Electronic Supplementary Information (ESI)

Heterogeneous adhesion of cells on polymer surface with underlying amorphous/crystalline phases

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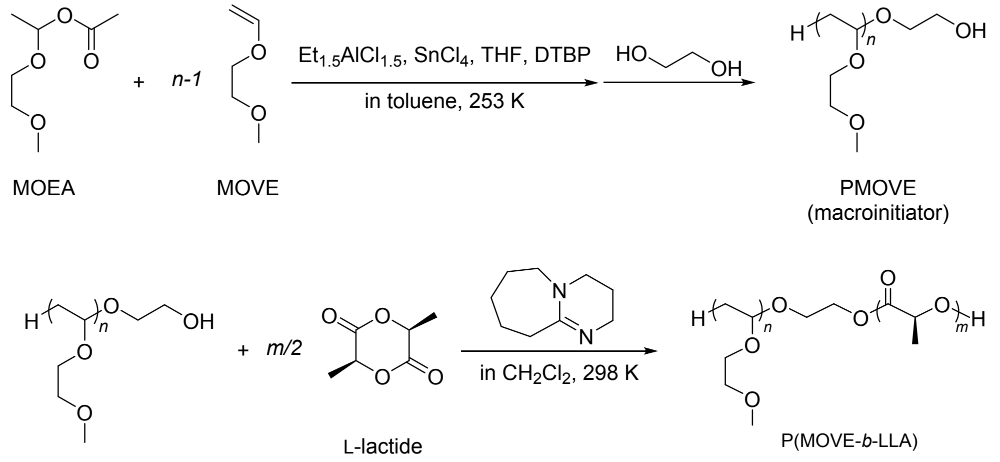
1. Polymer syntheses

Scheme S1 shows a synthetic route of poly(2-methoxyethyl vinyl ether-*b*-L-lactic acid) P(MOVE-*b*-LLA). Poly(2-methoxyethyl vinyl ether) (PMOVE) was first synthesized by base-assisting living cationic polymerization of 2-methoxyethyl vinyl ether (MOVE) using 1-(2-methoxyethoxy)ethyl acetate (MOEA) as an initiator. Polymerization reaction was terminated by ethylene glycol, resulting in an introduction of a hydroxy group at the chain end portion. Molecular weight characteristics of PMOVE were determined by gel permeation chromatography (GPC), as described below, and they were used as a macroinitiator for the following polymerization without purification.



Scheme S1. A synthetic route of P(MOVE-*b*-LLA). A macroinitiator having a hydroxy group (PMOVE) was synthesized by living cationic polymerization in toluene at 253 K under the presence of ethylaluminum sesquichloride ($Et_{1.5}AlCl_{1.5}$), $SnCl_4$, tetrahydrofuran (THF), and 2,6-di-*tert*-butylpyridine (DTBP). Subsequently, a diblock copolymer was synthesized by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)-catalyzed ring-opening polymerization of L-lactide in dichloromethane at 298 K.

Ring-opening polymerization of L-lactide was conducted under the presence of cyclic organic amidine catalyst, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), as shown in Scheme S1. Table S1 summarizes reaction conditions.

Table S1. Conditions for ring-opening polymerization of L-lactide using PMOVE as a macroinitiator. Reactions were conducted in dichloromethane at 298 K for 3 h. ^{a}A number-average molecular weight (M_n) and a polydispersity index (M_w/M_n), where M_w denotes a weight-average molecular weight, of each macroinitiator were determined by gel permeation chromatography (GPC) with polystyrene standards (PS). Chromatograms are shown in Fig. S1.

entry	macroinitiator	M_n^a	M_w/M_n^a	$[\text{macroinitiator}]_0$ / mM	$[\text{L-lactide}]_0$ / M	$[\text{DBU}]_0$ / mM
1	PMOVE-1	9.9k	1.09	4.0	0.40	5.0
2	PMOVE-2	10.3k	1.08	4.0	0.20	5.0
3	PMOVE-3	17.6k	1.07	4.0	0.16	5.0

Panels (a–c) of Fig. S1 show GPC curves for (upper) macroinitiator PMOVEs and (lower) P(MOVE-*b*-LLA)s obtained under different reaction conditions. For example, in the case of entry 1, a number-average molecular weight (M_n) and a polydispersity index (M_w/M_n), where M_w denotes a weight-average molecular weight, of PMOVE were 9.9k and 1.09, respectively, as shown in panel (a). Conversion of ring-opening polymerization was estimated to be 98% after 3 h-polymerization reaction. After purification using preparative GPC, the M_n and M_w/M_n values of P(MOVE-*b*-LLA) obtained were determined to be 25.2k and 1.12, respectively. Numbers of averaged MOVE (*n*) and LLA (*m*) repeating units were determined by ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectroscopy. Characteristics of three P(MOVE-*b*-LLA)s are summarized in Table S2.

