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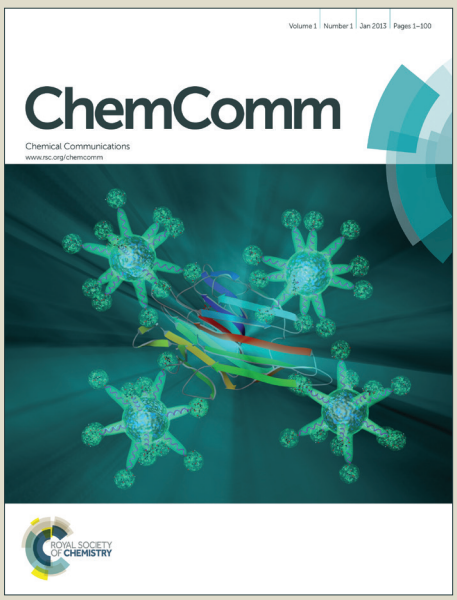

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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

A novel hydrophilic polymer-brush pattern for site-specific capture of blood cells from whole blood

Jianwen Hou,[ab] Qiang Shi,*[a] Wei Ye,[ab] Qunfu Fan,[c] Hengchong Shi,[a] Shing-Chung Wong,[d] Xiaodong Xu[c] and Jinghua Yin*[a]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

A novel hydrophilic PAMPS/PAAm brushes pattern is fabricated to selectively capture blood cells from whole blood. PAMPS brushes provide antifouling surfaces to resist proteins and cells adhesion while PAAm brushes effectively entrap targeted proteins for site-specific and cell-type dependent capture of blood cells.

Cell capture is a basic step to evaluate functions of living cells and detect infected and malignant cells in bodily fluids.[1] This is particularly true for dysfunctional platelet detection, immune cell analysis and cancer diagnosis.[2] Constructing a platform that allows site-specific and cell-type dependent capture and organization on localized areas is therefore highly desirable for isolating cells from whole blood, accessing cellular mechanism and developing diagnostic assays.[3]

Current strategies based on soft lithographic techniques, biochips and microfluidic devices offer multiple advantages for capture and analysis of cells.[4] However, most techniques are limited by potential harm to the captured cells because immobilization of targeted proteins mainly depends on hydrophobic interactions.[1d,2d,4c] The hydrophobic substrate is likely to form nonspecific linkages with cells even these cells are initially captured on immuno-affinity substrates via specific antibody-antigen binding, resulting in damage to cell viability and populations during living cell release or analysis.[1d,4c] In addition, the hydrophobic substrate encounters the fouling issue.[2d] This situation can be circumvented by robustly anchoring targeted proteins on hydrophilic substrate.[5] However, the anchoring of proteins can be lengthy and inefficient and the application of platform is significantly constrained to capture only one specific cell.[6] Thus, there is an urgent need to develop a new platform that is not only hydrophilic to reduce the damage to cells but also capable of immobilizing varied targeted proteins to capture cells flexibly.

Patterned surface with two-component polymer brushes entitles a structured surface with more abundant functions, such as biochips, biosensors and cell-growth regulation.[7] Recent advances in top-down and bottom-up techniques make a rapid progress in creation of two-component polymer-brush patterns.[8] The hydrophilic polymer brushes, one of which exhibits excellent antifouling behavior and the other has a capability to entrap targeted proteins, are expected to harvest varied cells from whole blood. Poly(N-isopropylacrylamid) brushes have been reported to have the ability to entrap proteins with hydrophobic interactions.[9] Recently, a novel hydrophilic polyacrylamide (PAAm) brushes is found to be able to entrap varied plasma proteins.[10] Therefore, the two-component polymer-brush pattern composed of this novel PAAm brushes and another hydrophilic polymer brushes with excellent antifouling property will offer a new avenue to specifically capture varied cells in the blood with reduced damage to the captured cells.

