Electronic Supplementary Information (ESI)

Bioinactive Semi-Interpenetrating Network Gel Layers: Zwitterionic Polymer Chains Incorporated in Cross-Linked Polymer Brush

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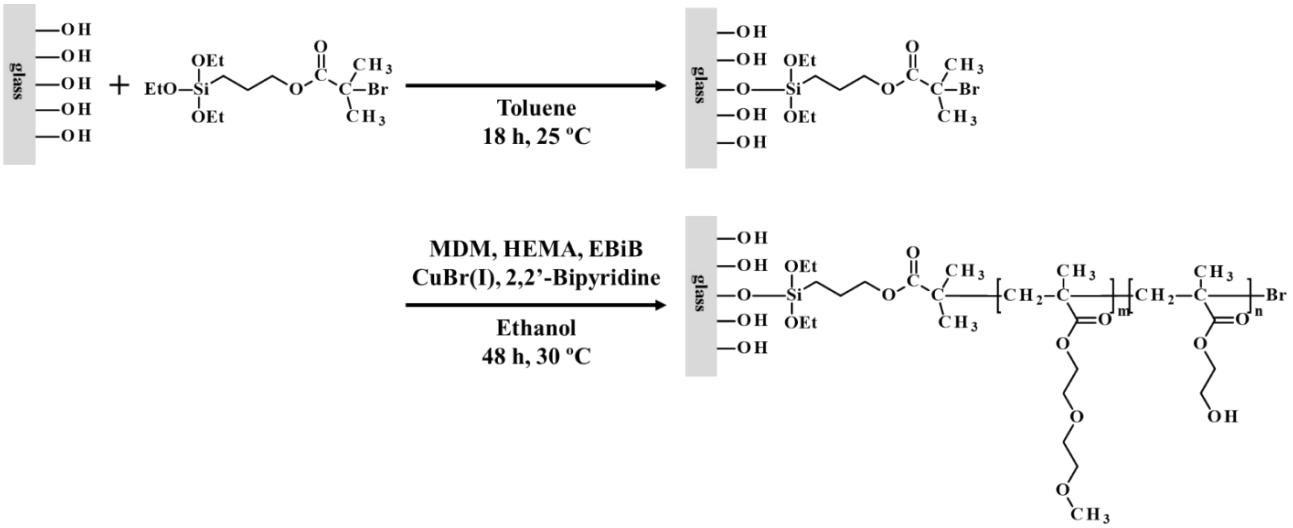
Experimental

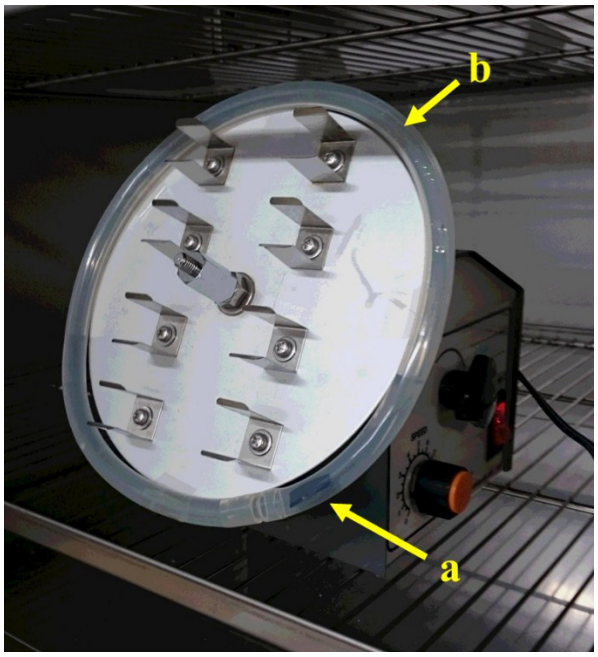
Surface Modification with BPE. BPE was dissolved in anhydrous toluene (30 mL, [BPE] = 4 mM). A glass substrate (26 × 20 mm) sequentially rinsed with water, MeOH, and acetone, and further washed with a UV/ozone cleaner (UV253E, Filgen, Nagoya, Japan) was incubated in the BPE solution at room temperature overnight (**Scheme 1**). The glass plate was immersed in toluene and rinsed. The plate was further rinsed with MeOH and acetone, and then, it was dried using N_2 gas. To vary the density of polymer brush, a feeding ratio of the initiator (BPE) and propyltrimethoxysilane (PTMS) was set to be [BPE] : [PTMS] = 1 : 10, 1 : 100, and 1 : 1000.

