SELF ASSEMBLED MONOLAYER PATTERNING USING CAPILLARY FORCE LITHOGRAPHY

Ieong Wong and Chih-Ming Ho
Department of Mechanical and Aerospace Engineering
University of California, Los Angeles, USA

ABSTRACT

A high performance, high throughput, low cost, and simple technique for patterning self-assembled monolayer (SAM) is successfully demonstrated in this report. Capillary force lithography is employed to generate micro features of protein resistant poly (ethylene glycol) (PEG) self-assembled monolayer on Si/SiO\textsubscript{2} substrate for accurate protein patterning. The monolayer shows significant reduction, >99%, of nonspecific protein adsorption compared to bare Si/SiO\textsubscript{2} substrate. Large area, in the order of centimeter, of high-density array (500 \times 500) of 2\textmu m features can be resolved with high fidelity. This method alleviates the difficulties and complexities in SAM patterning using micro contact printing and photolithography. To our knowledge, this is the first demonstration of capillary force lithography for patterning self-assembled monolayers.

1. INTRODUCTION

Self-assembled monolayers attracts substantial amount of studies involved in surface related nano and bio applications due to their spontaneous formation and thermodynamic stability [1,2]. Among a variety amount of applications of SAM, protein and cell resistant SAM is one of the most intensively studied candidates for biomedical applications such as drug discovery, biomaterials [3], and biosensors [4]. On the other hand, poly(ethylene glycol) (PEG) is the most well known and powerful biomaterial to provide protein and cell repellant properties, as well as its low toxicity and biological inertness [5]. Therefore, the capability to construct a high quality PEG SAM becomes an essential requirement for biomedical applications.

Most studies of PEG SAM patterning are performed on gold surfaces using micro contact printing (μCP) of alkanethiol terminated PEG derivative molecules [6]. However, the non-transparent optical property of gold surface largely reduces the compatibility of incorporating with optical measurement. Nonetheless, only very few of studies have been reported to pattern SAM on oxide surface due to the difficulties come from the intrinsic properties of the silane coupling chemistry. Therefore, the ability of forming SAM pattern directly on transparent glass surfaces becomes highly demanded. Besides, PEG SAM can be patterned by linking biotin-PEG molecules through streptavidin-biotin interaction on alkanethiol or alkanesilane precoated gold or oxide surfaces [7]. An alternative is to graft PEG on oxide surface using poly-L-lysine-g-PEG copolymer via electrostatic interaction between positively charged poly-L-lysine chain and negatively charged oxide surface [8]. Another method to pattern SAM is to use well characterized photolithography lift off technique to selectively pattern PEG-derivative silane molecules onto oxide substrate [9].

Recently, capillary force lithography [10] has been exploited to fabricate PEG microstructures for protein and cell patterning. Assisted with capillarity and wettability of polymer solution within the micro features of the PDMS mold, capillary force lithography can produce imprint lithography-quality microstructures with high pattern fidelity in nanoscale [12]. Polymer monolayer (PM) patterned on SiO\textsubscript{2} surface by capillary force lithography [11] also has been demonstrated. However, this requires complicated synthesis of the copolymer molecules. Moreover, the structure of PM is a layer of copolymer mesh instead of SAM of single molecules. Here, we present a low cost simple process to pattern SAM with inexpensive commercially available PEG-derivative molecules on SiO\textsubscript{2} surface.

2. PATTERNING CONCEPT

Self assembled monolayer patterning using capillary force lithography is a counter-intuitive method in the sense of surface chemistry and surface patterning technology because capillary force lithography is generally referred to the patterning of microstructures of polymeric materials with certain surface topographies, instead of interfacial patterning at the molecular level. The key innovation of our method combines the merits of capillary force lithography, i.e., photolithography-less imprint lithography-quality microfabrication process, and the virtue of self assembled covalent coupling chemistry. This advancement also proves the feasibility of using micro topographical molding technique to create surface patterns at the nano molecular level.

3. EXPERIMENTAL PROCEDURE

SELF ASSEMBLED MONOLAYER PATTERNING

A schematic of the capillary force lithography technique is shown in Figure 1. The process starts with highly polished Si <100> wafers cut into pieces of 2cm \times 2cm before any surface modification. The substrates are first plasma cleaned for 10min to generate enough hydroxyl functional groups for silane covalent procedure (Fig 1a). Immediately after the plasma clean, substrates are immersed into an epoxysilane (3-glycidoxypropyl trimethoxysilane, GPTS) (Sigma-Aldrich) solution in anhydrous toluene [13]. This is left undisturbed
for at least 16 hours to allow silanization to take place on the oxide surface (Fig 1b). After the silanization step, the modified substrate is ultrasonically cleaned with ethanol and DI water and dried with nitrogen gas. At this point, the substrate is terminated by a highly ordered SAM of epoxysilane molecules (Fig 1c). A small drop of methoxy-terminated PEG-amine (Nektar Therapeutics) is dispensed onto the dry silanized substrate (Fig 1d) and PDMS mold is immediately brought into conformal contact with the surface. The mold is left undisturbed at room temperature for 24 hours (Fig 1e). During the 24 hour period, water solvent evaporates through the edges of the PDMS mold and leaves behind a visible layer of dry PEG solid microstructures after PDMS peeled off (Fig 1f). This surface is then rinsed with DI water to dissolve all the PEG microstructures until no visible features can be seen on the substrate. At this point, the substrate is patterned with a densely covered PEG SAM (Fig 1g).

Static contact angle of the SAM surface is measured by an optical contact angle measurement system (FTÅ 4000, First Ten Angstroms). A droplet of 3µl of DI water is placed on the surface and measurement is made within 30sec.