Here, we fabricate a novel two-component polymer-brush pattern for capturing blood cells on the surface of a thermoplastic elastomer, styrene-b-(ethylene-co-butylene)-b-styrene elastomer (SEBS), with poly(2-acryl-amido-2-methylpropane sulfonic acid) (PAMPS) and polyacrylamide brushes. SEBS is used for substrate due to its unique nanostructures, good biocompatibility and outstanding stability under physiological conditions.[11] In spite of importance of blood cells in analysis and diagnosis, capture of blood cells, such as erythrocytes, remains challenging due to non-adherent property of blood cells.[1e,4c] Our strategy is based on construction of PAAm/PAMPS polymer-brush pattern combining surface-initiated photo-polymerization (SIPP) with surface-initiated atom transfer radical polymerization (SI-ATRP). The PAMPS brushes provide the antifouling surface to resist proteins and cells adhesion while polyacrylamide brushes entrap targeted proteins to capture the blood cells specifically. We demonstrate that the robust and well-defined two-component polymer-brush patterns are fabricated by top-down/bottom-up strategy; the PAMPS brushes exhibit superhydrophilic property with high resistance to proteins and cells adhesion while polyacrylamide brushes possess high capability to entrap varied targeted proteins. The ligand-cell interactions facilitate the capture of blood cells in a site-specific manner with reduced harmless. This two-component polymer-brush pattern is versatile and effective to entrap targeted proteins, offering a potential new approach for selective cell capture from whole blood.

A well-defined PAMPS/PAAm pattern on the SEBS substrate is generated with "top-down/bottom-up" strategy (Fig. 1). First, a homogeneous layer of hydrophilic PAMPS brushes is formed on the surface of SEBS substrate by SIPP. Compared with other typical hydrophilic polymer brushes, such as poly (ethylene glycol), poly (2-hydroxyethyl methacrylate) and poly (sulfobetaine methacrylate), PAMPS brushes are superhydrophilic (Video S1, ESI†) and maintain hydrophilic after subsequent treatments (Fig. S1, ESI†). Thus, PAMPS is selected

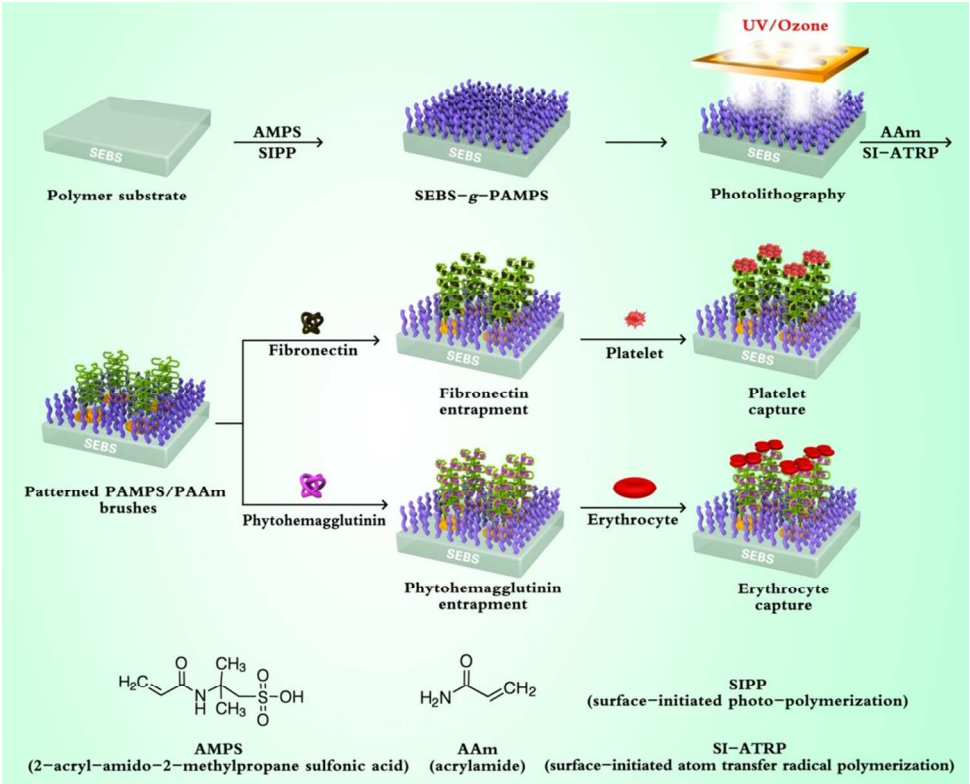

Fig. 1 Schematic diagram illustrating the patterning process of two-component polymer brushes for site-specific capture of blood cells via targeted proteins.

to form the initial hydrophilic layer. Then, a transmission electron microscopy (TEM) grid is used as a photomask to firmly cover on the surface of SEBS-*g*-PAMPS, and the PAMPS layer is exposed to UV/Ozone radiation through the photomask to create patterned hydroxyl groups, followed by immobilization of ATRP initiators using the aqueous-based method (Fig. S2, ESI†).[10] Next, PAAm brushes are grafted from patterned initiators (UV/Ozone-exposed areas) with SI-ATRP, and the second hydrophilic PAAm brushes are expected to entrap various targeted proteins to capture blood cells. After removal of the photomask, a two-component PAMPS/PAAm brushes pattern is created. Finally, targeted proteins including fibronectin (FN) and phytohemagglutinin (PHA) are entrapped by PAAm brushes to further capture blood cells specifically.