P(MDM-*co*-HEMA) Brush Constructed with BPE as Initiator. In a three-necked flask (100 mL), MDM (5.48 mL, 29.7 mmol), HEMA (36.4 μL, 0.30 mmol), and EBiB (11.1 μL, 0.075 mmol) were added, and it was dissolved in EtOH (24.48 mL). A vacuum was created in the flask, and CuBr (21.5 mg, 0.15 mmol) and 2,2'-bipyridine (46.9 mg, 0.30 mmol) were added under N_2 atmosphere. The BPE-modified substrate, a small stirrer tip (5 mm), and a sample plate stand made of Teflon® were placed in a sample vial, the reaction solution was added, and the vial was then sealed tightly. After 48 h at 30 °C, the substrate was recovered and sequentially rinsed with MeOH and acetone. The reaction solution was condensed by evaporation and passed through a silica gel column (Silica Gel 60N spherical neutral, diameter 40–50 μm) to remove Cu salt (eluate, MeOH) (**Scheme 1**). The polymer solution recovered was condensed by evaporation and it was dialyzed against MeOH for one week (Spectra/Por, Spectrum Laboratories, Inc., MWCO 3500). The purified polymer solution was condensed by evaporation and the obtained polymer product was analysed by GPC.



Scheme S1. Surface grafting of P(MDM-*co*-HEMA) via organosilane SAM and ATRP.

Evaluation of durability of thin gel layer using rotary flow system. The method and procedure are described in the main text. The set-up of system is shown in **Scheme S2**.



Scheme S2. Photograph of the homemade "rotary flow system" to investigate the durability of thin gel layer surfaces. A glass substrate (for investigating cell adhesion) or silicon wafer (for investigating thickness of polymer layer) modified with thin gel layer (a) was set in a silicon tube (b). PBS solution (8 mL) was added into the silicon tube. The silicon tube was rotated at 20 rpm. This system was set in the incubator at 37 °C.

Assay for Physical Adsorption of Proteins. The substrate (27 × 36 mm glass substrate) equipped with a frame (external diameter: D27 × W36 × H0.5 mm, inner window: D25 × W34 (8.50 cm^2)) derived from silicon sheet (AsOne Corporation, Tokyo, Japan) was immersed in PBS for 10 min for the priming treatment. The substrate was incubated for 2 h in the 2% bovine serum albumin (BSA)/ PBS solution at 37 ºC or 15 ºC. Then, the substrate was carefully washed with PBS, and the solution containing bicinchoninic acid (Micro BCA Protein Assay Kit, Thermo Fisher Scientific) was placed on the surface (230 μL/substrate) and it was color-reacted for 2 h at 37 ºC under the humidity of 95–100%. The absorbance of colorized solution at 570 nm was measured by the microplate reader (Multiskan FC, Thermo Fisher Scientific). The data obtained from six independent samples were averaged, and the amount of proteins was evaluated using the standard curve measured by the BSA solution at the known concentration (0–100 μg/mL).

Assay for Adhesion of Cells. The polymer-modified substrate was immersed in 70% ethanol solution and dried in the clean bench for the sterilization. The substrates were incubated in PBS for the priming treatment, and they were then incubated in the culture medium [Minimum essential medium (MEM) containing 10% FBS] until use in the experiment.

NIH3T3 cells (passage 138) were used in this assay. The cells pre-cultured for 2–3 days on the TCPS dish were harvested by 0.25% trypsin/1 mM EDTA solution and obtained by centrifugation (500×*g*, 3 min, 4 °C). These cells were suspended with the culture medium, and they were seeded on the polymer-modified surface with a density of 3×10^4 cells/cm^2. The cells were cultured for 3 days in 5% CO_2 at 37 °C.

To investigate the number of living cells adhered onto the surface, the cells were incubated with the medium containing 2 μg/mL Hoechst33342 (Dojindo Laboratories, Kumamoto, Japan) and 1 μg/mL Calcein-AM (Dojindo Laboratories) for 30 min in the CO_2 incubator (5% CO_2, 37 °C). Then, the cells were carefully washed with PBS warm-upped to 37 °C and observed using a fluorescence microscope (IX71, Olympus Corporation, Tokyo, Japan). The average number of adhering cells double-stained with Hoechst33258 (blue) and Calcein-AM (green) was determined by counting the pictures of 10-positions obtained randomly.

