Supplementary Information

Photoresponsive fiber scaffolds with core-sheath nanostructure for regulating cell behaviors

Kazuho Nagata, Tetsuya Kurebayashi, Keiichi Imato, and Naoya Takeda*

Department of Life Science and Medical Bioscience, Graduate School of Advanced Science and Engineering, Waseda University (TWIns)

2-2 Wakamatsu-cho, Shinjuku-ku, Tokyo 162-8480, Japan

*E-mail: ntakeda@waseda.jp

[Materials and Methods]

1. Materials

Nitrobenzospiropyran methacrylate (SPMA) and 4-cyanopentanoic acid dithiobenzoate (CPADB) were prepared according to the procedures published previously.[1] Methyl methacrylate (MMA) was purchased from Wako Pure Chemicals, purified via distillation under reduced pressure, and stored using molecular sieves. 1,4-Dioxane was purchased from Wako Pure Chemicals, purified via reflux and distillation under reduced pressure, and stored using molecular sieves. 4,4′-Azobis(4-cyanopentanoic acid) (ACPA) was purchased from Wako Pure Chemicals and washed with methanol. PMMA (M_w = 996,000) for the core layer was purchased from Sigma-Aldrich. Milli-Q water (resistivity > 18 MΩ cm^{-1}) was prepared using a Mill-Q integral water purification system (ZRXQ003JP, Merck Millipore). Dulbecco's modified Eagle's medium with high glucose (DMEM) and Dulbecco's phosphate-buffered saline (PBS) were purchased from Nacalai Tesque. Trypsin-ethylenediaminetetraacetic acid solution was purchased from Sigma-Aldrich. Penicillin and streptomycin (P/S) solution was purchased from Gibco Life Technologies. Fetal bovine serum (FBS) was purchased from Biosera. Bovine aortic endothelial cells (BAECs) were purchased from Cell

Applications. The other chemicals were purchased from Wako Pure Chemicals, Nacalai Tesque, or Tokyo Chemical Industry and used as received.

2. Characterization

^{1}H NMR spectroscopic measurements were recorded using a 400 MHz Varian spectrometer with tetramethylsilane as the internal standard in chloroform-*d*. Size exclusion chromatography (SEC) measurements were carried out at 40 °C using a TOSOH HLC-8220 SEC system. Tetrahydrofuran (THF) was used as the eluent, and polystyrene (PS) standards were used to calibrate the SEC system.

3. Preparation of photoresponsive polymers

Photoresponsive polymers (poly(SPMA-*co*-MMA): **P1** and **P2**) with different compositions were prepared by changing the monomer feed ratio of SPMA/MMA, 1/50 for **P1** and 1/3 for **P2**, using reversible addition–fragmentation chain-transfer (RAFT) polymerization.

In the typical preparation of **P1**, a solution of CPADB (50 mg, 0.18 mmol), ACPA (10 mg, 0.036 mmol), SPMA (170 mg, 0.40 mmol), and MMA (2.1 mL, 20 mmol) in 1,4-dioxane (3.6 mL) was prepared under an atmosphere of nitrogen gas and degassed in three freeze-thaw cycles. Polymerization was carried out at 68 °C for 72 h (Fig. S1). After the reaction, 1,4-dioxane was removed under reduced pressure. The crude product was dissolved in a small amount of THF and subsequently precipitated in methanol. The obtained polymer was dried under reduced pressure at 50 °C to obtain a powder (1.4 g, 65% yield). The monomer ratio of SPMA/MMA in the photoresponsive polymer was calculated using ^{1}H NMR with characteristic peaks for each monomer, δ = 6.63–8.09 ppm (SPMA, 6H, aromatic) and δ = 0.67–1.11 ppm (3H, CH_3 in methacrylic backbone). The M_n and M_w/M_n values of the polymer were measured using analytical SEC with PS standards, M_n = 42.8 kDa and M_w/M_n = 1.39.

