Supporting information for

Spin-Casting Polymer Brush Films for Stimuli-Responsive and Anti-Fouling Surfaces

Binbin Xu,[1] *Chun Feng*,[1,*] *Jianhua Hu*,[2,*] *Ping Shi*,[3] *Guangxin Gu*,[2]

Lei Wang,[2] *Xiaoyu Huang*[1,*]

[1] Key Laboratory of Synthetic and Self-Assembly Chemistry for Organic Functional Molecules, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032, People's Republic of China

[2] State Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science, Fudan University, 220 Handan Road, Shanghai 200433, People's Republic of China

[3] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, People's Republic of China

* To whom correspondence should be addressed, E-mail: xyhuang@mail.sioc.ac.cn (Tel: +86-21-54925310, Fax: +86-21-64166128); hujh@fudan.edu.cn (Tel: +86-21-55665280, Fax: +86-21-65640293); cfeng@mail.sioc.ac.cn (Tel: +86-21-54925606, Fax: +86-21-64166128)

Experimental Section

Materials

Poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, M_n = 500 g/mol, Aldrich, 99%) was passed through a basic alumina column to remove the stabilizer prior to use. Styrene (St, Aldrich, 99%) was washed with 5% aqueous NaOH solution and water, dried over $MgSO_4$, and distilled twice from CaH_2 under reduced pressure prior to use. 2,2'-Azobis(isobutyronitrile) (AIBN, Aldrich, 98%) was recrystallized from anhydrous ethanol twice. Copper(I) bromide (CuBr, Aldrich, 98%) was purified by stirring overnight over CH_3COOH at room temperature, followed by washing with ethanol, diethyl ether, and acetone prior to drying at 40°C *in vacuo* for one day. *tert*-Butyl 2-((2-bromo-propanoyloxy)methyl)acrylate (*t*BBPMA)[1] and cumyl dithiobenzoate (CDB)[2] were synthesized according to previous literatures. Toluene (Aldrich, 99.8%) and tetrahydrofuran (THF, Aldrich, 99%) were dried over CaH_2 and distilled from Na and benzophenone under N_2 prior to use. *N,N,N',N',N''*-pentamethyldiethylenetriamine (PMDETA, Aldrich, 99%) was used as received.

For protein adsorption experiments, bovineserum alumin (BSA, Sangon Biotech., China) and Cytochrome c (Sangon Biotech., China) were used as received. For cell adhesion experiments, ITO glass substrate (Kaivo optoelectronic Technology Co. Ltd., China), human keratinocyte cell line (HaCaT, ATCC), DMEM medium (GIBCO/ Invitrogen, USA), fetal bovine serum (FBS, BI Biological Industries Ltd., Isarel), and 1% penicillin-streptomycin (10,000 U/mL penicillin and 10 mg/mL streptomycin, Solarbio Life Science, China) were used as received.

Measurements

^{1}H NMR analyses were performed on a Bruker Avance 500 spectrometer (500 MHz) in $CDCl_3$ and acetone-d_6; tetramethylsilicone was used as internal standard. FT-IR spectra were recorded on a Nicolet AVATAR-360 spectrophotometer with a 4 cm^{-1} resolution. Relative molecular weights and molecular weight distributions were measured by conventional gel permeation chromatography (GPC) system equipped with a Waters 1515 Isocratic HPLC pump, a Waters 2414 refractive index detector, and a set of Waters Styragel columns (HR3 (500-30,000), HR4 (5,000-600,000) and HR5 (50,000-4,000,000), 7.8×300 mm, particle size: 5 μm). GPC measurements were carried out at 35^{o}C using THF as eluent with a flow rate of 1.0 mL/min. The system was calibrated with linear polystyrene standards. Atomic force microscopy (AFM) images were taken by a JPK NanoWizard Sense system in the tapping mode of spin-casting the sample solution onto ITO glass substrate. X-ray photoelectron spectroscopy (XPS) was recorded on a PHI 5000c ESCA photoelectron spectrometer. The contact angle of water on the film was measured by a JC2000C instrument at 20±1°C using a sessile drop method. Protein adsorption on film was monitored by a Biolin Q-Sense E1 quartz crystal microbalance with dissipation monitoring (QCM-D). Cells were imaged under a Nikon Eclipse TI inverted fluorescence microscope.