Results

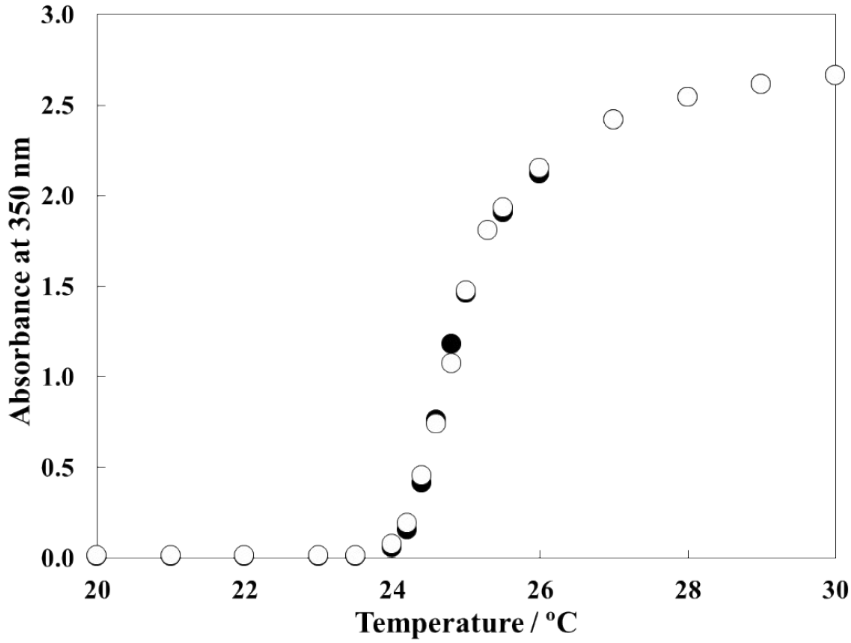


Figure S1. Temperature dependence of the absorbance of the aqueous solution of P(MDM-*co*-HEMA). ●: Heating, ○: Cooling. [polymer] = 1.0 mg/mL.

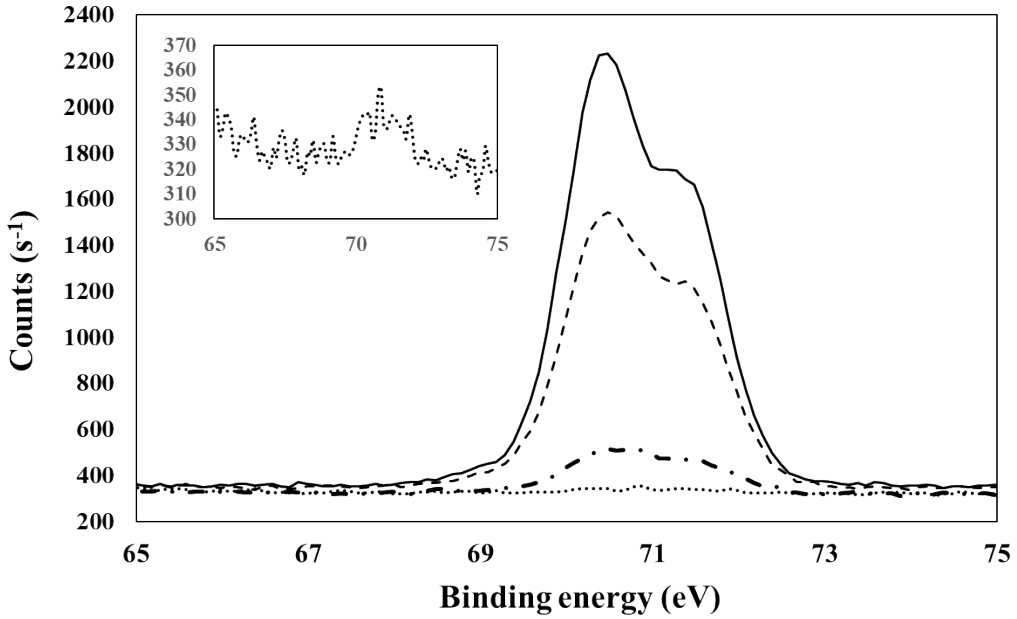


Figure S2. XPS spectra of Br3d of surfaces modified with BPE and PTMS at the various composition; BPE:PTMS = (solid line) 10 : 0, (broken line) 1 : 10, (chain line) 1 : 100, and (dotted line) 1 : 1000. Inset spectrum is magnification of BPE:PTMS = 1 : 1000.