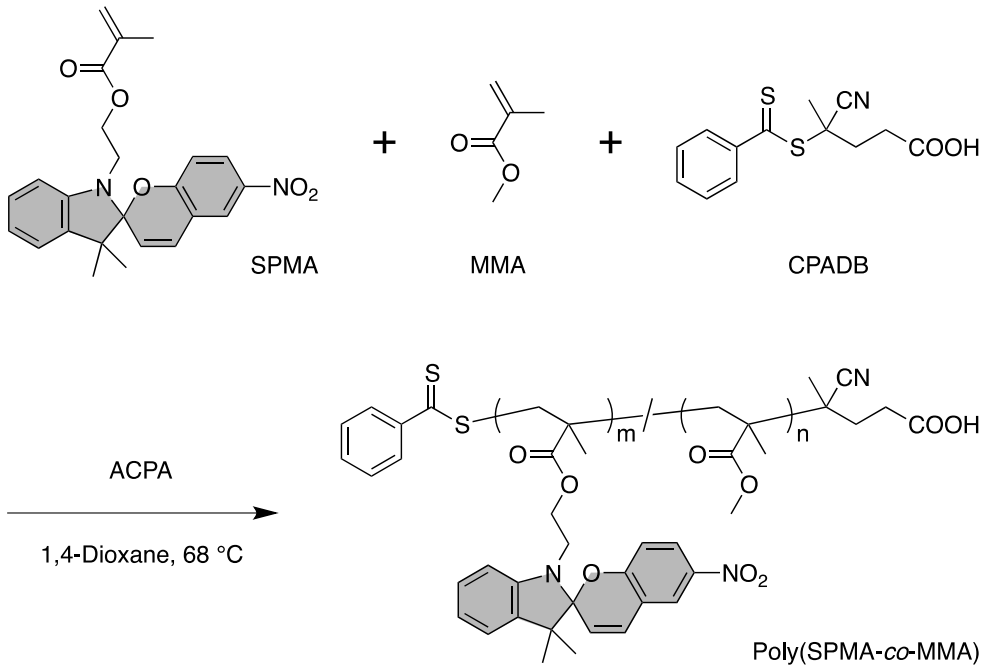

Fig. S1. Synthetic scheme for photoresponsive polymers (poly(SPMA-*co*-MMA): **P1** and **P2**) using RAFT polymerization.

4. Preparation of spin-coated films of photoresponsive polymers

Spin-coated films of the photoresponsive polymers were prepared on a hydrophobically modified glass surface to evaluate the photochemical properties of the copolymers. A silanol solution was prepared by stirring trimethoxy(metyl)silane (TMOMS) in 1 mM hydrochloric acid solution for 15 min at room temperature. Cover glasses (No. 1, 24 mm × 24 mm, thickness 0.12–0.17 mm, Matsunami) were exposed to air plasma (air pressure of 80 Pa and exposure time of 2 min) in a plasma cleaner (PIB-20, Vacuum Device). The cover glasses were immersed in the silanol solution and stirred overnight at room temperature to modify TMOMS on the cover glasses. The silanized cover glasses were washed with water and dried under reduced pressure at 50 °C for 12 h. The photoresponsive polymers were dissolved in toluene at a concentration of 0.5% (w/v). The glasses were spin-coated with the solutions at 3000 rpm for 30 s using a spin coater (ACT-300D, Active). The polymer-coated glasses were dried under reduced pressure at 50 °C for 12 h. The same method was used to prepare the control PMMA-coated glasses.

5. Photoisomerization in photoresponsive polymers

The photoisomerization of SP to merocyanine (MC) in the copolymers during UV light irradiation was evaluated using UV–vis absorption spectroscopy. Before exposure to UV light, the polymer-coated glasses were divided in half to load each half glass in a 1 cm quartz cell. Subsequently, they were exposed to 365-nm UV light with an intensity of 1.2 mW/cm^2 using a handheld UV lamp (UVGL-58, UVP) in water. The exposure distance was set to 1 cm. Immediately after UV irradiation for each exposure time, the glasses were loaded in a 1 cm quartz cell and subsequently, the photoisomerization was evaluated in water using a UV–vis spectrometer (V-630, JASCO).