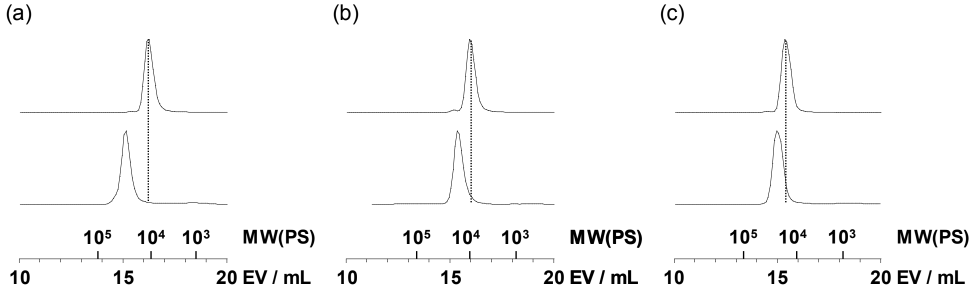
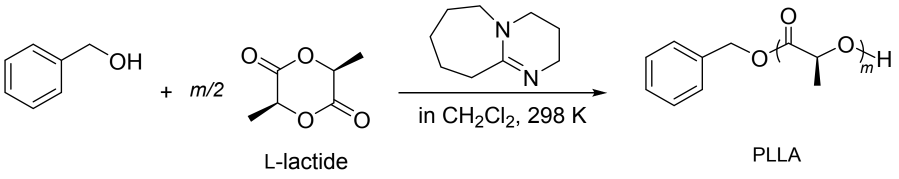


Fig. S1. GPC curves for PMOVEs and P(MOVE-*b*-LLA)s obtained under each reaction condition; (a) entry 1, (b) entry 2, and (c) entry 3 shown in Table S1. Upper and lower chromatograms show macroinitiator PMOVE and corresponding P(MOVE-*b*-LLA), respectively. EV: elution volume, MW(PS): molecular weight of polystyrene.

Table S2. Characteristics of P(MOVE-*b*-LLA)s determined by GPC with PS standards and ^{1}H-NMR spectroscopy.

Entry	M_n	M_w/M_n	*n*	*m*
1	25.2k	1.12	98	180
2	16.5k	1.08	107	74
3	24.1k	1.06	183	80

Scheme S2 shows a synthetic route of a PLLA homopolymer. Benzyl alcohol was used as an initiator for ring-opening polymerization of L-lactide in the presence of DBU. The product was purified by reprecipitation.



Scheme S2. A synthetic route for PLLA.

Fig. S2 shows a GPC chromatogram for PLLA. The M_n and M_w/M_n values were determined to be 18.0k and 1.20, respectively.

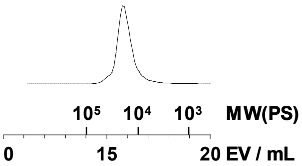


Fig. S2. GPC curve for poly(L-lactide) obtained in dichloromethane at 298 K. Polymerization condition: [L-lactide]$_0$ = 0.56 M, [benzyl alcohol]$_0$ = 4.0 mM, [DBU]$_0$ = 5.0 mM.

2. Bulk thermal property

Thermal properties of P(MOVE-*b*-LLA) and PLLA were examined by differential scanning calorimetry (DSC, EXSTAR6000 DSC6220, Hitachi High-Tech Science, Tokyo, Japan). Fig. S3 shows typical DSC thermograms for the samples. While the crystallization and melting temperatures for P(MOVE-*b*-LLA) were approximately 350 and 395 K, respectively, these temperatures of PLLA were approximately 375 and 413 K, respectively.

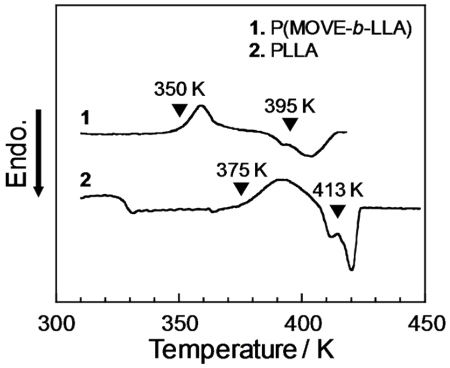


Fig. S3. DSC thermograms for P(MOVE-*b*-LLA) with the M_n of 25.2k and M_w/M_n of 1.12 and PLLA with the M_n of 18.0k and M_w/M_n of 1.20, respectively. The samples were heated up from 303 K to 423 K for P(MOVE-*b*-LLA) and from 303 K to 453 K for PLLA upon the first scan at 10 K·min^{-1}. The data were from the second scan at the heating rate of 2 K·min^{-1}.

