Polymer Brushes as Functional, Patterned Surfaces for Nanobiotechnology

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Polymer brushes have many desirable characteristics such as the ability to tether molecules to a substrate or change the properties of a surface. Patterning of polymer films has been an area of great interest due to the broad range of applications including bio-related and medicinal research. Consequently, we have investigated patterning techniques for polymer brushes which allow for two different functionalities on the same surface. This method has been applied to a biosensor device which requires both polymer brushes and a photosensitizer to be polymerized on a patterned gold substrate. Additionally, the nature of patterned polymer brushes as removable thin films was explored. An etching process has enabled us to lift off very thin membranes for further characterization with the potential of using them as Janus membranes for biological applications.

Keywords: Polymer brushes, patterning, multifunctional surfaces, thin films, lift off

1. Introduction

Polymer brushes have proven to be an effective interface between biosensors and biological media. They provide features such as scaffolding support, prevention of non-specific adsorption, tethering of molecules to surfaces, and respond to environmental changes such as pH [1–5]. Conventionally, polymer brushes are polymer chains tethered at one end to a surface or substrate [6]. They can be prepared by “grafting to”, in which the chain is first polymerized and then attached to the surface. Alternatively they can be “grafted from”, which is when the polymerization is carried out at the surface from an immobilized initiator monolayer. The structure and conformation of the brush layer is determined by the number of chains attached per unit area of surface, the grafting density. The brush is considered to be in a “mushroom state” when the neighboring chains are far enough apart that they

do not influence one another. At high grafting densities, on the other hand, the chains are close enough to each other that they experience steric and/or electrostatic repulsion which causes them to stretch away from the surface in a “brush-like” conformation [7].

Just as a photoresist, polymer brushes form patternable surfaces and depending on application, different patterning methods can be employed. The majority of biological substrates is used with structures in the micron range, therefore patterning the initiator through a mask before polymerization will provide feature sizes in suitable dimensions. One application of relevance is an electrochemical antibody detector. This particular biosensor takes advantage of the antibody catalyzed water oxidation pathway (ACWOP), a process which depends on the feature that all antibodies have a catalytic patch which converts singlet oxygen and water into hydrogen peroxide [8]. We can then

measure the concentration of hydrogen peroxide produced by the antibodies in an assay solution via square wave voltammetry. Our device utilizes patterned polymer brushes to prevent non-specific adsorption and to tether antibodies to a surface. In this case, the patterning process becomes complex due to the simultaneous polymerization of a photosensitizer, required for production of the singlet oxygen, on the same surface as the brushes. A multistep method allows for the functionalization needed to provide both signal amplification and background reduction.

Another application of interest is the production of Janus membranes. We have begun to explore means for detaching polymer brush membranes from a surface. Previous work on polymer brush nanochannels led us to question the robustness of the bridging polymer film that stretched over distances ranging from 100 nm to a few microns [9]. If these films can be removed and if they show good mechanical strength, then Janus membranes could be created for biological applications. To remove the films, we employ a hydrofluoric acid etching technique that has proven successful in producing durable films that we can further characterize with TEM and optical microscopy.

2. Experimental

2.1. Materials

Allyl-2-bromo-2-methylpropionate, chlorodimethyl-hydrosilane, Pt on activated carbon (10 wt %), 2,22bipyridine, N,N,N',N',N"-penta-methyldiethylenetriamine (PMDETA), pyridine, copper (I) bromide, copper (I) chloride, copper (II) dibromide, 2,22bipyridine, inhibitor remover, anhydrous toluene, hydrofluoric acid, oligo(ethylene glycol methacrylate) (OEGMA; $M_w = 360$), styrene (S) were purchased from Sigma Aldrich and used without purification unless stated otherwise. S was passed through basic alumina to remove the inhibitor before use. Deionized water with a resistivity of 18.2 M Ω cm at 25 °C was obtained from Millipore's Milli-Q Synthesis A10 system. All the other solvents were purchased from Fisher Scientific.

2.2. Synthesis of silane initiator and immobilization

Hydrosilylation of allyl 2-bromo-2-methylpropionate was carried out using a literature procedure to obtain the ATRP initiator, 3-(chlorodimethylsilyl)propyl 2-bromo-2-methylpropionate [10]. Wafers with a 2° m silicon oxide layer were immersed in toluene solution of the initiator (2 mM) and pyridine (0.05 mM) overnight. The substrates were then removed from the solution and washed with water, ethanol, and dichloromethane sequentially. They were blown dry under nitrogen gas and used for surface-initiated polymerization.