Preparation of P*t*BBPMA-*co*-PPEGMEMA Copolymer

In a typical procedure, AIBN (16.4 mg, 0.1 mmol) and CDB (81.6 mg, 0.3 mmol) were first added to a 25 mL Schlenk flask (flame-dried under vacuum prior to use)

sealed with a rubber septum for degassing and kept under N_2. Next, *t*BBPMA (0.88 g, 3 mmol), PEGMEMA (1.50 g, 3 mmol), and dry toluene (0.6 mL) were added via a gastight syringe. The flask was degassed by three cycles of freezing-pumping-thawing followed by immersing the flask into an oil bath set at 70°C. The polymerization was terminated by immersing the flask into liquid N_2 after 16 h. The solution was precipitated into cold *n*-hexane. The crude product was purified by repeated dissolution and precipitation followed by drying *in vacuo* overnight to give 1.97 g of pink powder. Next, AIBN was used to remove the dithiobenzoate moiety of the copolymer at 65°C in THF according to previous report.[3] Finally, 1.74 g of white powder, poly(*ter*t-butyl 2-((2-bromopropanoyloxy)methyl)acrylate)-*co*-poly(poly (ethylene glycol) methyl ether methacrylate) (P*t*BBPMA-*co*-PPEGMEMA) **1b**, was obtained after drying *in vacuo* overnight. GPC: M_n = 8,300 g/mol, M_w/M_n =1.19. ^{1}H NMR ($CDCl_3$): δ (ppm): 0.82, 0.98, 1.16 (3H, CH_2CCH_3), 1.44 (9H, $C(CH_3)_3$), 1.74, 1.85 (2H, CH_2C and 3H, CH_3CHBr), 3.37 (3H, OCH_3), 3.54, 3.64 (4H, OCH_2CH_2), 4.06 (2H, $CH_2CCH_2O_2C$ and 2H, $CH_2CCO_2CH_2$), 4.44 (1H, CH_3CHBr).

Table S1. RAFT Copolymerization of *t*BBPMA and PEGMEMA[a]

entry	[*t*BBPMA]:[PEGMEMA]:[CDB]:[AIBN]	$M_{n,GPC}$[b] (g/mol)	M_w/M_n[b]	$M_{n,NMR}$[c] (g/mol)	x:y[d]
1a	45:15:3:1	7,600	1.22	9,000	17.4/7.4
1b	30:30:3:1	8,300	1.19	9,000	10.7/11.4
1c	15:45:3:1	7,600	1.18	9,600	5.6/15.5

[a] Polymerization temperature: 70°C, polymerization time: 16 h, solvent: toluene. [b]

Measured by GPC at 35°C in THF. [c] Obtained from ^{1}H NMR. [d] x and y represent the number of *t*BBPMA repeated unit with an ATRP initiating group and PEGMEMA repeated unit without an ATRP initiating group, respectively, obtained from ^{1}H NMR.

Synthesis of (P*t*BA-*g*-PS)-*co*-PPEGMEMA Polymer Brush

In a typical procedure, CuBr (6 mg, 0.042 mmol) and P*t*BBPMA-*co*-PPEGMEMA **1b** ($M_{n,GPC}$ = 8,300 g/mol, M_w/M_n = 1.19, 40 mg, 0.042 mmol ATRP initiating group) were first added to a 25 mL Schlenk flask (flame-dried under vacuum prior to use) sealed with a rubber septum for degassing and kept under N_2. Next, styrene (1.9 mL, 16.8 mmol) and PMDETA (9 μL, 0.042 mmol) were charged via a gastight syringe. The flask was degassed by three cycles of freezing-pumping-thawing followed by immersing the flask into an oil bath set at 80°C. The polymerization lasted 2 h and was terminated by immersing the flask into liquid N_2. The mixture was diluted by THF and passed through an alumina column to remove the residual copper catalyst. The solution was concentrated and precipitated into cold methanol. After repeated purification by dissolving in THF and precipitating in methanol, (P*t*BA-*g*-PS)-*co*-PPEGMEMA **2d** polymer brush was obtained as a white powder after drying *in vacuo* overnight. GPC: M_n = 35,000 g/mol, M_w/M_n = 1.26. FT-IR (KBr): ν (cm^{-1}): 3026, 2924, 1732, 1598, 1488, 1446, 1143, 757, 696. ^{1}H NMR (acetone-d_6): δ (ppm): 0.87, 0.97, 1.10 (3H, CH_2CCH_3), 1.28, 1.45, 1.59 (9H, $C(CH_3)_3$, 2H, CH_2C, and 2H, $C_6H_5CHCH_2$), 1.93 (1H, $C_6H_5CHCH_2$), 3.27 (3H, OCH_3), 3.47, 3.57 (4H, OCH_2CH_2), 4.08 (2H, $CH_2CCH_2O_2C$ and 2H, $CH_2CCO_2CH_2$), 6.65, 7.08 (5H, $C_6H_5CHCH_2$).

Preparation of Polymer Brush Film on ITO Glass Substrate

ITO glass substrate was immersed in each of following solvents and sonicated for 1 h: aqueous solution of detergent, deionized water, acetone, and isopropanol, followed by drying *in vacuo* overnight. Next, chloroform solution of (P*t*BA-*g*-PS)-*co*-PPEGMEMA **2** polymer brush (7.5 mg/mL) was prepared by stirring for 30 min before filtering through a 0.45 μm syringe filter. The filtrate was then spin-cast onto the freshly cleaned ITO surface at a spin rate of 4500 rpm for 10 s and then 3000 rpm for additional 30 s. Freshly prepared film sample was dried *in vacuo* overnight.

Preparation of Polymer Brush Film on SiO_2-Coated QCM-D Sensor

SiO_2-coated QCM-D sensor was immersed in each of following solvents and sonicated for 1 h: deionized water, acetone, and isopropanol, followed by drying *in vacuo* overnight. Next, chloroform solution of (P*t*BA-*g*-PS)-*co*-PPEGMEMA **2** polymer brush (15 mg/mL) was prepared by stirring for 30 min before filtering through a 0.45 μm syringe filter. The filtration was then spin-cast onto the freshly cleaned QCM-D sensor at a spin rate of 2500 rpm for 30 s. Freshly prepared film sample was dried *in vacuo* overnight.

Solvent Treatment of Polymer Brush Film

For solvent treatment, film samples were placed in a sealed chamber with either methanol or cyclohexane under reduced pressure for 8 h. After solvent treatment, samples were removed from the sealed chamber and immediately dried under N_2 flow

before placing the samples under vacuum overnight to remove residual solvent.

Contact Angle Measurement

Contact angle measurements were carried out for (P*t*BA-*g*-PS)-*co*-PPEGMEMA **2** polymer brush films on ITO substrate. Water contact angle was measured after allowing the water droplet equilibrate on the surface for 30 s. Measurements were repeated on at least two substrates for each sample and at least on two different spots for every substrate. The final reported values represented an average of measurements.

Protein Adsorption Experiment

QCM-D sensors with (P*t*BA-*g*-PS)-*co*-PPEGMEMA **2** polymer brush films were placed in a flow cell and the films were allowed to hydrate for 1 h in flowing PBS (100 μL/min). A protein solution in PBS (10 mg/mL) was introduced into the cell at a rate of 100 μL/min for 1 h. Experiments were conducted for different PI values of protein (BSA, PI = 4.8; Cytochrome c, PI = 10.7). Finally, the films were rinsed with PBS (100 μL/min) again. The frequency of the harmonics and dissipation at these frequencies were monitored as the protein adsorbed to the film. The temperature of the crystal was maintained at 25^{o}C throughout the experiment.

Cell Adhesion Experiment

HaCaT cells were cultured at 37ºC under a humidified 5% CO_2 atmosphere in DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin.

Culture medium was replaced every day. After confluence, the cells were subcultured following trypsinization. Film samples were transferred to a new 6-well plate and sterilized through exposure to ultraviolet for 8 h. HaCaT cells were then seeded onto 6-well micro-well plates in 2 mL of DMEM medium at a density of 3×10^5 cells/well and cultured for 6 h. Cells were imaged *in situ*, after which the substrates were gently removed from the well and placed into new wells (pre-filled with cell culture medium) for further imaging. The new wells were then imaged under inverted fluorescence microscope.

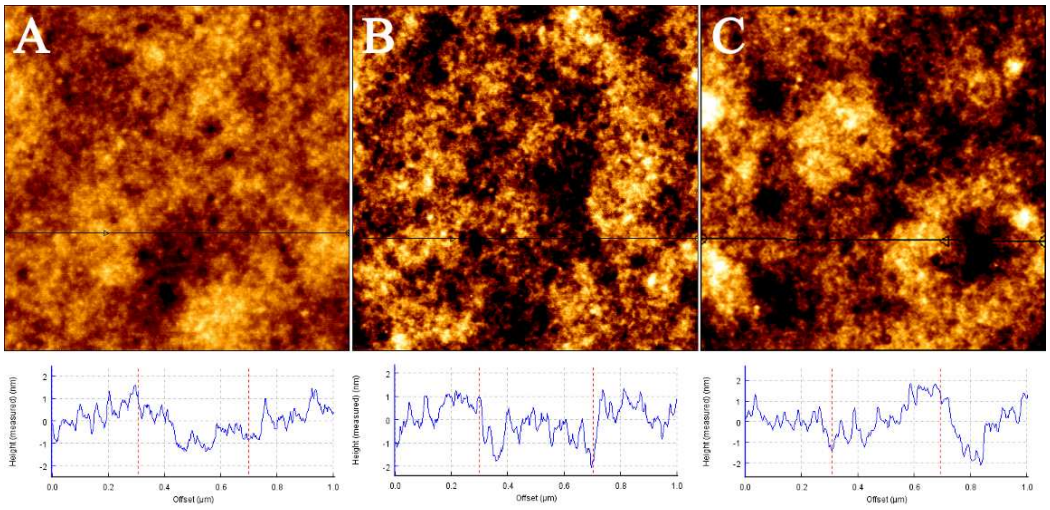

Figure S1. AFM height images for (P*t*BA-*g*-PS)-*co*-PPEGMEMA **2c** polymer brush film on ITO substrates under different conditions, (A) as-cast film, scale: 1 µm × 1 µm, (B) methanol-treated film, scale: 1 µm × 1 µm, and (C) cyclohexane-treated film, scale: 1 µm × 1 µm.

References and Notes

1. Zhang, Y. Q.; Shen, Z.; Yang, D.; Feng, C.; Hu, J. H.; Lu, G. L.; Huang, X. Y. Convenient Synthesis of P*t*BA-*g*-PMA Well-Defined Graft Copolymer with

Tunable Grafting Density. *Macromolecules* **2010**, *43*, 117-125.

2. Moad, G.; Chiefari, J.; Chong, Y. K.; Krstina, J.; Mayadunne, R. T. A.; Postma, A.; Rizzardo, E.; Thang, S. H. Living Free Radical Polymerization with Reversible Addition-Fragmentation Chain Transfer. *Polym. Int.* **2000**, *49*, 993-1001.
3. Perrier, S.; Takolpuckdee, P.; Mars, C. A. Reversible Addition-Fragmentation Chain Transfer Polymerization: End Group Modification for Functionalized Polymers and Chain Transfer Agent Recovery. *Macromolecules* **2005**, *38*, 2033-2036.