6. Contact angle measurements of spin-coated films

The photoresponsive changes in the surface wettability of the spin-coated films were examined by measuring the contact angles using the captive bubble method with a drop shape analyzer (DSA100S, Krüss). The polymer-coated glass surfaces were immersed in water to measure the contact angles with a 5-μL bubble. Subsequently, the surfaces were exposed to UV light for 5 min in water and the contact angles were measured again. Three films were employed for each polymer (n = 3).

7. Fabrication of co-axial core-sheath fiber scaffolds

PMMA for the core layer and poly(SPMA-*co*-MMA) for the sheath layer were separately dissolved in *N,N*-dimethylformamide (DMF) by stirring overnight at room temperature. The concentrations of PMMA and poly(SPMA-*co*-MMA) in the DMF solutions were 9 wt.% and 20 wt.%, respectively. Each core and sheath solution was loaded separately into a syringe. The core solution was fed through a needle tip (27 G) using a syringe pump. The sheath solution was set to an electrospinning machine (NANON, MECC) and fed through a spinneret (Fig. S2). The electrospinning parameters are listed as follows: needle tip to target distance of 23.5 cm, applied

voltage of 20 kV, spinning time of 1 min, flow rate of the core solution of 3.0 mL/h, and flow rate of the sheath solution of 0.3 mL/h. The same parameters were used for the electrospinning of control PMMA fibers. Fibers were collected on cover glasses (18 mm × 18 mm, thickness 0.17–0.25 mm, Matsunami) coated with Au sputtering (MSP-10, Vacuum Device) or aluminum foils. The fibers on aluminum foils were fractured, and their cross-sections were observed using field-emission scanning electron microscopy (FE-SEM; S-5500, Hitachi).

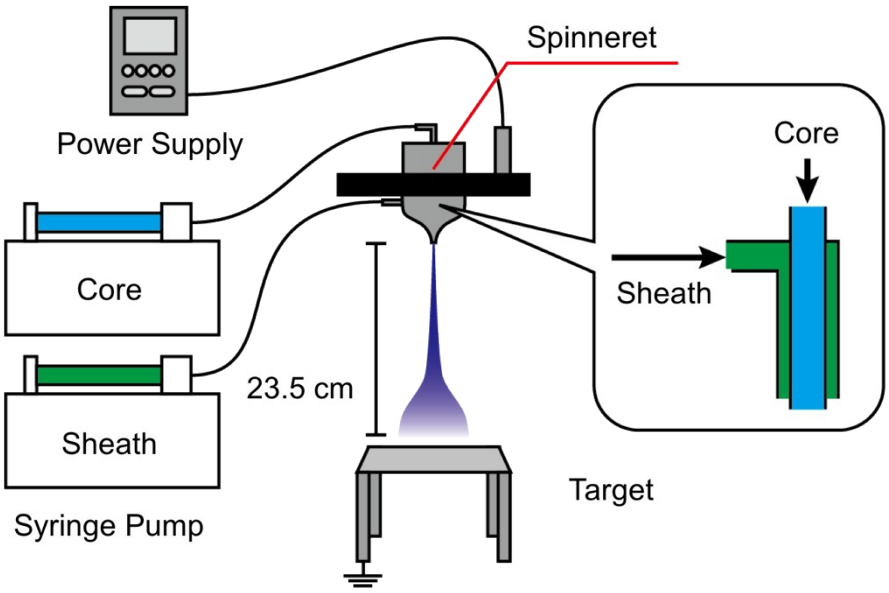

Fig. S2. Illustration of the employed electrospinning system for fabricating co-axial core-sheath fibers.

8. Photoisomerization in fiber scaffolds

The photoisomerization of SP to MC in the fibers was evaluated via quantitative analysis using Adobe Photoshop CS6. The fibers fabricated on aluminum foils were exposed to UV light for 1, 10, 20, 50, 200, 300, and 600 s in air and scanned using a scanner. The cyan intensities of the fiber mats were quantitatively analyzed using the software.

