Polym. E.*, **12**, 979 (2001).
(35) W. K. Son, J. H. Youk, and W. H. Park, *Biomacromolecules*, **5**, 197 (2004).
(36) H. Yoshimoto, Y. M. Shin, H. Terai, and J. P. Vacanti, *Biomaterials*, **24**, 2077 (2003).
(37) W. J. Li, C. T. Laurencin, E. J. Caterson, R. S. Tuan, and F. Ko, *J. Biomed. Mater. Res.*, **60**, 613 (2002).
(38) W. J. Li, K. G. Danielson, P. G. Alexander, and R. S. Tuan, *J. Biomed. Mater. Res. Part A*, **67A**, 1105 (2003).
(39) M. Shin, O. Ishii, T. Sueda, and J. P. Vacanti, *Biomaterials*, **25**, 3717 (2004).
(40) B. M. Min, G. Lee, S. H. Kim, Y. S. Nam, T. P. Lee, and W. H. Park, *Biomaterials*, **25**, 1289 (2004).
(41) Z. Zing, X. Y. Xu, X. S. Chen, Q. Z. Liang, X. G. Bian, L. X. Yang, and X. B. Jing, *J. Control. Release*, **92**, 227 (2003).
(42) K. Kim, M. Yu, X. H. Zong, J. Chiu, D. F. Fang, Y. S. Seo, B. S. Hsiao, B. Chu, and M. Hadjiargyrou, *Biomaterials*, **24**, 4977 (2003).
(43) C. M. Hsu and S. Shivkumar, *J. Mater. Sci.*, **39**, 3003 (2004).
(44) K. H. Lee, H. Y. Kim, M. S. Khil, Y. M. Ra, and D. R. Lee, *Polymer*, **44**, 1287 (2003).
(45) Y. K. Luu, K. Kim, B. S. Hsiao, B. Chu, and M. Hadjiargyrou, *J. Control. Release*, **89**, 341 (2003).
(46) I. S. Lee, O. H. Kwon, W. Meng, I.-K. Kang, and Y. Ito, *Macromol. Res.*, **12**, 374 (2004).
(47) L. S. Nair, S. Bhattacharyya, and C. T. Laurencin, *Expert Opinion on Biological Therapy*, **4**, 659 (2004).
(48) Z. M. Huang, Y. Z. Zhang, M. Kotaki, and S. Ramakrishna, *Compos. Sci. Technol.*, **63**, 2223 (2003).
(49) H.-J. Jin, D. S. Kim, M. L. Kim, I. M. Lee, H. S. Lee, and J. S. Yoon, *J. Appl. Polym. Sci.*, **81**, 2219 (2001).
(50) H.-J. Jin, D. S. Kim, B. Y. Lee, M. N. Kim, I. M. Lee, H. S. Lee, and J. S. Yoon, *J. Polym. Sci.; Part B: Polym. Phys.*, **38**, 2240 (2000).
(51) H.-J. Jin, B. Y. Lee, M. N. Kim, and J. S. Yoon, *J. Polym. Sci.; Part B: Polym. Phys.*, **38**, 1504 (2000).
(52) H.-J. Jin, J. K. Park, K. H. Park, M. N. Kim, and J. S. Yoon, *J. Appl. Polym. Sci.*, **77**, 547 (2000).
(53) M. Colonna, T. E. Banach, C. Beri, M. Fiorini, E. Marianucci, M. Messori, F. Pilati, and M. Toselli, *Polymer*, **44**, 4773 (2003).
(54) Y. Wan, W. Chen, J. Yang, J. Bei, and S. Wang, *Biomaterials*, **24**, 2195 (2003).
(55) S. I. Lee, S. C. Yu, and W. S. Lee, *Polym. Degrad. Stab.*, **72**, 81 (2001).
(56) K. M. Huh and Y. H. Bae, *Polymer*, **40**, 6147 (1999).
(57) Y. Teramoto and Y. Nishio, *Polymer*, **44**, 2701 (2003).
(58) J. D. Stitzel, K. J. Pawlowski, G. L. Bowlin, G. E. Wnek, D. G. Simpson, and G. L. Bowlin, *J. Biomater. Appl.*, **16**, 22 (2001).
(59) E. D. Boland, G. E. Wnek, D. G. Simpson, K. J. Pawlowski, and G. L. Bowlin, *J. Macromol. Sci.-Pure Appl. Chem.*, **A38**, 1231 (2001).
(60) W. Tan, R. Krishnaraj, and T. A. Desai, *Tissue Eng.*, **7**, 203 (2001).
(61) K. E. Kadler, D. F. Holmes, J. A. Trotter, and J. A. Chapman, *Biochem. J.*, **316**, 71 (1996).