Supporting information for *A surface decorated with diblock copolymer for biomolecular conjugation*

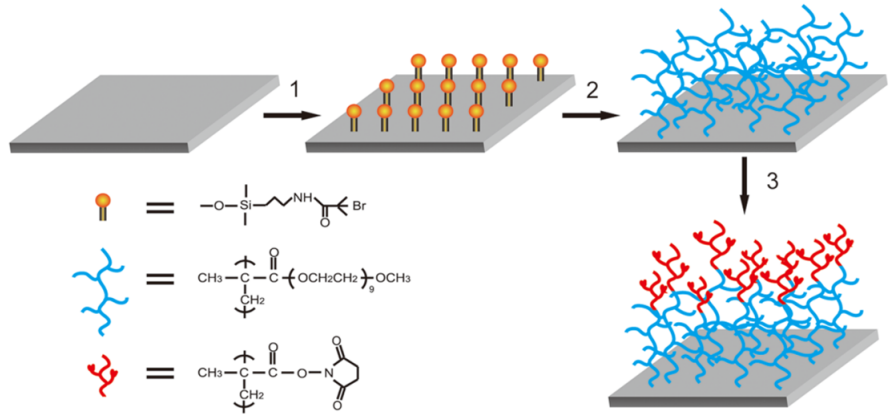
Yanxia Zhang, Qian Yu, He Huang*, Feng Zhou, Zhaoqiang Wu, Lin Yuan, Dan Li, Hong Chen*

Materials

Oligo(ethylene glycol) methacrylate (OEGMA, Mn=475 g/mol, Aldrich) was distilled over CaH_2 under vacuum prior to use. Copper (I) bromide (CuBr, Fluka, 98%), N-hydroxysuccinimide (NHS, Aldrich), 3-aminopropyltriethoxysilane (APTES, Aldrich), bromoisobutyryl bromide (BIBB, Fluka), 2,2′-bipyridyl (Bpy, Aldrich), 1,1,4,7,7-pentamethyldiethylenetriamine (PMDETA, Aldrich), 2-(2-aminoethoxy) ethanol ($(EG)_2NH_2$, Aldrich), biotin hydrazide (Biotin-NH_2, Sigma), collagen (type I, Sigma), and heparin sodium salt (Sigma) were used as received. Fluorescein isothiocyanate-labeled avidin (FITC-avidin) was purchased from Wuhan Boster Biological Technology, LTD. Fibrinogen (Fg) and antithrombin III (ATIII) were obtained from Enzyme Research Laboratories. Human serum albumin (HSA) was obtained from Sigma Chemical Co. and was labeled with FITC [1]. L929 cells were supplied by China Center For Type Culture Collection (CCTCC). The active ester monomer N-hydroxysuccinimidyl methacrylate (NHSMA) was synthesized according to the literature [2].

Preparation of POEGMA-b-PNHSMA grafted silicon wafers

The general reaction procedure for POEGMA-b-PNHSMA brushes grafting from silicon wafer surfaces was illustrated in **Scheme S1**. The pretreatment of silicon wafers for immobilization of initiator was done following the procedures reported in our previous work [3]. SI-ATRP grafting of OEGMA was carried out in a glovebox purged with argon. OEGMA (7.93g, 16.7mmol), Bpy(312mg, 2mmol) and CuBr (143mg, 1mmol) were dissolved in a 4:1 mixture of methanol and water (15 mL) to give a reaction solution. The reaction solution was sonicated for 5min and then added to a glass vessel in which the initiator-functionalized wafers were placed. The polymerization was carried out at room temperature for 4h and stopped by adding a methanol solution of $CuBr_2$/Bpy. The obtained POEGMA-grafted silicon wafers were removed from solution, cleaned ultrasonically in methanol and rinsed thoroughly, and then dried under a argon flow. SI-ATRP of NHSMA from Si-POEGMA surfaces was carried out according to the previous report [4] with some improvements. Briefly, the NHSMA monomer (0.5g, 2.73mmol) and CuBr (9mg, 0.063mmol) were dissolved in 4 mL anisole. After the mixture was degassed by two freeze–pump–thaw cycles, PMDETA (26μL, 0.126mmol) was added and degassing was continued for two cycles. The solution was then transferred to a 50 mL Schlenk flask containing the Si-POEGMA surfaces. The polymerization was carried out at 90^{o}C for 2h, and then the resulting surfaces were rinsed vigorously with dimethylformamide and dried under a argon flow.