9. Contact angle measurements of fiber scaffolds

The photoresponsive changes in the surface wettability of the fiber mats were examined by measuring the contact angles in a similar manner to the spin-coated films. The mats were immersed in Mill-Q water overnight prior to the measurements. The mats were immersed in water to measure the contact angles with a 5-μL bubble. Subsequently, the mats were exposed to UV light for 5 min in water and the contact angles were measured again. Three mats were employed for each fiber (n = 3).

10. Cell culture and photoregulation of cell adhesion

The cell adhesion and proliferation behaviors on the fiber scaffolds were examined using BAECs. DMEM, containing 10% FBS and 1% P/S, was used as the culture medium. The fiber scaffolds were placed in 35-mm cell culture dishes. Subsequently, silicon rings (inner diameter of 0.8 cm, outer diameter of 2.0 cm, and height of 1.0 cm) were placed on the scaffolds. The scaffolds were pre-incubated overnight in the culture medium at 37 °C and 5% CO_2 to remove the residual solvent. After pre-incubation, the scaffolds were washed with PBS and BAECs were seeded at 1.0×10^5 cells cm^{-2} and incubated overnight at 37 °C with 5% CO_2 on the UV-irradiated and non-UV-irradiated scaffolds. The UV-irradiated scaffolds were exposed to UV light for 5 min in PBS before cell seeding.

11. Evaluation of cell adhesion and proliferation

The cell adhesion and proliferation behaviors of BAECs on the photoresponsive fiber scaffolds were evaluated using a fluorescence microscope (ECLIPSE TE2000-U, Nikon) and SEM (VE-9800, Keyence). After one-day incubation, the cells were fixed with 4% paraformaldehyde, and the nuclei were fluorescently stained with Hoechst 33342 (0.1% in PBS) for 15 min. The cell densities were determined by counting the number of nuclei using ImageJ software (NIH). Subsequently, the samples were immersed in 20%, 50%, 75%, and 100% ethanol in a stepwise manner and finally in *tert*-butyl alcohol. The samples were lyophilized and observed using SEM. Before observation, the

samples were coated with Au sputtering. Single cell areas on the fiber scaffolds were estimated from the cell densities in Fig. 4A–C and total cell area in SEM images (Fig. S7).

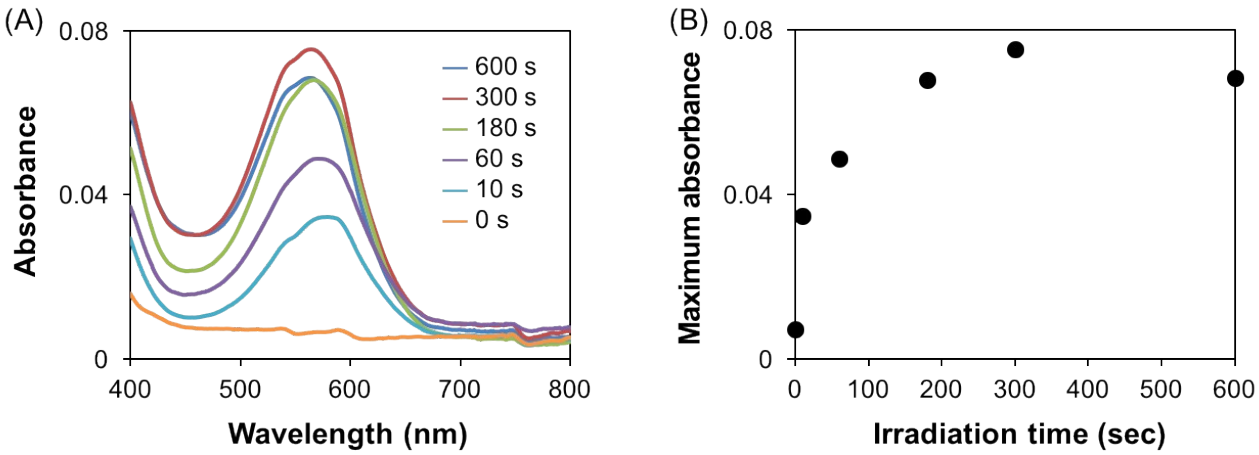

Fig. S3. (A) UV–vis absorption spectra of **P2** film spin-coated on hydrophobically surface-modified glass substrates during UV exposure. (B) Maximum absorbance changes of the film with UV exposure time.