3. Bulk crystalline structure

Aggregation structures of P(MOVE-*b*-LLA) were examined by small angle X-ray scattering (SAXS) analyses. Bulk films were prepared on polyimide substrates by a solvent-casting method. The films were melted at 423 K and then isothermally crystallized at 353 K. The SAXS measurements were performed at the BL03XU beamline of SPring-8 (Japan Synchrotron Radiation Research Institute, Hyogo, Japan). The X-ray wavelength was 0.1 nm and the camera distance was 2,224 mm. An imaging plate was used to record two-dimensional SAXS patterns. The calibration of the scattering angle was carried out using diffraction from a regular structure in silver behenate. Fig. S4 shows typical one-dimensional SAXS profiles obtained by integration of the full range of angles of patterns for P(MOVE-*b*-LLA) films without/with thermal annealing. For the non-annealed film, one peak and one slight shoulder with a relative *q* position of 1:2 were discernible, suggesting that a microphase-separated lamellar structure was formed. From the position *q* of the first-order peak (0.266 nm^{-1}), the repeating period (*d*) was simply calculated to be 23.6 nm using $2\pi/q$. After the thermal annealing, the *q* value decreased to 0.220 nm^{-1}, or d = 28.6 nm, and the shoulder became clear. This result means that the crystallization of PLLA was promoted due to the thermal annealing.

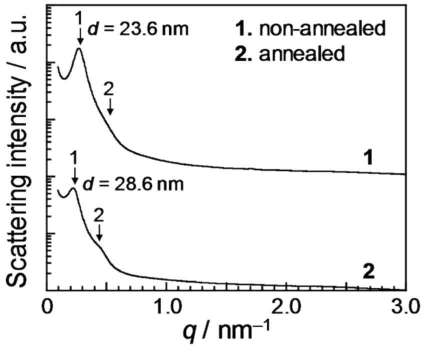


Fig. S4. One-dimensional SAXS intensity profile for a bulk film of P(MOVE-*b*-LLA) with M_n of 16.5k and M_w/M_n of 1.08, which was isothermally crystallized at 353 K. As a reference, a non-annealed film was also measured.

4. Surface structure

Thin films of P(MOVE-*b*-LLA) and PLLA were prepared on Si-wafers with a native oxide layer and borosilicate glasses by a spin-coating method from its toluene solution. The thickness of the films estimated by an M-150 ellipsometer (JASCO, Tokyo, Japan) was 150 nm. The surface morphology of the films was observed with an optical microscope VHX-700F (Keyence, Osaka, Japan). In addition, a polarized optical microscope ECLIPSE ME600 (Nikon Instech, Tokyo, Japan) was used to observe the surfaces under the crossed-Nicols condition. Static contact angle measurements against the films were performed using a Drop Master 500 (Kyowa Interface Science, Niiza, Saitama, Japan) at room temperature. A droplet of water purified by a Milli-Q system (Merck KGaA, Darmstadt, Germany) was used as a probe liquid. The surface of the P(MOVE-*b*-LLA) film was further observed by atomic force microscopy (AFM, E-sweep with a SPI3800 controller, Hitachi High-Tech Science, Tokyo, Japan) using an intermittent contact mode under ambient atmosphere at room temperature. A cantilever tip micro-fabricated from Si was used for the observation. The spring constant (k_c) and resonance frequency were 0.9 $N \cdot m^{-1}$ and 28 kHz, respectively. The tip radius (R) was approximately 10 nm. The mechanical properties in the surface region of the P(MOVE-*b*-LLA) film were examined on the basis of force-distance curves by AFM (SPA-300HV, Hitachi High-Tech Science) using a contact mode at room temperature. The tip indentation rate was 100 $nm \cdot s^{-1}$. The surface chemical composition of P(MOVE-*b*-LLA) and PLLA films was examined by X-ray photoelectron spectroscopy (XPS) using a PHI 5800 ESCA system (Physical Electronics, Chanhassen, MN, USA) with a monochromatized AlK_{α} source. The emission angle was 15º. The C_{1s} peak was calibrated to a binding energy of 285.0 eV for neutral carbons to correct the charging energy shifts.

5. Cell culture

P(MOVE-*b*-LLA) films isothermally crystallized for 1 hour were used as a scaffold for fibroblast cell culture. The P(MOVE-*b*-LLA) films prepared on borosilicate glasses were placed on the basement of 24-well culture dishes filled with normal Roswell Park Memorial Institute 1640 culture media (Life Technologies, Carlsbad, CA, US) with 10% fetal bovine serum (FBS, Life Technologies). L929 fibroblasts from mouse (RIKEN BRC Cell Bank) were seeded at 5.0×10^4 cells/well. Cultures were maintained at 310 K (37 °C) under a humidified atmosphere containing 5% CO_2. Cell adhesion states were evaluated by phase-contrast microscopy (BZ-8100, Keyence) after 4 h-culturing.