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Creation of antifouling microarrays by photopolymerization of zwitterionic compounds for protein assay and cell patterning

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ABSTRACT

Nonspecific binding or adsorption of biomolecules presents as a major obstacle to higher sensitivity, specificity and reproducibility in microarray technology. We report herein a method to fabricate antifouling microarray via photopolymerization of biomimetic betaine compounds. In brief, carboxybetaine methacrylate was polymerized as arrays for protein sensing, while sulfobetaine methacrylate was polymerized as background. With the abundant carboxyl groups on array surfaces and zwitterionic polymers on the entire surfaces, this microarray allows biomolecular immobilization and recognition with low nonspecific interactions due to its antifouling property. Therefore, low concentration of target molecules can be captured and detected by this microarray. It was proved that a concentration of 10 ng mL^{-1} bovine serum albumin in the sample matrix of bovine serum can be detected by the microarray derivatized with anti-bovine serum albumin. Moreover, with proper hydrophilic-hydrophobic designs, this approach can be applied to fabricate surface-tension droplet arrays, which allows surface-directed cell adhesion and growth. These light controllable approaches constitute a clear improvement in the design of antifouling interfaces, which may lead to greater flexibility in the development of interfacial architectures and wider application in blood contact microdevices.

1. Introduction

Microarray technology offers high throughput, low reagent consumption and fast assay speed for the investigation of biomolecular interaction, drug screening and diagnostic assay (Saliba et al., 2014; Bai et al., 2013; Melamed et al., 2012). Of the diverse microarray tools used, protein microarray and cell microarray are analogues because both require micropatterning of protein repulsive surfaces as background to achieve specific interactions between biological components and array. A key challenge for gaining control over specific interaction is the suppression of protein nonspecific adsorption (Rodriguez-Emmenegger et al., 2013; Sauer, 2017). To some extent, the sensitivity of protein microarrays, especially antibody microarray can hardly meet the requirements in clinical diagnostics nowadays (Bai et al., 2013; Jung et al., 2013; Ratner and Bryant, 2004). One of the reasons is nonspecific binding/adsorption between biomolecules and surfaces, which greatly reduced the density of antibody coverage, specificity of biomolecular reaction and accuracy of analytical results (Sauer, 2017; He et al., 2012; Yoshimoto et al., 2010; Huang et al., 2015). Water-mediated hydrophobic and hydration forces as well as electrostatic interactions are regarded as an important factor determining protein adsorption (Vogler, 1998; Chandler, 2005; Wei et al., 2014). Thus, major efforts have been made toward constructing hydrophilic and ultra-low fouling microarrays for in vivo and in vitro biomedical applications (Bertok et al., 2013; Feng et al., 2015).

Poly(ethylene glycol) (PEG) polymers (Yamaguchi et al., 2012; Harbers et al., 2007; Yang et al., 2014; Ding et al., 2012) and PEG-like materials (Hirschbiel et al., 2015; Lei et al., 2016) became mainstream in the fabrication of nonfouling surfaces. Surface initiated atom transfer radical polymerization (SI-ATRP) method is widely utilized to pattern PEG polymers. For example, Yamaguchi et al. (2012) exploited nitrobenzyl photocleavable PEG for dynamic patterning of non-adherent surfaces for cell culturing. Hirschbiel et al. (2015) employed (SI-ATRP) of oligo(ethylene glycol) methyl ether methacrylate to pattern antifouling brushes for controlled cell adhesion. Lei et al. (2016) developed poly(glycidyl methacrylate-*co*-2-hydroxyethyl methacrylate) brushes for protein microarrays. However, these ATRP methods involve the initiator immobilization and deactivation, which complicated the array fabrication process. Self-assembled monolayer with PEG structures were also tried to achieve antifouling arrays (Cattani-Scholz et al., 2009; Larsson et al., 2007), but its stability in different pH and ionic strength during surface derivatization is still a problem. Despite their

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excellent nonfouling performance, problems existing in PEG based protein microarray include: (1) Derivatization of hydroxyl groups on PEG molecules to link probe molecules requires complicated procedures. (2) Each PEG polymer chain only linked with one hydroxyl group, which resulted in relatively low density of reactive sites on functional surfaces. (3) PEG is easily to be oxidatively degraded (Ostuni et al., 2001), therefore, the storage and life time of PEG based microarray are limited, and the antifouling performance of PEG coatings decrease over time due to its inability to resist bacteria (Ding et al., 2012; Kane et al., 2003).