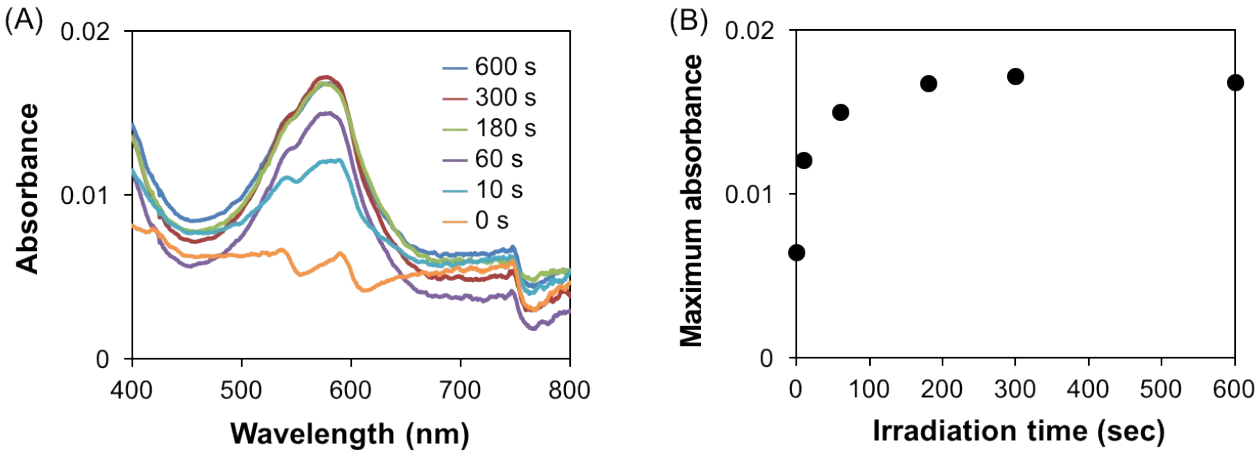

Fig. S4. (A) UV–vis absorption spectra of **P1** film spin-coated on hydrophobically surface-modified glass substrates during UV exposure. (B) Maximum absorbance changes of the film with UV exposure time.

