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Adjacent assembly of self-assembled monolayers for the construction of selective bio-platforms

Amit Ron[a,*], Gwan Hyoung Lee[a], Levy Amar[b], Saba Ghassemi[a], James Hone[a]

[a] Department of Mechanical Engineering, School of Engineering and Applied Science, Columbia University, New York, NY, 10027 USA
[b] Department of Chemical Engineering, School of Engineering and Applied Science, Columbia University, New York, NY, 10027 USA

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ABSTRACT

Selective patterning of bio-substances onto solid platforms is of increasing importance in many areas and widely used for various applications ranging from bio-sensing to cell and tissue engineering. In this study, a new fabrication scheme for the construction of highly selective bio-platforms is presented. The method is based on a direct patterning of poly(ethyleneglycol) (PEG) bio-inert layers on a conducting indium tin oxide (ITO) substrate using electron beam lithography and subsequent assembly of modified amine reactive layers onto the exposed areas. The process is found to create very high "surface contrast" between adhesive and repulsive regions onto the substrate. The platforms are shown to be enable efficient for selective adsorption of a variety of bio-substances including protein arrays, latex beads, and single cells. The high resolution of the technique makes it also applicable for the construction and deposition of bio-structures at the sub-micron scale. The reported technique employs standard lithography and surface chemistry processes, which makes it useful and easy to adopt for a variety of applications and other conductive substrates.

1. Introduction

The interactions of bio-substances with solid surfaces are of critical importance to a wide variety of fields ranging from biology through chemistry, physics and engineering [1,2]. During the years, many patterning techniques have been employed to study these interactions and to demonstrate the utility of different surface scaffoldings for variety of bio-applications [3–6]. One of the more interesting techniques is based on self-assembled monolayers (SAMs) [7]. Those have been greatly demonstrated to use as excellent fabrication building blocks mainly due to the minute structure of their unit molecules and the ability to selectively promote or resist the adherence of bio-substances [8,9]. One effective use of SAMs is as a sacrificial resist material for various forms of lithography (e.g. UV, electron-beam, atomic force) [10–16]. Additionally, SAM can be effectively used in overcoming fabrication difficulties such as interface and integration gaps especially at the nanometer scale [17,18].

A major challenge in patterning of bio-substances is dealing with the selective binding of these materials onto patterned areas [1,2]. This issue can be qualitatively characterized by the stability of the patterned surface and the selectivity of adherence of bio-materials to the designated regions. Common modification schemes involve the construction of physical and chemical barriers [1,2,4]. A well known strategy to promote and repel the adsorption of bio-materials is based on the chemistry of amine terminated SAM (Amino-SAM) and poly(ethyleneglycol) SAM (PEG-SAM), respectively. Different derivatives of these materials can be grafted onto variety of surfaces (e.g. metal, silicon, glass) and be served as an effective adhesion and inert layers [19,20]. This approach has been applied for a variety of application including bio-sensing, μ-fluidic devices and cellular platforms [21–26]. However, a major obstacle lies in the simultaneous integration of these materials especially for the construction of patterned surfaces. This is mainly due to fabrication complexity and the need for multiple processing steps [4,8,9].

Nowadays, many bio-platforms are primary designed for immobilization purposes and require the construction of shaped features [4,21,24,26]. Therefore, development of simple and rapid fabrication schemes, for selective and formational immobilization of bio substances, are required. This study is focused on developing a SAM-based electron-beam (E-beam) lithography technique which could provide a reliable and systematic approach for targeted immobilization of variety of bio-substances. This method is based on an adjacent assembly of reactive (amino-silane) and inert (PEG-silane) SAM layers to construct highly defined, adhesive and repulsive bio-regions, onto a conducting indium tin oxide (ITO) substrate. This approach is supported by previous work that has successfully demonstrated the ability to construct SAM-based plat-

* Corresponding author. Tel.: +1 212 8542966; fax: +1 212 8543304.
E-mail address: ar3038@columbia.edu (A. Ron).

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forms including the assembly of mixed SAM derivatives [27–29]. The choice of ITO is mainly due to its electrical and optical properties; ITO is of increasing importance in the area of bio-fabrication and is now widely used in many biological applications [30,31]. The presented fabrication scheme is found to be highly applicable for micro patterning of variety of bio substances including latex beads, protein arrays, and single cells. Moreover, preliminary findings indicate that the process may be scalable to the sub-micron range.

2. Experimental

The process flow diagram is presented in Fig. 1. Briefly, a PEG-silane layer is first adsorbed onto an ITO coated glass slide. The sample is then patterned by an electron beam, which breaks the carbon chains within the exposed PEG-silane layer. Next, the sample is immersed in a dilute solution of amino-silane (APTES) which forms a monolayer onto the patterned regions. The amine-reactive layer is then activated by glutaraldehyde (Glu) which promotes the adhesion of bio-substances by reacting with free amine groups on their surface. At last stage, the sample is immersed in the desired bio-solution when the substances selectively adhere to the APTES-Glu regions.