2.3. Synthesis of thiol initiator, patterning and immobilization

Surface initiator was synthesized as described [11]. Gold quartz crystal microbalance (QCM) substrates were cleaned using a Harrick Plasma Cleaner for 5 minutes and then spin-coated with positive tone photoresist S1813 (Shipley) and baked for 90 seconds at 115°C. The samples were then exposed to UV light ($\lambda = 405$ nm) for ten seconds using an ABM contact aligner. After a post exposure bake (PEB) for the same length of time and temperature as before, the photoresist was developed and then etched using a Branson Resist Strip with a pattern descumming recipe. The initiator was immobilized on the surface in a 1 mM solution in anhydrous hexanes overnight under argon. Afterwards, the remaining photoresist was stripped off using acetone, rinsed with ethanol, and dried before electropolymerizing the photosensitizer. For the electropolymerization, a solution of 0.5 mM [Ru(v-bpy)(bpy)₂](PF₆)₂ in 0.1 M Bu₄NClO₄/acetonitrile is bubbled in nitrogen for 15 minutes. The Pt electrode is then cycled between -0.7 and -1.7 V vs. Ag/AgCl for 10 cycles at a scan rate of 100 mV/s [12]. After immobilization of the photosensitizer, polymerization of the polymer brushes can be carried out.

2.4. Polymer Brush Polymerization

POEGMA brushes were prepared using a literature procedure [13]. Polystyrene was prepared by degassing via vacuum freeze-pumping a solution of styrene 14 mL (inhibitor removed), PMDETA 130 μ L, and anhydrous anisole 5 mL for 15 min four to five times. The solution was then

transferred to an argon-purged Schlenk tube loaded with CuBr (50 mg). The mixture was stirred for 15 mins before transferred to another Schlenk tube with initiator wafers. The reaction was kept at 110°C for a certain time before stopped and rinsed in solvents.

2.5. PS membrane detachment

Wafers with PS brushes were spin coated, exposed, and developed the same way as mentioned above with a pattern placing 20 μm holes 190 μm apart. Substrates were etched for 2 minutes with a PT72 Etcher using a standard oxygen etch recipe. To crosslink the rest of the polymer brushes, the wafer was flood exposed with the same UV source but for 10 minutes and the PEB/development was the same as before. The brush films were removed from the surface by etching the silicon oxide layer with a 50% solution of HF for 1.5 minutes and rinsing with water without disturbing the detached membranes.

3. Results and Discussion

3.1. Antibody biosensor

A major obstacle to device fabrication was determining how to polymerize both the polymer brushes and the photosensitizer on the same gold surface. Without an initial patterning step, the brushes or the photosensitizer would occupy the entire surface providing no room for the second component. We therefore needed to designate specific areas for each function. First we modified the surface of the QCM by adding a thin layer of silicon oxide and patterning regions for the polymer brushes to be grown from. However, this resulted in decreasing the detection signal. A QCM can detect a mass by measuring the change in frequency of its quartz crystal resonator. As a mass is deposited on the surface, the oscillation frequency of the crystal decreases and the change can be quantified and correlated precisely to the mass change using Sauerbrey's equation [14]. When a mass is too large, however, it will cause inaccuracies in the output signal. Thus adding additional films to change the gold surface is a poor solution. Instead, we patterned a photoresist and immobilized the polymer brush initiator in the exposed regions to create a grid pattern of polymer brush "islands" surrounded by the photosensitizer

directly on the gold surface (Figure 1). We found significantly reduced electrochemical polymerization of the photosensitizer if the polymer brushes were first grown from the patterned initiator. However if the initiator was first immobilized then the photosensitizer was electrochemically attached followed by polymer brush growth, all components were effectively bound to the surface and well integrated. The specific order of this method and the importance of patterning are key factors for creating a biosensor device with multiple elements on the same surface.

Fig. 1. Polymer brushes and a photosensitizer were functionalized on the same surface in a checkerboard pattern. The lighter squares are the areas where polymer brushes were grown and the darker gold was electrochemically polymerized with the photosensitizer.

3.2. Patterned PS brush membranes

A 2 μm silicon oxide layer was thermally grown on a regular silicon wafer and an ATRP initiator was immobilized on the surface by standard methods. PS brushes were grown on substrates that ranged in thicknesses of 30-70 nm. The patterning, crosslinking and HF etching as detailed above lead to the production of very thin membranes. HF was chosen for a number of reasons. First, HF only etches silicon oxides and silicon nitrides; it does not harm polymers and therefore will not affect the polymer brush membranes. Second, the thick silicon oxide layer is the same as the surface used conventionally to grow polymer brushes. Since immobilization conditions and various polymerization environments will not remove the oxide film, we

are not limited to the type of brushes that can be grown.

After brush membranes were lifted off of the substrate, they were characterized by TEM and optical microscopy. As seen in figure 2, the membranes retain a significant amount of strength. They hold together and either fold, wrinkle, or bend instead of tearing or falling apart. This implies these membranes can potentially undergo further functionalization methods and remain intact. They can also provide more information about the initiator immobilization and brush polymerization process and answer questions about the nature of the brush films. If we make changes to the initial growth and processing steps, does this alter the outcome of the membranes and if so, perhaps the overall polymer brush procedure can be improved?

Fig. 2. Optical image of 30nm PS membrane on TEM grid.

4. Conclusion

Polymerizing two components on the same surface can become a complex issue, but patterning and attention to the specific order of immobilization steps can lead to multifunctionalized surfaces which in this case have biological implications. Additionally, polymer brush films can be patterned and removed from the surface by an etching process. The strength and durability of these extremely thin films could present information about the initiator immobilization and brush polymerization process as well as be explored as potential Janus membranes for biological applications.

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