Electronic Supporting Information

Micropatterning of hydrophilic polyacrylamide brushes to resist cells adhesion but promote proteins retention

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1. Experimental Section:

Materials: SEBS copolymer with 29 wt% styrene (Kraton G 1652, M_n=74800) was purchased from Shell Chemicals (USA). Acrylamide (99.9%), Copper(II) bromide (CuBr₂, 99%) and 2,2'-bipyridine (Bpy, >99%) were purchased from Alfa Aesar. Copper(I) bromide (CuBr, 98%) were obtained from Sigma-Aldrich. FITC-bovine serum fibrinogen (FITC-BFg), FITC-lysozyme (FITC-Lyz), FITC-limulus polyphemus hemocyanin (FITC-Hc), rhodamine-bovine serum albumin (RBITC-BSA), and rhodamine-bovine hemoglobin (RBITC-BHb), rhodamine-transferrin (RBITC-Tf) were purchased from Beijing biosynthesis biotechnology (China). Sodium dodecyl sulfate (SDS) was provided by Dingguo Bio-technology (China). Protein assay reagent kit (Micro BCATM) was from Boster Biological Technology (AR1110, Wuhan, China). Phosphate buffered saline (PBS, 0.1 mol L⁻¹, pH 7.4) solution was freshly prepared. Other chemicals were analytical grade and used without further purification. Milli-Q water (18.2 MΩcm) was used in all experiments.

Immobilizing patterned ATRP initiator on SEBS: The basic strategy for the fabrication of the patterned ATRP initiator using the aqueous-based method was adapted from our previous study.¹ Copper grids with different shapes and sizes served as photomasks and transparent substrates incorporating copper grids were prepared in a cleanroom environment. Then they were exposed to UV/O₃ for 30 min in a cleaning chamber and were subsequently immersed in HBr/H₂SO₄ (5/1, v/v) mixture solution for 24 h at 60 °C. After that, these treated films were washed drastically with deionized water to remove physically adsorbed HBr and dried overnight under

vacuum at 25 °C before use. The so-obtained films with immobilized patterned initiator were referred as patterned brominated SEBS samples (SEBS-Br).

Patterning of PAAm brushes: Briefly, CuBr (29.2 mg, 0.2 mmol), CuBr₂ (4.6 mg, 0.02 mmol) and patterned SEBS-Br samples were placed in a 100 mL round-bottom flask equipped with a glass stopper and then was subjected to laboratory vacuum followed by high pure argon gas in-flow for 30 min. A deoxygenated aqueous solution (water and methanol in a 1:1 volume ratio, 30 mL) with acrylamide (2 g, 28.14 mmol) and PMDETA (0.125 mL, 0.60 mmol) was introduced into the flask under argon protection. The resultant mixture was degassed through three freeze-pump-thaw cycles before the reaction started. The grafting process proceeded at 60 °C for a certain period of time. After the desired reaction time, substrates were removed from the polymerization solution, exhaustively rinsed with Milli-Q water to remove all traces of the polymerization solution, and subsequently dried under vacuum overnight at 25 °C.

ATR-FTIR measurements: ATR-FTIR measurements of virgin SEBS and SEBS-*g*-PAAm films were performed on a Bruker FTIR spectrometer Vertex 70 equipped with an Attenuated Total Reflection (ATR) unit (ATR crystal 45°) at a resolution of 4 cm⁻¹ for 32 scans.

XPS measurements: The surface composition was determined via X-ray photoelectron spectroscopy (XPS) by using VG Scientific ESCA MK II Thermo Avantage V 3.20 analyzer with Al/K (hv = 1486.6 eV) anode mono-X-ray source. All the samples were completely vacuum dried before characterization. The take-off

angle for photoelectron analyzer was fixed at 90°. All binding energy (BE) values were referenced to the C_{1s} hydrocarbon peak at 284.6 eV. The atomic concentrations of the elements were calculated by their corresponding peak areas.

POM: The morphologies of the patterned surface were observed by polarized optical microscopy (Zeiss Axio Imager A2m, Carl Zeiss, Germany) equipped with a video CCD camera.

SEM: The surface of the film was observed with field emission scanning electron microscopy (FESEM) by using a XL 30 ESEM FEG (FEI Company) instrument equipped with an EDX spectroscopy attachment.

CLSM: Images were acquired by using a confocal laser scanning microscope (CLSM) (LSM700-Zeiss, Germany) equipped with an InGaN semiconductor laser (405 nm), an Ar laser (488 nm), and a He-Ne laser (555 nm). All samples were visualized using the same acquisition settings and analyzed using Zen 2011 software (Carl Zeiss).

AFM: The film morphologies were characterized by tapping-mode using a SPI 3800/SPA 300HV AFM (Seiko Instruments Inc., Japan). All measurements were carried out at room temperature in ambient air conditions.

Contact angle/Surface energy analysis: Surface wettabilities of unmodified and PAAm-modified SEBS were evaluated by the sessile drop method with a pure water droplet (ca. 3 μ L) using a contact angle goniometer (DSA, KRUSS GMBH, Germany). The average value of five measurements made at different surface locations on the same sample was adopted as the contact angle. Surface energy of

SEBS films was then calculated according to the following equation $\cos\theta = -1 + 2\sqrt{\frac{\gamma_s}{\gamma_l}}e^{-\beta(\gamma_s - \gamma_l)^2}$ (1)

where γ_s and γ_1 represent the solid (SEBS) and liquid (water) surface energy, respectively; β is the constant coefficient relating to a specific solid surface. Herein, a value of 1.247×10^{-4} is adopted according to instrument handbook; θ is the contact angle of water on a solid SEBS surface.

BCA assay for protein quantitation: To quantitatively evaluate the protein adsorption on the virgin and PAAm-modified SEBS surfaces, the bicinchoninic acid (BCA) assay was adopted and six model proteins (BFg, Lyz, Hc, BSA, BHb and Tf) in PBS were prepared with the concentration of 1 mg mL⁻¹, respectively.. The general procedure is described in detail elsewhere,¹ and only a short description is given here. After being equilibrated with PBS overnight, the film specimens (1 cm×1 cm) were moved into PBS solution containing model protein (1.0 mg/mL) at 37 °C for 2 h. Then each sample was rinsed five times by gentle shaking in the fresh PBS solution. Subsequently, the samples were immersed in 1 mL of PBS solution containing 1 wt% of sodium dodecyl sulfate (SDS), and the protein adsorbed on the surface was completely desorbed by sonication for 20 min. A micro BCATM protein assay reagent kit based on the bicinchoninic acid (BCA) method was used to determine the concentration of the protein in the SDS solution. The concentrations were determined using a TECAN (TECAN GENIOS, Austria) operating at 562 nm. Independent measurements were performed in triplicate samples and the total adsorbed protein values were calculated from the concentration of standard protein solutions.

Multiple proteins micropatterning: We have developed a novel and simple method for the production of multiple proteins micropattern on SEBS surface. To fabricate micropattern of multiple proteins, six model proteins (FITC-BFg, FITC-Lyz, FITC-Hc, RBITC-BSA, RBITC-BHb and RBITC-Tf) in PBS were prepared with the concentration of 1 mg mL⁻¹, respectively. Subsequently, the patterned substrates were incubated in the as-prepared protein solutions for 60 min at 37 °C in the dark. Following the incubation, protein solutions were removed and the samples were gently washed twice with pre-warmed PBS, double distilled water, respectively. Then the samples were dried under a stream of nitrogen and observed with a CLSM (LSM700-Zeiss, Germany). As for the characterization of CLSM, the FITC and RBITC dyes were excited with an argon ion laser at 488 nm and 555 nm, respectively.

Platelet micropatterning: The micropatterned samples were placed into cell culture plates and equilibrated with PBS solution for 1 h at 37 °C. Fresh blood from healthy white rabbits was extracted via venipuncture through a 19-gauge Butterfly needle into a standard blood collection tube containing 3.8 wt% sodium citrate [9:1 (v/v) blood/anticoagulant]. The first portion of blood drawn was discarded to avoid contamination by tissue thromboplastin caused by puncture with the needle. The citrated whole blood was immediately centrifuged (1000 rpm, 15 min, 25 °C) to obtain platelet rich plasma (PRP). After equilibration with PBS, the micropatterned samples were placed in contact with PRP and incubated for 60 min at 37 °C under static conditions. Then PRP was removed with an aspirator, and the samples were rinsed three times with PBS to remove any non-adhered platelets. Subsequently,

adhered platelets were fixed using 2.5 vol% glutaraldehyde in PBS for 10 h at 4 °C. Finally, the samples were freeze-dried and the platelets adhered on the micropatterned sample surfaces were observed using POM, FESEM and CLSM, respectively.