Scheme S1. Synthesis procedure for POEGMA-b-PNHSMA brushes on silicon surface: (1) Immobilization of initiator; (2) SI-ATRP grafting of OEGMA; (3) ATRP of NHSMA initiated from OEGMA grafts.

Preparation of bioactive surfaces via covalent attachment of bioactive molecules onto POEGMA-b-PNHSMA brushes

The immobilization of three typical bioactive molecules (biotin hydrazide, heparin and collagen) on the surfaces via covalent amide bonds were carried out according to the respective procedure reported previously [5-7] and shown as follows. The resulting bioactive surfaces were immersed in the ethanolic solution of $(EG)_2NH_2$ (0.1 mg/mL) for 2h at room temperature to deactivate the remaining NHS ester groups and then they were rinsed with ethanol and dried in a stream of argon.

Biotin-NH_2 attachment

The surfaces were immersed in absolute ethanol solution of biotin-NH_2 (1mg/mL) for 24 h at room temperature. The resulting substrate was washed with ethanol, followed by drying with a stream of argon.

Heparin attachment

The surfaces were immersed in phosphate buffered saline (PBS, pH 8.0) containing the heparin (10mg/mL) for 6 h at room temperature and subsequently rinsed three times with fresh PBS, followed by drying with a stream of argon.

Collagen attachment

The surfaces were immersed in phosphate buffered saline (PBS, pH 7.4) containing the collagen (1mg/mL) for 24 h at 4^{o}C and subsequently rinsed three times with fresh PBS, followed by drying with a stream of argon.

Surface characterization

The chemical compositions of the modified silicon surfaces were determined with an ESCALAB MK II X-ray photoelectron spectrometer (XPS) (VG Scientific Ltd.). The thickness of the polymer grafts on the silicon substrate was measured by an M-88 spectroscopic ellipsometer (J. A. Woollam Co., Inc.).

Protein adsorption and elution

Fibrinogen adsorption on the surfaces from phosphate buffered saline (PBS) (pH 7.4) was determined via radiolabeling method [3] using a Wizard 3″1480 Automatic Gamma Counter (Perkin-Elmer Life Sciences). Adsorption was allowed to proceed for 3 h under static conditions at room temperature. Elution tests were performed to test protein-binding affinity. These samples with adsorbed radiolabeled proteins were transferred to solution of sodium dodecyl sulfate (SDS)(wt 2%) and soaked for 3h at room temperature. Then the amount of residual protein on the surfaces was measured.

Functional characterization for respective bioactive surfaces

For the bioactive surfaces immobilized with different bioactive molecules, the respective characterizations were carried out to confirm the synthesis process and the biofunctionality of these surfaces. The details are shown as follows.

Biotin conjugated surface [5]

The biotin conjugated surfaces were incubated in solution containing FTIC-avidin (0.1 mg/mL) or FTIC-HSA (0.1 mg/mL) for 3h at room temperature. After the processes, the surfaces were thoroughly rinsed with the reaction solvents and then dried in a stream of argon. The adsorbed avidin or HSA were determined by confocal laser scanning microscopy (CLSM).

Heparin conjugated surface [6]

Antithrombin III (ATIII) adsorption on the Si-POEGMA and heparin conjugated surfaces from phosphate buffered saline (PBS) (pH 7.4) were determined via radiolabeling method followed the procedures reported in our previous work using a Wizard 3″1480 Automatic Gamma Counter (Perkin-Elmer Life Sciences). Adsorption was allowed to proceed for 3 h under static conditions at room temperature (23 °C).

Collagen conjugated surface [7]

The Si-POEGMA and collagen conjugated surfaces were placed into the wells of a 48-well culture plate. L929 cells were cultured in RPMI medium 1640 (Gibco) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 100 U/mL penicillin and 10 mg/mL streptomycin. L929 cells were seeded at a density of 3.0×10^4 cells/cm^2 and incubated at 37 ^oC in a humidified atmosphere of 5% CO_2 for 2 days. Then the samples were washed twice in PBS (pH 7.4) solution at 37^oC to remove the unattached cells, then fixed by 2.5% glutaraldehyde and dehydrated in a series of ethanol solutions (30-100%), each for 10 min. The morphology of cells on these surfaces was observed by scanning electron microscope (SEM, JSM-5610LV) after coating a thin platinum layer.

XPS data

The chemical composition of the modified surfaces from XPS analysis is summarized in **Table S1**. The presence of sulfur elements confirms successful immobilization of biotin and heparin. The increase of N/C ratio indicates the successful progress block of PNHSMA and further immobilization of collagen.

