Electronic Supporting Information

Guided proteins/cells patterning on superhydrophilic polymer brushes functionalized with mussel-inspired polydopamine coatings

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S1
1. **Experimental Procedures:**

1.1 Materials.

SEBS copolymer with 29 wt% styrene (Kraton G 1652E, $M_n=74800$) was purchased from Shell Chemicals (USA). Benzophenone (BP) was supplied by Peking Ruichen Chemical (China). 2-Acrylamido-2-methylpropane sulfonylic acid (AMPS) was purchased from Yuyuan Chemistry Company (Shandong, China). Dopamine hydrochloride (98%), tris(hydroxymethyl)aminomethane (Tris) and poly-L-lysine coated glass slides were purchased from Sigma-Aldrich Chemical Co (Shanghai, China). Fluorescein isothiocyanate labeled bovine serum fibrinogen (FITC-BFg), rhodamine B isothiocyanate labeled bovine serum albumin (RBITC-BSA) and fluorescein isothiocyanate labeled lysozyme (FITC-Lyz) were purchased from Beijing Biosynthesis Biotechnology (China). Phosphate buffered saline (PBS, 0.1 mol L$^{-1}$, pH 7.4) and isotonic saline (NaCl solution, 0.154 mol L$^{-1}$, pH 7.4) were freshly prepared. Other chemicals were analytical grade and used without further purification. Milli-Q water (18.2 MΩcm) was used in all experiments.

1.2 Characterization.

**ATR-FTIR Measurements.**

The infrared spectra were measured in 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\textsubscript{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.**

Obtained substrates were 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).

**Water Contact Angle Measurement.**
Water contact angles were determined at different temperatures (37°C and 20°C) by the sessile drop method with a pure water droplet (ca. 3 μL) 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.

1.3 Preparation of SEBS Films.

The SEBS film was prepared by dissolving 7 g of SEBS powder in 50 mL of dimethyl benzene. The polymer solution was then cast onto a glass substrate, and the solvent was removed by controlled evaporation at room temperature over a period of 48 h. And the thickness of the film was about 200 μm.

1.4 Preparation of Poly(AMPS)-Grafted SEBS.

For the surface-initiated photo-polymerization (SIPP) with AMPS, flat SEBS films were immersed in an ethanol solution of BP (1.5 wt %) for 30 min and then dried in vacuo under dark condition for 1 h at 25 °C. Monomer solution was prepared prior to the experiments by mixing AMPS monomer (3.2 g) in degassed DI water (36.8 mL) and then argon was bubbled for 30 min to eliminate any oxygen. The BP-preadsorbed SEBS films were put on the slide glasses and coated with 8 wt % aqueous solution of AMPS, followed by covering with another quartz plate (0.8 mm thick). Then the sandwiched system was exposed to UV illumination (high-pressure mercury lamp, 400 W, main wavelength 380 nm) at a distance of 15 cm for 8 min at ambient temperature (22 °C). After SIPP, the samples were vigorously rinsed with deionized water and ethanol for 24 h to remove unreacted monomers and unreacted
initiators. The samples were then dried in a vacuum oven for 24 h at room temperature. The so-obtained poly(AMPS)-grafted film was referred to as SEBS-g-PAMPS samples.

1.5 Formation of Patterned Polydopamine (PDA) Coating on SEBS-g-PAMPS Samples.

For the preparation of patterned PDA coating using the oxidative self-polymerisation,⁠¹ copper grids with different shapes and sizes served as masks. SEBS-g-PAMPS samples incorporating copper grids were immersed in a freshly prepared dopamine solution (2 mg/mL in 10 mM Tris-HCl, pH 8.5) for 24 h. The PDA-coated SEBS-g-PAMPS films were finally rinsed extensively with ultrapure water to remove the unattached dopamine and dried under nitrogen flow. Finally, the copper grids were separated from the substrates and the obtained samples were denoted as PAMPS-PDA pattern on SEBS for simplicity. Non-patterned SEBS-g-PAMPS-PDA samples were obtained without using the copper grids when the above procedure was conducted. These model samples were denoted as SEBS-g-PAMPS-PDA.

1.6 Formation of Proteins Arrays.

FITC-BFg, RBITC-BSA and FITC-Lyz were dissolved in PBS at a concentration of 1 mg mL⁻¹ and the patterned SEBS-g-PAMPS-PDA substrates (PAMPS-PDA pattern) 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 (37 °C). Then the
samples were dried under a stream of nitrogen and fluorescent images were 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.