RBC micropatterning: To isolate and purify the red blood cells (RBCs), we used the method described by Brooks et al.² Briefly, the whole blood sample was centrifuged at 1000 rpm for 15 min to separate red blood cells, white blood cells, and platelet rich plasma (PRP). Then the plasma and buffy coat layers (platelets and white cells) were carefully removed and discarded. RBCs concentrates were washed three times with isotonic saline [0.9% (w/v) of aqueous NaCl solution, pH 7.4]. Afterward, the RBC pellets were resuspended in normal saline to obtain an RBC suspension at 20% (v/v) hematocrit. Subsequently, 80 μ L of RBC suspension was dropped on the PBS-equilibrated micropatterned substrate surface and incubated at 37 °C for 60 min under static conditions to provide enough time to adhere RBCs. After the incubation, the samples were carefully rinsed twice with pre-warmed PBS, followed by immersing in 3 mL of 2.5 vol% glutaraldehyde in PBS for 10 h at 4 °C to fix the adhered RBCs. Finally, the samples were freeze-dried and the morphologies of adhered RBCs on the sample surfaces were visualized using POM, FESEM and CLSM, respectively.

2. Supplemental Figures:

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Figure S1. (a) XPS survey scan and (b) ATR-FTIR spectra of SEBS, SEBS-OH, SEBS-Br and SEBS-*g*-PAAm surfaces.

XPS and ATR-FTIR were employed to detect the chemical changes of the modified SEBS surface. In comparison with virgin SEBS, the peak at 532.0 eV corresponding to the binding energy of O_{1s} became much stronger, indicating that rich oxygen-containing functional groups had been introduced onto SEBS surface after UV/O₃ treatment (Figure S1a). After the subsequent reaction in the HBr/H₂SO₄ mixture, the O_{1s} peak became weak and additional peaks at 69.0 and 182.0 eV, corresponding to the binding energies of Br_{3d} and Br_{3p}, respectively, were detected from the spectrum of the initiator-immobilized SEBS surface (Figure S1a).³ These results demonstrated that many oxygen-containing groups had been substituted by -Br in the HBr/H₂SO₄. However, for the PAAm-modified film, an additional emission peak (400.1 eV) of N_{1s} was detected (Figure S1a), additional four absorption peaks at 3340 and 3191 cm⁻¹(N-H stretching of NH₂ moieties), 1659 cm⁻¹ (C=O stretching), 1605 cm⁻¹ (N-H deformation vibration of NH₂ moieties) were found in the ATR-FTIR spectra (Figure S1b).⁴ All these results proved that a dense PAAm layer was established on the SEBS surface.



Figure S2. C_{1s} high resolution XPS spectra of (a) virgin SEBS, (b) SEBS-OH, (c) SEBS-Br, (d) SEBS-*g*-PAAm surfaces. Peaks corresponding to different carbon bonds are indicated.

The high-resolution spectra corresponding to C_{1s} were shown in Figure S2 to distinguish the different types of functional groups on the surfaces. In comparison with virgin SEBS film (Figure S2a), the high-resolution spectrum of C_{1s} confirmed the presence of C–OH (285.8 eV) and O–C=O (289.0 eV) bonds, indicating the UV/O₃ treated sample were rich in hydroxyl and carboxylic acid groups on the surfaces (Figure S2b).⁵ Successful anchoring of initiator onto the film surface was substantiated by the appearance of a doublet peak in the corresponding C_{1s} spectrum



(Figure S2c). The presence of PAAm brushes grown from the ATRP initiator was evidenced by the amide carbon $O=C-NH_2$ peak at 288.0 eV (Figure S2d).⁶

Figure S3. AFM images $(50 \times 50 \ \mu\text{m}^2)$ of the micropatterned SEBS surfaces and their corresponding height profiles along the line shown in the images. (a) SEBS-Br surface, (b) SEBS-*g*-PAAm surface (ATRP time: 2 h), (c) SEBS-*g*-PAAm surface (ATRP time: 6 h), (d) SEBS-*g*-PAAm surface (ATRP time: 10 h). The scale bar is 10 μ m in all images.