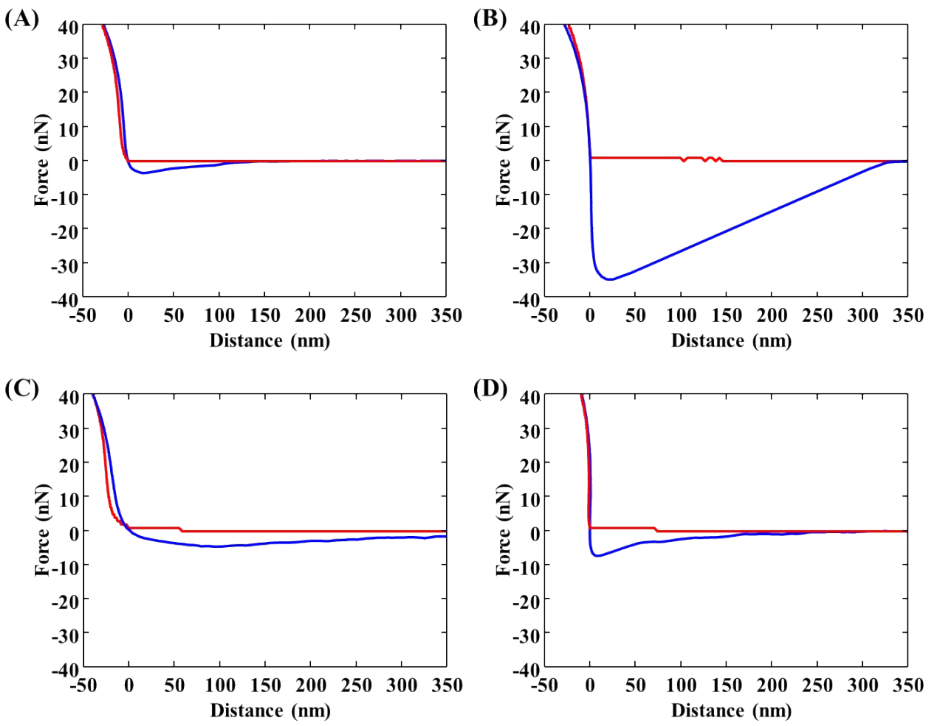


Figure S3. Force curve of (A) cross-linked polymer brushes surface and thin gel layer surfaces interpenetrating (B) PDMAEMA, (C) PMA and (D) PCMB; red line: approach curve, blue line: Retract curve.

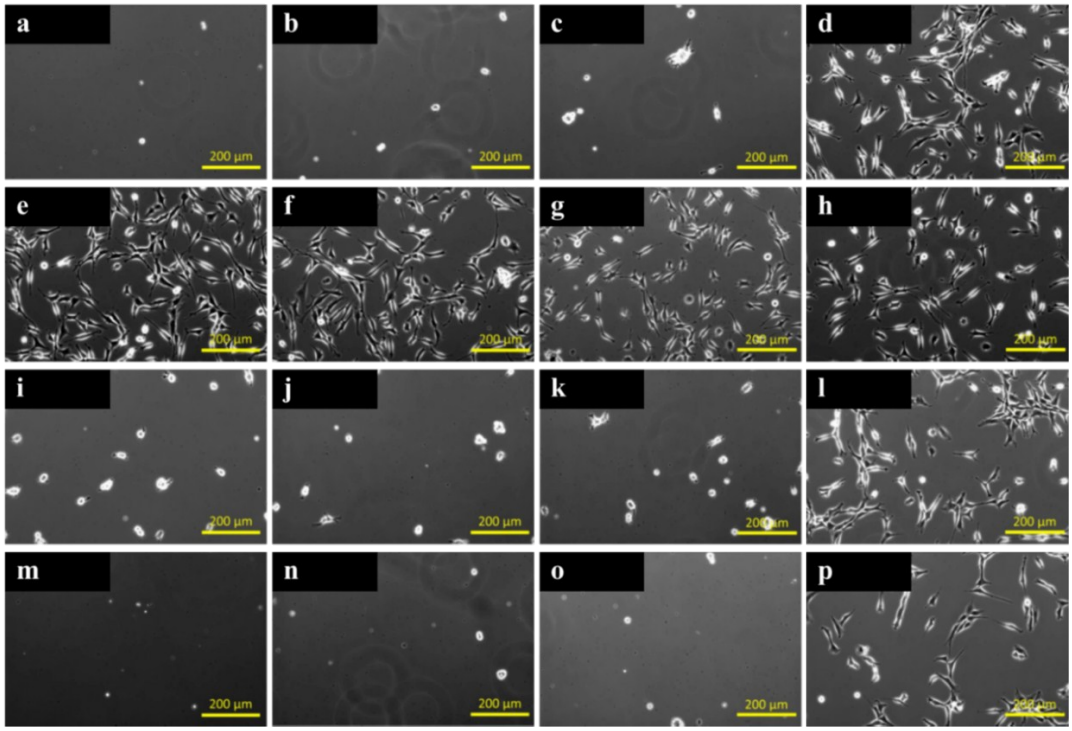


Figure S4. Photographs of 3T3 cells on the surfaces modified with (a-d) crosslinked P(MDM-*co*-HEMA) brushes, and the thin gel layer formed with interpenetration by (e-h) DMAEMA, (i-l) PMA, and (m-p) PCMB into the polymer brush. The polymer brushes at the graft density of (a, e, i, m) 0.37, (b, f, j, n) 0.27, (c, g, k, o) 0.16, and (d, h, l, p) 0.03 chains·nm^{-2} were used in this experiment.