1.7 Formation of Platelets Arrays.

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] (Blood collection from animals was carried out in accordance with the guidelines issued by the Ethical Committee of the Chinese Academy of Sciences.). 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, patterned SEBS-g-PAMPS-PDA substrates (PAMPS-PDA pattern) 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 gently rinsed three times with PBS to remove non-adhered platelets. Subsequently, captured 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 captured on the patterned sample surfaces were observed using FESEM and CLSM, respectively.

1.8 Formation of Erythrocytes Arrays.
To isolate and purify the erythrocytes, we used the method described by Brooks et al. Briefly, the whole blood sample was centrifuged at 1000 rpm for 15 min to separate erythrocytes, white blood cells (WBCs), and PRP. Then the plasma and buffy coat layers (PRP and WBCs) were carefully removed and discarded. Erythrocytes concentrates were washed three times with isotonic saline (0.9% w/v of aqueous NaCl solution, pH 7.4). Afterward, the erythrocyte pellets were resuspended in normal saline to obtain an erythrocyte suspension at 20% (v/v) hematocrit. Subsequently, 80 μL of erythrocyte suspension was dropped on the surface of patterned SEBS-g-PAMPS-PDA (PAMPS-PDA pattern) and incubated at 37 °C for 60 min under static conditions to provide enough time to capture erythrocytes. 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 captured erythrocytes. Finally, the samples were freeze-dried and the morphologies of captured erythrocytes on the sample surfaces were visualized using FESEM and CLSM, respectively.

1.9 Blood Cells Adhesion on the Non-Patterned Samples.

The adhesion of blood cells (platelets and erythrocytes) on non-patterned surfaces, including SEBS, SEBS-g-PAMPS, SEBS-g-PAMPS-PDA and poly-L-lysine coated glass slides, was performed according to the above procedures described in platelets/erythrocytes adhesion.
2. Supplementary Figures:

Figure S1. ATR-FTIR spectra of (a) SEBS, (b) SEBS-g-PAMPS and (c) SEBS-g-PAMPS-PDA samples.
Figure S2. XPS survey scan of (a) SEBS, (b) SEBS-g-PAMPS and (c) SEBS-g-PAMPS-PDA samples.
Figure S3. POM images showing PDA patterns on the surface of SEBS-g-PAMPS sample with varied geometries.
Figure S4. SEM images showing PDA particles on the surface of SEBS-g-PAMPS sample under different magnifications (a), 80000× (b), 40000× (c), 10000× and (d) 2500×.
Figure S5. (a, d) FITC, (b) bright field, and (c) merged images of patterned SEBS-g-PAMPS-PDA surfaces after FITC-BFg adsorption. The scale bar is 100 μm in all images.
Figure S6. CLSM image of patterned SEBS-g-PAMPS-PDA surface before (a) and after (b) FITC-Lyz adsorption. The scale bar is 100 μm in all images.
Figure S7. RBITC, bright field, and merged images of the RBCs adhered on the patterned SEBS-g-PAMPS-PDA samples. The scale bar is 200 μm in all images.
Figure S8. CLSM images of adhered platelets (upper part) and erythrocytes (down part) on the surface of (a, d) virgin SEBS, (b, e) SEBS-g-PAMPS, and (c, f) SEBS-g-PAMPS-PDA, respectively. The scale bar is 50 μm in all images.
**Figure S9.** SEM images of adhered platelets (upper part) and erythrocytes (down part) on the surface of (a, c) virgin SEBS and (b, d) SEBS-g-PAMPS-PDA, respectively. The scale bar of the insets is 2 \( \mu \text{m} \).
Figure S10. CLSM images and statistical number of adhered platelets (upper part) and erythrocytes (down part) on the surface of SEBS-g-PAMPS-PDA (a, c) and poly-L-lysine coated glass slides (b, d), respectively. The scale bar is 50 μm in all images.
3. Supplementary Table:

Table S1. Chemical Composition of the Functionalized SEBS Surfaces.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Compositions (at.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C(%)</td>
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<tr>
<td>SEBS</td>
<td>96.13</td>
</tr>
<tr>
<td>SEBS-g-PAMPS</td>
<td>64.08</td>
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<tr>
<td>SEBS-g-PAMPS-PDA</td>
<td>75.01</td>
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</tbody>
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4. Supplementary Movie:

Video S1. The video shows the superhydrophilicity of SEBS-\textit{g}-PAMPS sample.
5. References:
