Synthesis and Antiplatelet Adhesion Behavior of a Poly(L-lactide-*co*-glycolide)-Poly(1,5-dioxepan-2-one) Multiblock Copolymer

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Materials and methods

Materials

1,5-Dioxepan-2-one (DXO) was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan), and purified by distillation. L-Lactide was purchased from Musashino

Chemical Laboratory, Ltd. (Tokyo, Japan) and was purified by crystallization in dry toluene and subsequent sublimation prior to use. Benzyl alcohol was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan) and purified by distillation. Diphenyl phosphate (DPP), dehydrated toluene, and 4-(dimethylamino)pyridine (DMAP) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan) and used as received. Dehydrated dichloromethane and dehydrated tetrahydrofuran were purchased from Fujifilm Wako Pure Chemical Co. and used without purification. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) was purchased from Watanabe Chemical Ind., Ltd. and purified by distillation. Palladium on activated carbon (10 wt%) was purchased from Merck Millipore (Tokyo, Japan) and used as received. *N*,*N*′-Diisopropylcarbodiimide (DIPCI) was purchased from Kokusan Chemical Co., Ltd. (Tokyo, Japan) and purified by distillation. 4-Dimethylaminopyridinium 4-toluenesulfonate (DPTS) was prepared according to a previously described procedure.[1] Quick eye partner tubes containing sodium citrate (3.8%) were purchased from Nipro Co. (Osaka, Japan). Glutaraldehyde (20%, FUJIFILM Wako Pure Chemical Industries, Ltd., Osaka, Japan) was diluted to 1% with phosphate-buffered saline (PBS, pH = 7.4). ActinGreen 488 Ready Probes were purchased from

Thermo Fisher Scientific K. K. (Tokyo, Japan). Perm/wash buffer was purchased from BD Bioscience (San Jose, CA, USA). Fibrinogen from human plasma was purchased from FUJIFILM Wako Pure Chemical Industries, Ltd. Two monoclonal anti-fibrinogen IgG antibodies, anti-Aα529-539 and anti-γ86-411, were kindly provided by D. K. Galanakis in Stony Brook University hospital, NY. AffiniPure Goat Anti-Mouse IgG (H + L) Dylight 649 (Jackson ImmunoResearch) used as a secondary antibody for confocal microscopic measurements was purchased from FUJIFILM Wako Pure Chemical Industries, Ltd. Goat Anti-Mouse IgG_1-HRP (Southern Biotech.) and ABTS Microwell Peroxidase Substrate containing 2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonate) at a concentration of 0.3 d L^{-1} and H_2O_2 (0.01 %) (KPL Inc.) for enzyme-linked immune sorbent assay (ELISA) were purchased from Funakoshi Co., Ltd, Tokyo, Japan. Tris-buffered saline (TBS, pH = 7.4) was used for the experiments to investigate the adsorption of fibrinogen on polymer surfaces. Protein assay bicinchoninic acid (BCA) kit (NakaraiTesque, Inc.) was used for microBCA protein assay.

Characterization

^1H and ^{13}C NMR spectra were recorded using a Jeol JNM-ECX 500 NMR spectrometer

(Tokyo, Japan). Size exclusion chromatography measurements (Shodex LF-804 column, Tokyo, Japan) were conducted using chloroform as the solvent, and the molecular weight was calculated against polystyrene standards in the range 1,241-648,800. The contact angle of a water droplet (1 μL) on the film was measured using a Phoenix P300 (Meiwafosis co., Ltd, Tokyo, Japan). The contact angle data were corrected in every ten seconds. Tapping mode atomic force microscopy (AFM) measurements were carried out using a Nano Navi S-image SPM system (Hitachi High-Tech Science, Tokyo, Japan) with an SI-DF20 cantilever (Epolead Service, Tokyo, Japan). Fluorescence microscopic measurements were conducted using a DMI 4000B fluorescence microscope (Leica, Tokyo, Japan) equipped with a Leica Cooled Color Digital camera (DFC450C, Leica Microsystems K. K., Tokyo, Japan). Confocal laser microscopic measurements were conducted using a LSM780 (Zeiss). Scanning electron microscopic measurements were conducted using a JSM-7800F Schottky Field Emission Scanning Electron Microscope (Jeol, Tokyo Japan). Tensile tests were performed using an EZ-Test EZ-LX 50N tensile tester (Shimadzu Co., Kyoto, Japan) with a crosshead speed of 20 mm min^{-1}. Dumbbell-shaped five test pieces, whose short bar is 12 mm × 2 mm × ca. 50 μm, were

measured to determine the strength, modulus, and elongation at break.