The formation of two-component polymer brushes on the model surface is confirmed by ATR-FTIR and XPS spectra (Fig. S2, S3, ESI†). New peaks at 1743 cm^{-1} (C=O stretching vibration of PAMPS) and 1659 cm^{-1} (C=O stretching vibration of PAAm) appear after grafting the initial PAMPS and the second PAAm brushes, respectively. Similarly, two new peaks at binding energies of 399.7 (C-N groups) and 401.8 eV (N-C=O groups) in the N_{1s} spectra of SEBS-*g*-PAMPS are observed. But only a binding energy of 399.3 eV (N-C=O groups) appears after formation of PAAm brushes. The changes are due to the etching of PAMPS brushes by UV/Ozone radiation and generation of PAAm brushes. The thickness of PAMPS layers and PAAm layers is determined to be ~300 nm and 700 nm with atomic force microscopy, respectively (Fig. S4, ESI†). The formation of two-component polymer-brush pattern is further supported by the change of chemical composition, static water contact angle and nitrogen contents of the functionalized SEBS surfaces (Table S1, ESI†). The two-component PAMPS/PAAm brushes pattern exhibits hydrophilic with WCA of 27°. As SIPP and SI-ATRP are versatile and effective, the strategy presented here facilitates fabrication of large-scale, high-throughput patterned surfaces.

Fig. 2 shows optical images of patterned surfaces. The patterned PAMPS/PAAm brushes show a very clean and well-defined pattern, demonstrating the excellent retention of the size and shape of the photomask (Fig. 1a). Furthermore, the size of PAAm domains can be tunable by manipulation of hole size of the photomask (Fig. S5, ESI†). The uniform of patterned surface with high resolution facilities the detection and analysis of protein adsorption and cell capture. Fibronectin and phytohemagglutinin are then selected to be entrapped by PAAm brushes for capturing platelets and erythrocytes, respectively (Fig. S6, ESI†). FN is a multifunctional protein present in plasma and extracellular matrix to mediate platelet-matrix and platelet-platelet interactions, which can bind glycoproteins on the surface of platelet membrane.[12] PHA is a glycoprotein that can agglutinate all human erythrocytes by ligand-cell reactions.[13] Fig. 2 b-c show fluorescence images of protein entrapment on the patterned surface. The PAAm regions exhibit strong and homogeneous green and red fluorescence, confirming fluorescein isothiocyanate-labeled FN (FITC-FN) (Fig. 2 b-1, b-2) and rhodamine-labeled PHA (RBITC-PHA) (Fig. 2 c-1, c-2) are selectively entrapped on the PAAm regions. In contrast, the PAMPS regions are almost dark, indicating that there is nearly no nonspecific adsorption of labeled proteins due to the inherent

antifouling nature of PAMPS brushes.[14] Further examination of the uniform protein patterning by fluorescence intensity profile shows the similar signal intensity on the different patterned surfaces (Fig. S7, ESI†), showing the sizes of micropatterned polymer brushes have slight effects on the amount of protein entrapment.

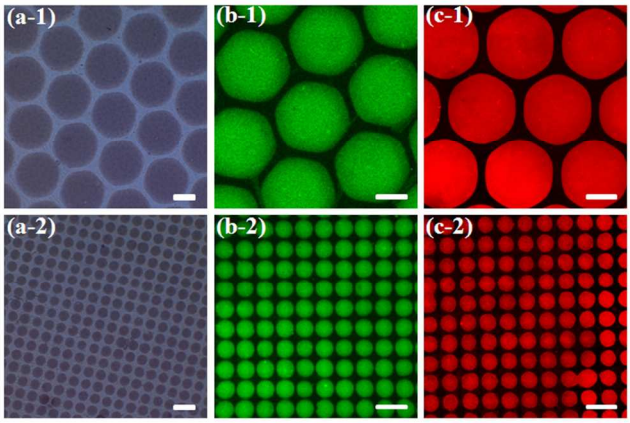

Fig.2 Optical images (a-1, a-2) of two-component PAMPS/PAAm brush patterns and fluorescence images of FITC-FN (b-1, b-2) and RBITC-PHA (c-1, c-2) entrapment on PAMPS/PAAm patterned surfaces. The scale bar is 200 μm in all images.