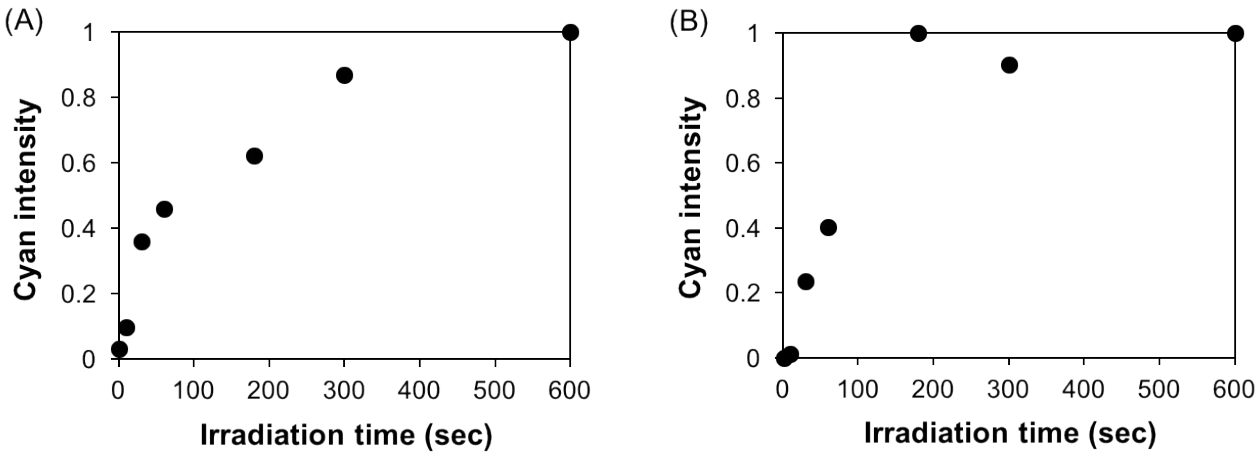

Fig. S5. UV-induced changes in cyan intensity in PMMA/**P1** (A) and PMMA/**P2** (B) fiber mats. The intensities were normalized to those after UV irradiation for 600 s.

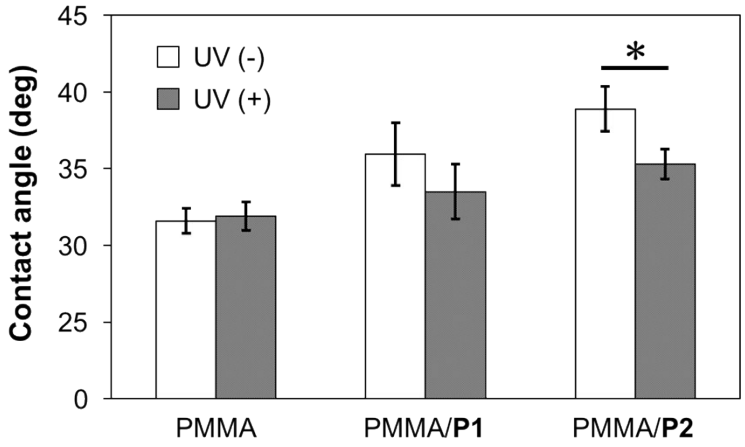

Fig. S6. Wettability changes of fiber mats with photostimulation. Static contact angles of PMMA, PMMA/**P1**, and PMMA/**P2** mat surfaces before (UV (-): open bars) and after (UV (+): closed bars) UV exposure. Mean ± S.D. (n = 3). Statistical significance, *$p < 0.05$ (Student's *t*-tests).

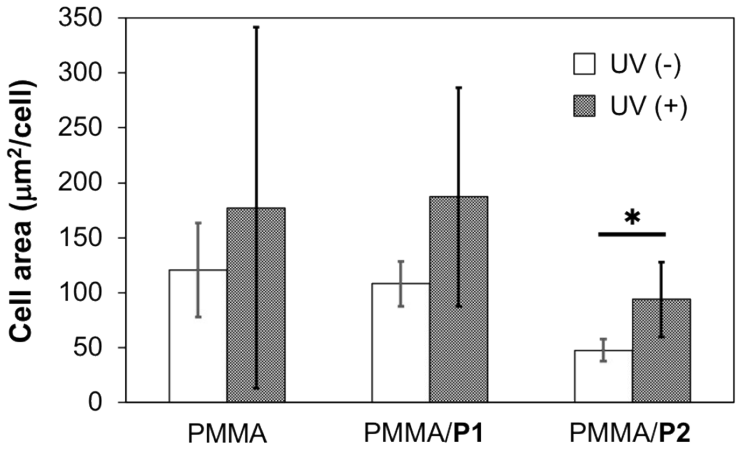

Fig. S7. Single cell areas on non-UV-irradiated (UV (-)) and UV-irradiated (UV (+)) PMMA, PMMA/**P1**, and PMMA/**P2** scaffolds. Mean ± S.D. (n = 3). Statistical significance, *$p < 0.05$ (Student's *t*-tests).

[Reference]

[1] D. He, Y. Arisaka, K. Masuda, M. Yamamoto and N. Takeda, *Acta Biomater.*, 2017, **51**, 101.