Hydrolysis of PLGA-PDXO MBC film

The films were hydrolyzed by immersing the films in PBS (pH = 7.4) at 37°C. The PBS was freshly replaced in every week. The residual weights and contact angles of the films were averaged by two samples.

Micro bicinchoninic acid (BCA) protein assay

Micro BCA protein assay was carried out for PLGA-PDXO and PLGA-PCL MBCs. Fibrinogen solution (4 mg mL^{-1}, 50 μL) in PBS was poured to each well of the polymer-coated microplate and incubated at 37 °C for 1 h. After washing with PBS three times, the mixture of solution A (BCA) and solution B (copper (II) sulfate) in the ratio 50:1 (100 μL) was poured to each well and incubated at 37 °C for 30 min. The absorption at 570 nm was measured using a microplate reader.

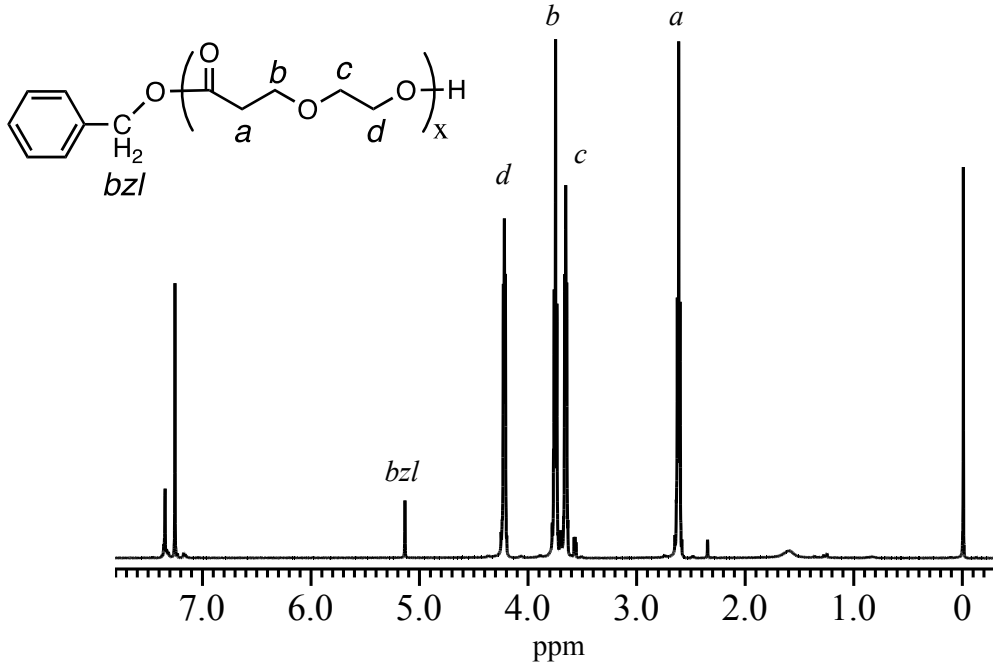

Figure S1. ^{1}H NMR spectrum of PDXO oligomer.