The high capacity of PAAm brushes to entrap proteins is believed to be the hydrogen bonds formation between PAAm brushes and proteins.[10] It is well known that PAAm behaves as both hydrogen bond donors and acceptors.[15a] In addition, the peptide bonds of the protein can form multiple-point hydrogen bonds with AAm, which results in the strong interaction force between the protein and AAm.[15b] Furthermore, a hierarchical architecture is formed on the surface of SEBS driven by physical crosslinking in polymer brushes (Fig. S8, ESI†). And this hierarchical architecture will offer more contact sites for the formation of multiple-point hydrogen bonds between the proteins and PAAm brushes. Thus, a combination of hydrogen-bond interactions and surface topography is considered to jointly entrap the proteins in the PAAm brushes.[15]

The patterned surfaces are used to capture blood cells with the aid of pre-absorbed targeted proteins. Capture of platelets in a harmless way is important because there are big differences between normal platelets and activated platelets in the process of thrombosis and hemostasis.[16] Fig. 3a-1, a-2 show fluorescence microscopy images of platelets capture from the platelet-rich plasma on the patterned surface with pre-adsorbed FN. Most platelets are captured on PAAm domains with the round morphology (Fig. 3a-3). Similarly, most erythrocytes are specifically captured from the concentrated erythrocytes on the patterned PAAm domains with the entrapped PHA (Fig. 3b). The unique biconcave discoidal morphology of erythrocytes is clearly observed in magnified images of CLSM (Fig. 3b-1) and SEM (Fig. 3b-3), confirming the erythrocytes maintain their normal morphology and functionality, which is significant in erythrocyte-based detection, diagnosis of diseases and target drug delivery.[17]

The harvest of blood cells from whole blood on the model surfaces shows the selectivity of cell capture. After incubation with whole blood, some platelets and erythrocytes adhere randomly on the surface of virgin SEBS due to the hydrophobic surface of SEBS (Fig. 3c). In contrast, a large number of platelets are captured on the SEBS-*g*-PAMPS-*g*-PAAm surface pre-adsorbed with FN, and no other blood cells are observed (Fig. 3d). Similarly, a large amount of erythrocytes are captured on the surface of SEBS-*g*-PAMPS-*g*-PAAm with the aid of PHA and almost no other blood cells appear (Fig. 3e). The patterned surface thus shows a binding specificity and an ability to selectively capture cells from whole blood. As PAAm brushes can entrap varied plasma proteins,[10] and hydrophilic PAMPS/PAAm brushes reduce the damage to captured cells, the two-component polymer-brush pattern constructed here offers new way to specifically capture varied cells in the blood for disease diagnosis and therapy.

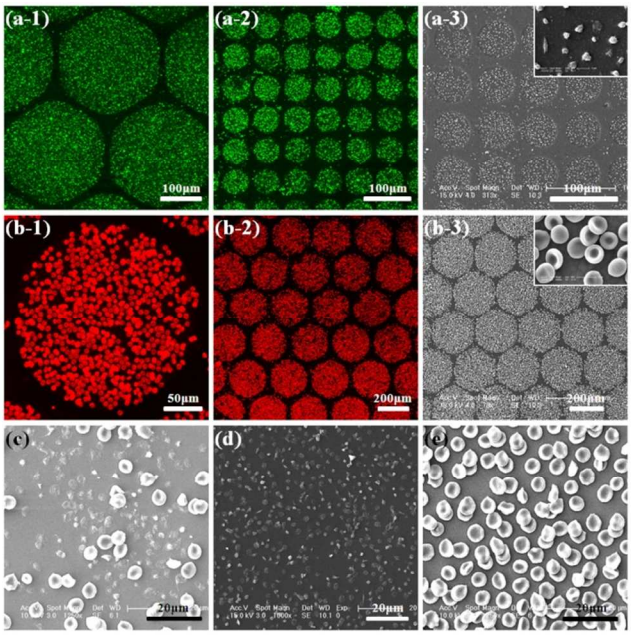

Fig.3 CLSM (left and middle) and SEM (right) images of captured purified platelets (a1-a3) and erythrocytes (b1-b3) on the patterned surface of SEBS-*g*-PAMPS-*g*-PAAm pre-adsorbed with FN and PHA, respectively. SEM images of blood cell capture from whole blood on the surface of virgin SEBS (c), non-patterned surface of SEBS-*g*-PAMPS-*g*-PAAm pre-adsorbed with FN (d) and PHA (e), respectively.