Figure S3 presented the AFM images and the corresponding cross-section analysis of the SEBE-Br and SEBS-g-PAAm surfaces with different ATRP time. As can be seen from the AFM image of SEBS-Br (Figure S3a), the relative height difference between the UV-unexposed domains and UV-exposed domains appeared to be approximately 230 nm. This phenomenon was mainly caused by the etching process of UV/O₃ treatment. Photoirradiation led to decomposition of the SEBS substrate in the exposed regions.⁷ It can be clearly seen that the polymer brushes grew from the patterned initiator, resulting in regularly distributed microstructures. After performing the subsequent SI-ATRP of AAm, the overall height, as well as the height-profile, changed significantly. The thickness of the PAAm brushes was about 235.1 nm, 651.2 nm and 1099.8 nm when the polymerization time was 2 h, 6 h and 10 h, respectively. The above results indicated that there was an approximately linear increase in PAAm thickness with polymerization time, indicating that the chain growth from the SEBS– Br surface was consistent with a "controlled" and well-defined process.⁸



Figure S4. (a) SEM image of micropatterned PAAm brushes grown from SEBS substrates. (b) Higher-magnification views of section (B) in (a) image, showing the morphology of cross-linked PAAm brushes. (c, d) The EDX spectra of (A) and (B)

section in (a) image, respectively. The Au peak in c and d was attributed to gold granules. The sharp carbon peak was due to the SEBS substrate.

We performed SEM and EDX line scanning analyses to reveal the compositional variation over micropatterned SEBS-*g*-PAAm surface. Figure S4b showed an enlarged SEM image of section (B) in Figure S4a, indicating the PAAm brushes were highly physically entangled. And the spot-profile EDX spectra from specific regions of the patterned surface were shown in Figure S4c and S4d. The EDX spectrum from the (A) regions (Figure S4c) showed that it was composed primarily of carbon and a small percentage of oxygen. By contrast, the existence of N signal, thus clearly indicated the presence of the PAAm brushes on (B) region of Figure S4a. It is interesting to note that there are no PAAm brushes on the regions between two circular apertures, indicating a high degree of fidelity of our method. This result also clearly showed that the surface chemistry was precisely modified.



Figure S5. Water contact angle and surface energy of virgin SEBS, SEBS-Br, and SEBS-*g*-PAAm surfaces obtained with different ATRP time.

The water contact angle (WCA) and surface energy values of the virgin SEBS, SEBS-Br, and SEBS-*g*-PAAm samples with different ATRP time were shown in Figure S5. The WCA decreased from 106° (virgin SEBS) to 99° (SEBS-Br), due to the remained polar groups after UV/O₃ treatment and brominating reaction. The grafting of PAAm brushes on the surface led to an abrupt enhancement of surface energy from 20 mJ m⁻² (virgin SEBS) to 68 mJ m⁻² (SEBS-*g*-PAAm-24h), and sharp drop of water contact angle from 99° to 21°.



Figure S6. POM images of patterned PAAm brushes with varied geometries (stripe, square, and circle).



Figure S7. Fluorescent micrographs of micropatterned PAAm brushes before and after FITC-BSA adhesion (a, b), and before and after RBITC-BSA adhesion (c, d). The scale bar is 200 µm in all images.

Initial studies of protein adhesion on the patterned surface were performed using BSA labeled with FITC and RBITC, respectively. And Figure S7 showed the fluorescent micrographs of micropatterned SEBS-*g*-PAAm surfaces before and after BSA adhesion. To our surprise, the intensity of background signals associated with SEBS substrates was very low, and the patterned regions covered with PAAm brushes showed strong fluorescence signals after FITC-BSA adhesion (Figure S7b). Additionally, we found weak fluorescence emission at 488 nm was an intrinsic property of the PAAm brushes (Figure S7a). To avoid the influence of autofluorescence background caused by PAAm brushes, we selected BSA labeled with RBITC as model protein because as-grown PAAm brushes did not show any fluorescence in the detection wavelength range of RBITC (Figure S7c). Interestingly, spatially well-ordered, two-dimensional protein patterns were observed with high selectivity and fluorescence intensity (Figure S7d). The fluorescence intensity was assumed to be proportional to the density of the bound proteins. These results demonstrated that the amount of BSA adsorbed by the PAAm brushes was much higher than that adsorbed by the hydrophobic interaction between BSA and SEBS substrate.