Besides, most of the reports focused on suppressing nonspecific adsorption of biomolecules on arrays instead of background. As for the background of a microarray, simple procedures such as physical adsorption of some surfactants or proteins as protective layer were usually adopted (Perrino et al., 2008; Murthy et al., 2013). As a matter of fact, the non-covalently linked layer is easily to be desorbed during experiment, which usually resulted in increasing background noises, poor sensitivity and reproducibility of a protein microarray (Sauer, 2017; Huang et al., 2015; Hao et al., 2014; Currie et al., 2003). Thus, microarrays with entire surfaces antifouling are crucially important to achieve sensitive and reliable detection of target biomolecules. Shlyapnikov et al. (2014) tried carboxymethyl cellulose film as substrates to fabricate magnetic bead arrays. Feng et al. (2015) used low surface energy fluorinated polymer as background material to develop ultra-low fouling microarray, but both the fabrication process and the analytical procedure are conducted on a polydimethylsiloxane microfluidic channel, in other words, the surrounding material of the microarray is still protein adhesion.

Recently, materials with biomimetic zwitterionic groups, such as carboxybetaine (CB), sulfobetaine (SB) and phosphocholine (PC), have received increasing interest owing to their superior antifouling property (Gao et al., 2010; Zhang et al., 2008; Ishihara et al., 1991), as reported of < 5 ng/cm^2 of nonspecifically adsorbed protein in the presence of 100% human blood plasma and serum (Zhang et al., 2008), as well as their hydrophilicity and biocompatibility. Of which, polycarboxybetaine methacrylate (pCBMA) not only highly resists nonspecific protein adsorption (Gao et al., 2010), but also has abundant functional groups for biomolecular immobilization. In view of the presence of reactive double bond in betaine methacrylate molecule, more flexible fabrication methods in addition to ATRP could be employed to pattern protein resistant surfaces.

We herein described a photopolymerization method for the fabrication of ultra-low fouling microarrays for protein detection, which included functionalizable pCBMA grafted arrays and nonfunctionalizable polysulfobetaine methacrylate (pSBMA) grafted background. Bovine serum albumin (BSA), as a model protein, was used to test the analytical performance of this antifouling microarray. With properly designed hydrophilic-hydrophobic surfaces, this method can be broadened to fabricate droplet arrays, which was proved to be useful in surface-directed cell adhesion and growth. As we know, SI-ATRP method was generally employed to graft zwitterionic polymers on/to surfaces (Gao et al., 2010; Kyomoto et al., 2010; Steinbach et al., 2012). However, SI-ATRP method can hardly pattern protein sensing and background surfaces selectively. Additionally, it requires heavy metal catalyst, oxygen free environment and long reaction time. In contrast, the photopolymerization method investigated in this study is cleaner, faster and much simpler to perform at ambient conditions as well as more controllable, these characters are more favorable for the fabrication of in vitro diagnostic microdevices. As is well known, bioassay microarrays can be generated via commercially available techniques, such as inkjet printing (Saliba et al., 2014; Patwa et al., 2009), contact printing (Huang et al., 2015), electron-beam lithography (Manfrinato et al., 2013), nanolithography (Laing et al., 2013), etc. In comparison with these arraying techniques, the photopolymerization method described in this study is based on chemical reactions to generate arrays, which is less dependent on expensive facility and more feasible to be conducted in lab. In summary, this study provided a new route to fabricate protein and cell microarrays, and possible application to various bioassays may be implicated.