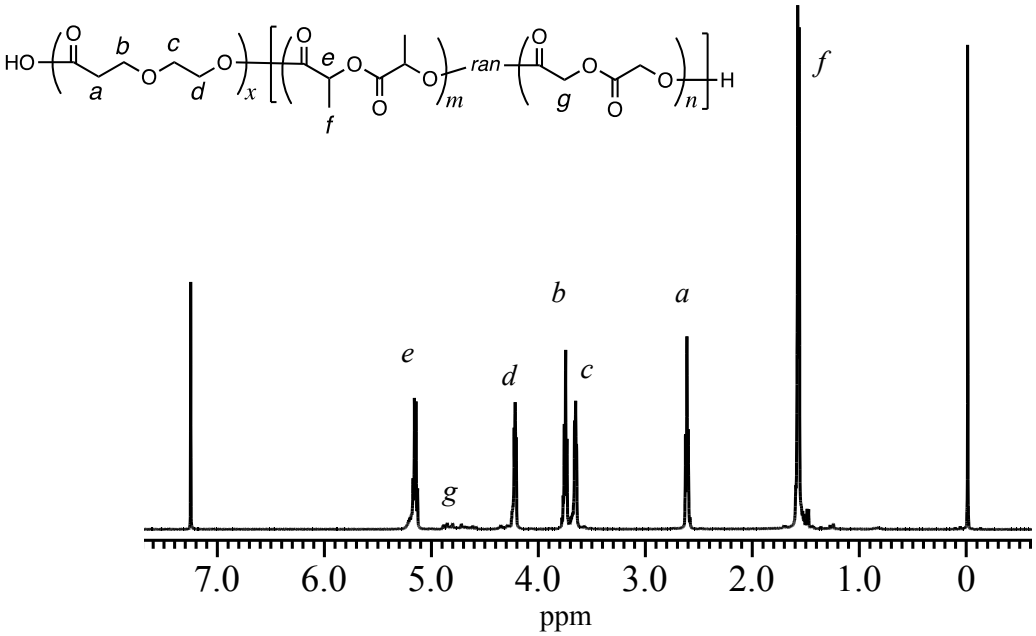

Figure S2. ^{1}H NMR spectrum of PLGA-PDXO diblock copolymer.

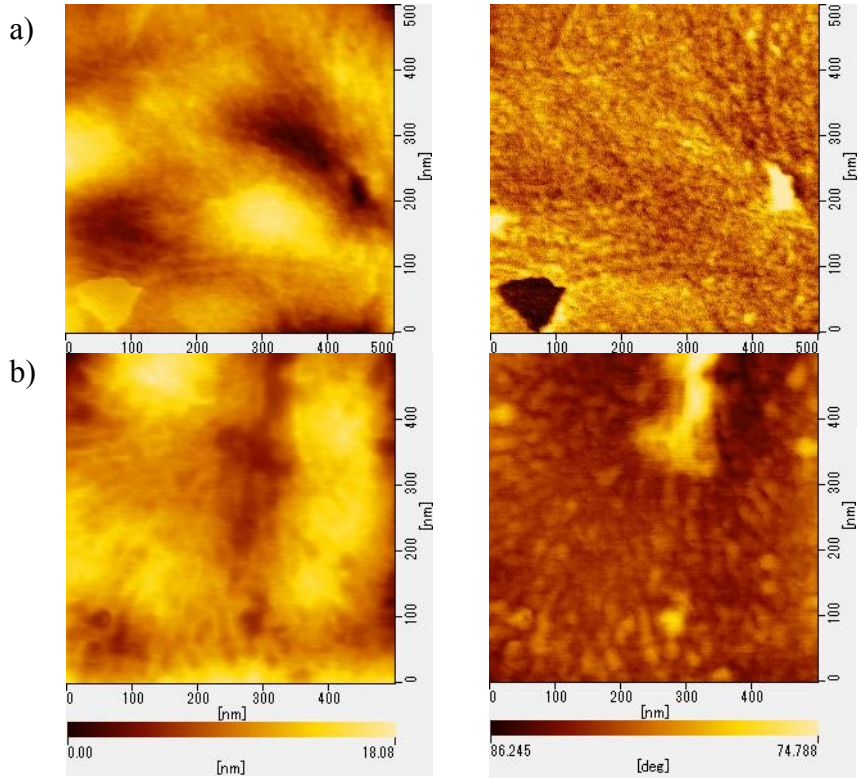

Figure S3. AFM images of PLGA-PDXO MBC (a) and PLGA-PDXO RC (b).

Left: topography, Right: phase image.

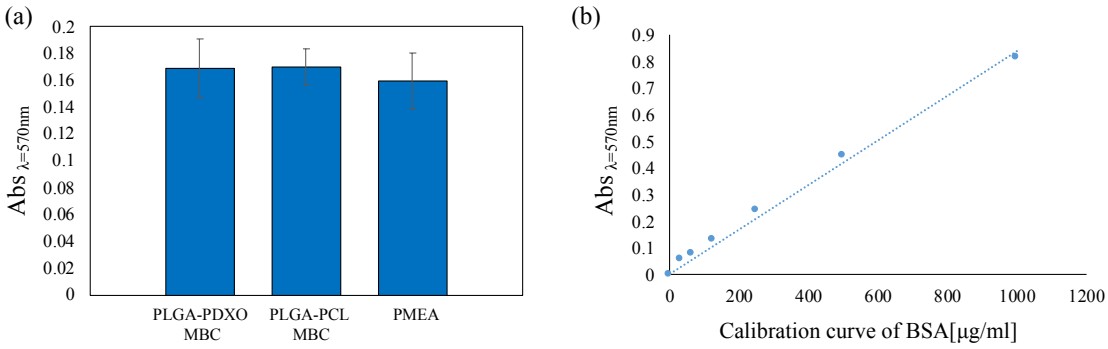

Figure S4. (a) MicroBCA tests of fibrinogen (n = 8), (b) calibration curve of BSA.

1. Moore, J. S.; Stupp, S. I., Room temperature polyesterification. *Macromolecules* **1990,** *23*, 65-70.