Table S1 Elemental composition of the modified silicon surfaces determined by XPS using 90° takeoff angles

Surface code	C1s (%)	O1s (%)	N1s (%)	N/C	Br3d (%)	S2p (%)
Si-POEGMA	69.0	29.4	1.2	0.017	0.4	-
Si-POEGMA-PNHSMA	60.9	35.2	3.8	0.062	0.1	-
Biotin conjugated Si-POEGMA-b-PNHSMA	62.5	31.8	2.9	0.046	-	2.8
Heparin conjugated Si-POEGMA-b-PNHSMA	62.4	34.5	1.9	0.03	-	1.2
Collagen conjugated Si-POEGMA-b-PNHSMA	60.1	35.6	4.3	0.071	-	-

CLSM data

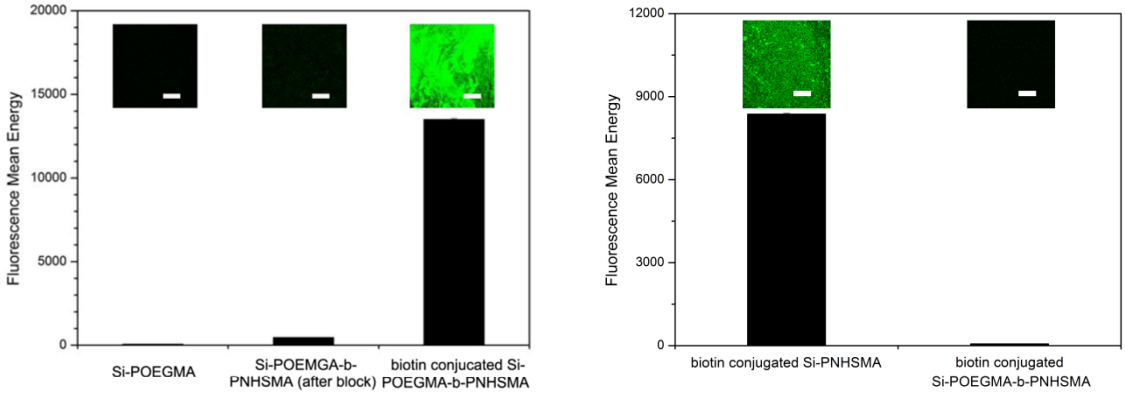


Figure S1 Representative CLSM images of surfaces after been exposed to FITC-avidin (left) and FITC-HSA (right) for 3h. (Scale bar in all images = 20μm). The corresponding fluorescence mean energy is also shown. Data are means ± the standard error (n=3)

The adsorption of avidin and HSA was examined with a confocal laser scanning microscopy (CLSM) and the respective images were shown in **Figure S1.** For avidin adsorption, no obviously fluorescence was observed on the Si-POEGMA surface due to the good protein-resistance of POEGMA. The scattered fluorescence was also observed on the Si-POEGMA-b-PNHSMA surface (after blocking by $(EG)_2NH_2$), indicating that most NHS active esters have been passivated. On the contrary, uniform and strong fluorescence was detected on biotin conjugated Si-POEGMA-b-PNHSMA surface and the relative fluorescence intensity increased significantly, which is suggested a significant amount of avidin on the

surface has been conjugated with the biotin. On the other hand, it is shown that a amount of HSA adsorbed on this surface without POEGMA layer was detected, suggesting the lack of nonspecific protein resistance. By comparing the CLSM images and the corresponding fluorescence mean energy, we believed that it is the POEGMA layer endows the modified surface with the non-specific protein resist property.

References

1 H. Chen, W. Song, F. Zhou, Z. Wu, H. Huang, J. Zhang, Q. Lin, and B. Yang, *Colloids Surf., B*, 2009, **71**, 275.

2 H. G. Batz and J. Koldehoff, *Makromol. Chem.*, 1976, **177**, 683.

3 Q. Yu, Y. Zhang, H. Chen, Z. Wu, H. Huang, and C. Cheng, *Colloids and Surfaces B: Biointerfaces*, 2010, **76**, 468.

4 J. M. Rathfon and G. N. Tew, *Polymer*, 2008, **49**, 1761.

5 B. S. Lee, Y. S. Chi, K.-B. Lee, Y.-G. Kim, and I. S. Choi, *Biomacromolecules*, 2007, **8** 3922.

6 H. Chen, M. A. Brook, H. D. Sheardown, Y. Chen, and B. Klenkler, *Bioconjugate Chem.*, 2006, **17**, 21.

7 F. Zhang, F. J. Xu, E. T. Kang, and K. G. Neoh, *Ind. Eng. Chem. Res.*, 2006, **45**, 3067.