Figure S8. Fluorescent micrographs showing FITC-BSA adhesion on micropatterned polymer brushes with varied sizes. The scale bar is 50 µm in all images.



Figure S9. 3D reconstruction of reflection-mode CLSM images of the micropatterned SEBS-*g*-PAAm surfaces after absorption of different fluorescently labeled proteins. (a) FITC-BFg, (b) FITC-Lyz, (c) FITC-Hc, (d) RBITC-BSA, (e) RBITC-BHb, (f) RBITC-Tf.



Figure S10. SEM images showing PLTs (a1-a3) and RBCs (b1-b3) adhesion on the micropatterned films with different pore sizes. The scale bar is 50 µm in all images.



Figure S11. (a) Total amount of adsorbed proteins (BFg, Lyz, Hc, BSA, BHb and Tf) onto the virgin SEBS and non-patterned SEBS-*g*-PAAm surfaces. (b) Statistical number of adherent PLTs and RBCs on the virgin SEBS and non-patterned SEBS-*g*-PAAm surfaces. Error bars represent a standard deviation for n = 5.

In this work, BCA protein assay kit method was adopted to quantitatively determine adsorbed proteins on the model surfaces. The adsorption of different proteins on the virgin SEBS and non-patterned SEBS-g-PAAm surfaces was carried

out by incubation at 37 °C for 2 h using the same initial protein concentration of 1 mg/mL. Fig. S11a showed the total amount of various adsorbed proteins on SEBS surfaces before and after grafting with PAAm brushes. Non-patterned SEBS-g-PAAm showed the highest adsorption of Tf (5.5 μ g/cm²) and the lowest adsorption of Lys $(3.1 \,\mu\text{g/cm}^2)$ among all the proteins. The amount of proteins entrapped on the nonpatterned SEBS-g-PAAm surface were 1.5-3.5 times of that adsorbed on virgin SEBS surface, indicating that the PAAm brushes obtained here could entrap plasma proteins and the encapsulating capacity was much higher than the hydrophobic interaction between proteins and hydrophobic materials. Figure S11b displayed the statistical results of adhered PLTs and RBCs on the virgin and non-patterned SEBS-g-PAAm surface, respectively. The density of adhered RBCs on the virgin SEBS surface was $(7.4 \pm 2.6) \times 10^3$ cells/mm², which was much lower than that of adhered PLTs (5.4 ± 0.4) \times 10⁴ cells/mm². This result could be explained by two primary factors. On the one hand, RBCs were non-adhesive compared with PLTs, which was a typical adhesive cell; on the other hand, the size of RBCs (6-8 µm in diameter) was much larger than that of PLTs (2-3 μ m diameter). There was an average of (6.6 \pm 2.3) \times 10² RBCs /mm² and (2.0 \pm 0.5) \times 10³ PLTs /mm² on the SEBS-g-PAAm surface, respectively. The PAAm-modified SEBS film could reduce about 91% and 96% of adhering RBCs and PLTs, respectively, as compared with the virgin samples. Figure S11a and S11b provided the direct evidence that the fabricated PAAm brushes have the capability to resist cells adhesion but promote proteins retention.

3. Supplemental Tables:

 Table S1. Elemental composition (at %) of the functionalized SEBS surfaces as

 determined by XPS survey spectra.

Sample	Compositions (at.%)					
	C(%)	O(%)	Br(%)	N(%)		
SEBS	96.32	3.68				
SEBS-OH	74.08	25.92				
SEBS-Br	84.99	12.9	2.11			
SEBS-g-P(AAm)	82.28	9.84	0.12	7.76		

 Table S2. Properties of the six model proteins.⁹⁻¹⁶

Protein	Plasma Concentration	Molecular Weight	Isoelectric	Size
	$(mg mL^{-1})$	(g mol ⁻¹)	Point	(nm ³)
BSA	40	66,000	4.8	14×4×4
BFg	2~3	340,000	5.5	45×9×9
Hb	110~140	510,000~640,000	6.8	6.4×5.5×5.0
Lyz	10~15	14,300	11.0	4.5×3.0×3.0
Hc	40	3300,000	6.4	35×13×11
Tf	2~4	77,000	5.2	9.5×6.0×5.0

4. References:

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