2.1. PEG silanization

ITO slides ($\sigma_e = 8–12\,\Omega/m^2$, $10\,mm \times 10\,mm \times 1\,mm$) were cleaned using sonication in a series of solvents: isopropanol, methanol and deionized water (5 min each). After cleaning, the slides were oxidized using oxygen plasma (200 W 5 min, Technics series 800 RIE) and transferred into a 1% (v/v) solution of 2-[methoxy(polyethyleneoxy)propyl]trichlorosilane (PEG-silane, Gelest Inc.) in anhydrous toluene containing 1% triethylamine for 3 h. After silanization, the slides were sonicated in toluene, ethanol and deionized water (5 min each) in order to remove silane remainders.

2.2. E-beam patterning

The silanized slides were patterned using a scanning electron microscope (LEO Electron Microscopy) equipped with a pattern generator system (NPGS, JC Nabity Lithography Systems). Unless otherwise noted, patterns were written at an accelerating voltage of 10 kV and a dose of 2000 $\mu C/cm^2$. After exposure, the slides were sonicated for 5 min in ethanol in order to remove organic residues.

2.3. Amino silanization

The patterned slides were transferred into a 1% (v/v) solution of (3-aminopropyl)triethoxysilane (APTES, SIGMA) in isopropanol at 65 °C for 20 min. After silanization, the slides were sonicated in methanol and deionized water (5 min each) and immediately incubated in a 2% (v/v) solution of glutaraldehyde (Grade II, 25% in aqueous solution) in phosphate buffered saline (PBS 250 mM, pH 7.0) for 1 h at room temperature. Following incubation, the slides were rinsed with PBS to remove glutaraldehyde remainders.

2.4. Protein adsorption

Prior to the adsorption, slides were rinsed with deionized water and PBS. The slides were transferred into a dilute streptavidin solution (0.3 mg/ml in PBS, Invitrogen) for 1 h at room temperature. Following incubation, the slides were rinsed with PBS to remove unattached proteins.

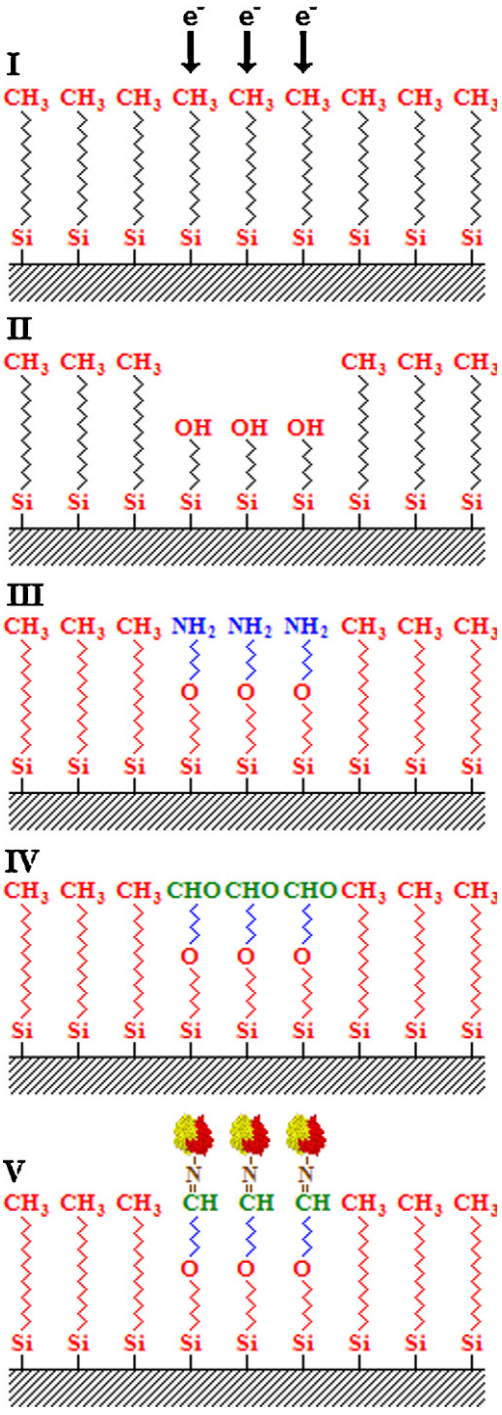

Fig. 1. Fabrication scheme. (I) The PEG-silane layer is patterned using electron beam resulting in breaking of the carbon chains within the layer (II). (III) A monolayer of APTES is formed onto the patterned regions. (IV) The amine-reactive layer is activated by glutaraldehyde. (V) The bio-substances are loaded onto the APTES-Glu regions.

2.5. Polystyrene latex beads adsorption

Amine-modified beads (2.5% solids, SIGMA) with an average diameter of 100 nm were pasteurized for 24 h at 70 °C in order to remove microbes from the latex preparation. Before each use, beads were sonicated and centrifuged (5 min each) in order to remove clusters from the suspension. The slides were then immersed in a 2-(N-morpholino)ethanesulfonic acid (MES) buffer (25 mM, pH 6.1) consisting of 50 μl beads for 2 h. Following incubation, the slides were rinsed with deionized water to remove unattached beads.