Fig. 4a and 4b display the statistical results of captured platelets and erythrocytes on different model surfaces based on the SEM images (Fig. S9, ESI†). Compared with virgin SEBS, the number of captured platelets and erythrocytes is drastically reduced on the hydrophilic surfaces. In contrast, after FN and PHA entrapment, the numbers of platelets and erythrocytes increase about 22% and 28%, respectively, on the surfaces of SEBS-*g*-PAMPS-*g*-PAAm in comparison with that on the surface of virgin SEBS, demonstrating the high capture ability of the patterned surfaces. In addition, 81% of adhered platelets on virgin SEBS surface are highly activated (Fig. 4c), as evidenced by their spread-dendritic shape and presence of pseudopodia (Fig. S9a, ESI†). In contrast, only ~7% of platelets become activated on PAAm brushes, confirming the advantage of PAAm brushes in maintaining the normal morphology and function of captured platelets (Fig. S9e, ESI†). For the virgin SEBS sample, 63% of erythrocytes are transformed into echinocytes and lost their

normal shape (Fig. 4d, Fig. S9a', ESI†), whereas just ~4% of erythrocytes lost their normal discoid shapes on PAAm brushes (Fig. S9e', ESI†). These results confirm site-specific blood-cell capture is achieved with substantially reduced damage.

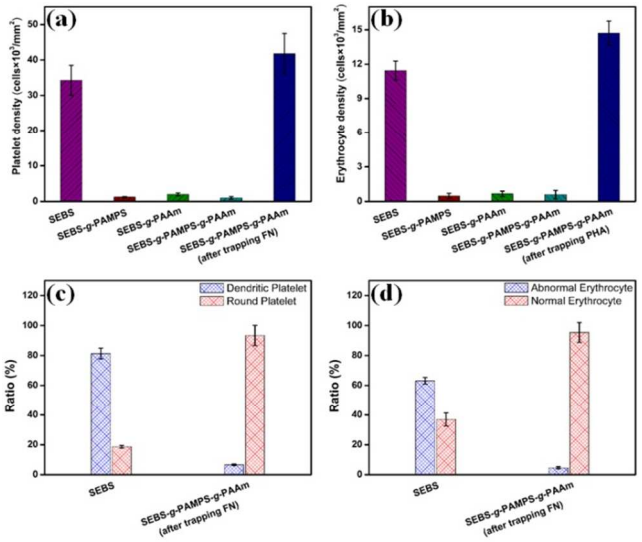

Fig.4 Statistical number of adhered platelets (a) and erythrocytes (b) on different model surfaces. Analysis of dendritic and round platelets (c), and normal and abnormal erythrocytes (d) on the surfaces of different samples. Error bars represent a standard deviation for n = 5.

In summary, we constructed a two-component polymer-brush pattern on the surface of SEBS with PAMPS and PAAm brushes. The PAMPS brushes provided the antifouling surface to resist proteins and cells adhesion while PAAm brushes could entrap targeted proteins to capture the blood cells specifically. We demonstrated that the robust and well-defined two-component polymer-brush pattern were fabricated by top-down/bottom-up strategy; the PAMPS brushes exhibited superhydrophilic property with high resistance to proteins and cells adhesion while PAAm brushes possessed high capability to entrap varied targeted proteins. The ligand-cell interactions facilitated the capture of blood cells in a site-specific and harmless manner. This novel two-component polymer-brush pattern was versatile and effective, which offered a potential approach in in diagnosis and therapy for generation of multiple-binding sites with different specificity on one support and selective cell capture from whole blood.

This work was supported by the National Natural Science Foundation of China (51273199, 21274150 and 51103030).

Notes and references

[a] State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, P. R. China.
E-mail: shiqiang@ciac.ac.cn; yinjh@ciac.ac.cn
[b] University of Chinese Academy of Sciences, Beijing 100049, P. R. China.
[c] Polymer Materials Research Center, College of Materials Science and Chemical Engineering, Harbin Engineering University, Harbin 150001, P. R. China.
[d] Department of Mechanical Engineering, University of Akron, Akron, Ohio 44325-3903, USA.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

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