Electronic Supplementary Information

Effective Cell Sheet Preparation using Thermoresponsive Polymer Brushes with Various Graft Densities and Chain Lengths

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S.1 Materials

N-isopropylacrylamide (NIPAAm) was obtained from KJ Chemicals (Tokyo, Japan), and purified through reprecipitation from *n*-hexane. Formic acid, formaldehyde, sodium hydroxide, magnesium sulfate, *n*-hexane, methanol, acetone, dichloromethane, toluene, 2-propanol, copper (I) chloride, and copper (II) chloride were obtained from Fujifilm Wako Pure Chemicals (Osaka, Japan). Tris(2-aminoethyl)amine (TREN) was obtained from Acros Organics (Pittsburgh, PA, USA). Tris[2-(dimethylamino)ethyl]amine was synthesized from TREN. (Chloromethyl)phenylethyl-trimethoxysilane (CPTMS) and phenethyltrimethoxysilane (PETMS) were purchased from Gelest (Morrisville, PA, USA). Cover glass (24 × 50 mm, thickness: 0.17–0.25 mm) was obtained from Matsunami Glass Industry (Osaka, Japan).

Bovine carotid artery endothelial cells (ECs), mouse fibroblasts (NIH/3T3), and Madin–Darby canine kidney cells (MDCKs) were obtained from the JCRB cell bank (Osaka, Japan). Human lung carcinoma cells (A549) were obtained from the American Type Culture Collection (Manassas, VA, USA). Dulbecco's Modified Eagle's Medium (DMEM), penicillin–streptomycin solution (AB; 100 unit/mL penicillin, 100 μg/mL streptomycin), and trypsin-EDTA solution (0.1% trypsin-1.1 mM EDTA) was obtained from Sigma Chemicals (St. Louis, MO, USA). Fetal bovine serum (FBS) was obtained from Bioserum Co. Ltd. (Victoria, Australia). Polystyrene dishes and tissue culture polystyrene dishes were obtained from BD Bioscience (Franklin Lakes, NJ, USA).

S.2. Cell culture

S.2.1 Culture of endothelial cells

Growth medium was prepared in a clean bench by adding 56 mL of FBS and 5.6 mL of AB to 500 mL of DMEM. PBS was prepared by diluting 10 × PBS with sterile water. These prepared solutions were stored at 4°C. DMEM containing FBS was used within 1 week.

Frozen suspensions of endothelial cells were thawed and cultured on TCPS culture dishes (φ 60 mm Falcon 3002) until subconfluent. Subconfluent endothelial cells were passaged as follows. The frozen suspensions of endothelial cells were thawed and subsequently cultured on TCPS culture dishes (φ 60 mm Falcon 3002) until they reached the subconfluent stage. Subconfluent endothelial cells were passaged in accordance with the following procedure. The prepared solution and trypsin-EDTA solution were heated in a thermostatic bath at 37°C. The medium of the subconfluent endothelial cells was removed and washed, and 5 mL of PBS(−) was prepared in accordance with 4.2.8.1 and added in its place. Then, 1 mL of trypsin-EDTA solution was added, and an enzymatic treatment was performed at 37°C for 2 min. This was done in order to degrade the intercellular junctions and ECM proteins present on the basement membrane side, with the aim of collecting the endothelial cells in suspension. The recovered endothelial cells were diluted with 10 mL of prepared growth medium in order to halt the enzymatic reaction. Subsequently, the cells were transferred into a

pipette and dispensed into a suspension. The cell suspension was subjected to centrifugation at 800 rpm for a period of 5 min at a temperature of 4°C. Following this, the supernatant was aspirated. The cell suspension was then diluted with 5 mL of medium, and the number of cells in this 10 μL cell suspension was counted on a blood cell counting board, resulting in a concentration of 2.0×10^4 cells/mL.

S.2.2 NIH/3T3, MDCK, and A549 cell culture

Growth medium was prepared in a clean bench by adding 56 mL of FBS and 5.6 mL of AB to 500 mL of DMEM. PBS was prepared by diluting 10 × PBS with sterile water. These prepared solutions were stored at 4°C. DMEM containing FBS was used within 1 week.

Suspensions of cryopreserved cells (NIH/3T3, MDCK, A549) were thawed and cultured on TCPS (φ 60 mm Falcon 3002) dishes until subconfluent. Cells that became cosubconfluent were passaged according to the following procedure. The prepared solutions and trypsin-EDTA solution were subjected to heating in a thermostatic bath at 37°C. The medium of subconfluent cells was removed and replaced with 5 mL of PBS(−) prepared in 5.2.3.1, and the cells were washed with an additional 5 mL of the same solution. Then, 1 ml of trypsin-EDTA solution was added, and the cells were collected in suspension by enzymatic treatment at 37°C for 2 min for NIH/3T3, 10 to 15 min for MDCK, and 5 min for A549. This was done to degrade intercellular bonds and the extracellular matrix present on the basement membrane side. The recovered cells were diluted with 10 mL of the medium prepared in 5.2.3.1 to halt the enzymatic reaction. Subsequently, the cells were transferred into a new vessel by pipetting, and this constituted the cell suspension. The cell suspension was subjected to centrifugation at 4°C and 800 rpm for a period of 5 min, after which the supernatant was aspirated. The cell suspension was then diluted with 5 ml of medium, and the number of cells in 10 μL of the cell suspension was counted on a blood cell counting board to reach a concentration of 2.0×10^5 cells/mL.

Table S1 Estimated PNIPAAm brush length and density based on previously reported data

Code	Initiator (CPTMS):PETMS	Monomer concentration (mmol/L)	Amount of grafted PNIPAAm (μg/cm^2) [a)]	Chain length [b)]	Graft density (chains/nm^2) [c)]
I100-PN250	100:0	250	0.91 ± 0.16	15300	0.36
I100-PN500		500	1.65 ± 0.21	27800	0.36
I50-PN250	50:50	250	1.30 ± 0.17	37700	0.21
I50-PN500		500	1.56 ± 0.25	45249	0.21
I25-PN250	25:75	250	0.83 ± 0.42	28400	0.18
I25-PN500		500	0.85 ± 0.11	29100	0.18

a) Measured by ATR–FTIR. b) Calculated using the data of Ref [S1]. b) Calculated using the data of Refs [S1] and [S2]. c) Calculated using the data of Refs [S1] and [S2].

References

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