2. Experimental

2.1. Reagents and materials

Sulfobetaine methacrylate (SBMA, 97%) was from Sigma-Aldrich (Milwaukee, WI, USA). Carboxybetaine methacrylate (CBMA) was synthesized following the procedures published previously (Kyomoto et al., 2010). The structures of pCBMA and pSBMA were shown in Fig. S1. Glycidyl methacrylate (GMA) and (γ–aminopropyl)trimethoxysilane (APTMS, purity > 99%) were from Aladdin Chemicals (Shanghai, China). Toluene, isopropanol, methanol, dimethyl sulfoxide, sodium azide, sulfuric acid, hydrogen peroxide, sodium chloride, potassium chloride, disodium monohydrogen phosphate, sodium carbonate, sodium bicarbonate, ethylenediaminetetraacetic acid (EDTA) and borosilicate glasses were from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Bovine serum albumin (BSA), goat anti-BSA and fluorescein 5-isothiocyanate (FITC) labeled BSA were from Amresco (USA). Insulin, 2-(N-morpholino)ethanesulfonic acid (MES), N-hydroxysuccinimide (NHS) and (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride) (EDC) were from Shanghai Yuanye Biotechnology Co., Ltd.(Shanghai, China).

2.2. Fabrication/functionalization of pCBMA/pSBMA grafted antifouling microarrays

Borosilicate glasses were cut to a size of 0.5 × 0.5 cm^2 and cleaned by piranha solution, water and isopropanol sequentially. The cleaned glass substrate was heated on a hot plate for 1 min at 200 °C to remove residual solvents, then transferred into 2% γ–aminopropyltrimethoxysilane (APTMS) solution in toluene for 4 h at room temperature. Next, the silanized glass substrate reacted with 10% glycidyl methacrylate (GMA) for 5 h at 50 °C. After the reaction, the glass substrate was cleaned thoroughly with isopropanol to remove the residual GMA. A concentration of CBMA solution was then applied on GMA immobilized glass surfaces, and UV light was illuminated to polymerize CBMA as arrays. Immediately after, the glass substrate was washed with isopropanol for 1 min to fully expose the unreacted double bonds. Finally, a concentration of SBMA solution was applied on surfaces. The photopolymerization process was repeated to obtain antifouling background of a microarray. Therefore, after two consecutive photopolymerization reactions, the overall surfaces of the microarray are covered with zwitterionic polymers, while only the array surfaces are functionalizable. The fabricated microarray was rinsed in 1 M NaCl overnight to remove the unbonded pCBMA from surfaces before testing. X-ray photoelectron spectroscopy (XPS) analysis was performed on a Kratos AXIS Ultra DLD instrument (Shimadzu) to get the surface chemical compositions. Elemental compositions present on surfaces were identified from a survey scan. Atomic force microscope (AFM) experiment was performed on an Agilent 5100 SPM under tapping mode to map the morphological difference of GMA and zwitterionic polymer covered surfaces. Fluorescent measurements were performed on a BX 53 F upright fluorescent microscope (Olympus).

Biomolecules were functionalized on pCBMA grafted surfaces following the reported methods (Gao et al., 2010). The carboxylate groups on array surfaces were activated by a freshly prepared solution of 0.05 M NHS and 0.2 M EDC in MES buffer for 10 min at a pH of 5.0. Then, anti-BSA with a concentration of 10 mg mL^{-1} in diluted NaOH (pH = 9.5) reacted with the activated surface for 30 min. Finally, the anti-BSA functionalized microarray was rinsed with 10 mM phosphate buffer-0.5 M NaCl (pH 8.2) to remove all noncovalently bound molecules. The fabricated microarray was used to detect BSA in bovine serum. Briefly, a volume of 50 μL of FITC labeled BSA was placed on the