CHARACTERIZATION OF DRY PEG STRUCTURE

Atomic force microscopy (AFM) is used to characterize the topography of the dried PEG microstructure after PDMS is peeled off. Measurements are taken in tapping mode on a Nanoscope III Dimension instrument in air, using RTESP tips at a scan rate of 0.5Hz.

4. RESULT

After the water solvent of the PEG solution dries out through evaporation from the edges of the PDMS mold, the PDMS mold is peeled off and a visible layer of dried PEG microstructures resided on the surface. SEM image (Fig 2) clearly shows that the patterns of this PEG microstructures (array of 3µm recessed wells) form a negative replica of the PDMS mold (array of 3µm protruding cylinders), with very high pattern fidelity. The image also shows a highly smooth exposed substrate surface at the bottom of the recessed wells where no PEG resides. This exposure of the substrate surface is essential for later selective protein and cell deposition.

Figure 2. SEM image of the dry solid PEG structure after PDMS mold is peeled off. The microwells have a diameter of 3µm. Wavy distortion of the rim on PEG layer is produced during PDMS peeling (inset)

Scanning the PEG microstructure with AFM reveals that the microstructures exhibit a periodical wavy topography of PEG mass pileup (Fig 3), instead of a flat uniform topography as the PDMS mold. During the molding process, small volume of solution is dispensed on the substrate and is squeezed into the void region by capillary force exerted by the PDMS mold. Due to the dewetting of the water on the wall of the PDMS mold, the water meniscus detaches from the PDMS wall and tends to adhere to the relatively more hydrophilic GPTS surface. The cross section image (inset) of the AFM measurement clearly reveals the water dewetting phenomenon in the PDMS mold. According to the AFM measurement, the bottom exposed substrate of the recessed well has a diameter of 3µm and a height, (h in inset) around 200nm.

SURFACE CHARACTERIZATION OF PEG SAM

To investigate the protein adsorption of the PEG SAM, fluorescence labeled proteins are used in optical microscopy characterization. A few drops of 50µg/ml of Texas-Red labeled bovine serum albumin (BSA) (Invitrogen) and 1mg/ml of fluorescein isothiocyanate-labeled poly-L-lysine (PLL) (Sigma) are distributed onto the patterned SAM surface and incubated at room temperature for 10min. The surface is then washed with PBS and analyzed with fluorescence microscope (DMIRB, Leica).
Figure 3. AFM images of dry solid PEG microstructure after PDMS is peeled off. The size of the wells (3\( \mu \)m) can be realized by the width of the bottom of the valley in the inset. The height of the structure is ~200nm

CONTACT ANGLE MEASUREMENT

To understand the SAM grafting mechanism, contact angle measurements are carried out to characterize the two layers of SAMs (GPTS and PEG) on the oxide substrate. As illustrated above, the Si oxide surface is first silanized with GPTS to terminate the surface with a layer of epoxy functional groups for subsequent grafting of PEG SAM. Therefore, constructing a high quality GPTS SAM is crucial for construction of high quality PEG SAM. Fig 4(a) and 4(b) shows that the contact angles of the SAM surfaces of GPTS and PEG are 52° ± 1° and 31° ± 1°, respectively. The contact angle of GPTS confirms a highly ordered and densely packed GPTS SAM is produced, as revealed by previous study [13]. This assures the PEG SAM is built upon a high quality functionalized GPTS SAM platform. After the GPTS SAM is formed, PEG SAM is subsequently grafted on top, and switches the contact angle from 52° to 32°. This contact angle change ensures a covalent epoxy/amine reaction taken place on the surface. As demonstrated in previous study [14], a contact angle of 32° corresponds to a highly packed and well oriented PEG SAM.

Figure 4. Contact angle measurement of the two SAM layers. GPTS SAM layer has a contact angle of 52° before PEG coating (a). After PEG coating, the contact angle changes to 32°(b).

One intuitive thought can also be elucidated from the contact angle measurement. Since the PDMS mold is applied after the PEG solution is dispensed on the surface, certain amount of PEG molecules is supposed to immediately react with the surface and affect the accuracy of the PDMS patterning technique. However, from our contact angle measurement on GPTS surface after a 20-min immersion in PEG-derivative solution, no change in contact angle is observed. This slow reaction comes from the intrinsic chemical reaction kinetics between the epoxy and amine functional groups, which is ingeniously exploited in this method.

FLUORESCENCE MICROSCOPY

To demonstrate the protein repellant ability of the PEG SAM, surfaces are incubated with two fluorescence-labeled proteins: BSA and PLL. Fluorescence microscopy images (Fig. 5a and b) and quantitative analysis (Fig. 5c) demonstrate that proteins are only selectively adsorbed to the exposed glass substrate (bright circles) while only extremely low level of protein adhesion (<3%) is present on the PEG SAM (dark area). As shown in Fig 5a and c, the adsorption of BSA is significantly reduced by PEG SAM (>99%) compared to the exposed glass control surface. The images also shows patterning with this method can create micro protein features, as small as 3\( \mu \)m, in a large area, in the order of centimeter, with high pattern fidelity.

Figure 5. Fluorescence microscopy images of high density array patterning of both BSA (a) and PLL (b). The protein adsorption on PEG surface is substantially reduced to 1% for BSA and 3% for PLL (c). Each bright dot is 3\( \mu \)m in diameter.

Interestingly, increasing the protein concentration or the incubation time for 2 or 3 folds does not increase the protein nonspecific adsorption on the PEG SAM, which gives a good evidence that the PEG surface is indeed a highly ordered and densely packed PEG SAM surface. In additional, only a brief washing step (less than 10 sec) is required to rinse off the unbound proteins, compared to most convention method which requires prolonged and strong rinsing step. Similar results are obtained from the tests on PLL (Fig 5b), where more than 97% of PLL is repelled by the PEG SAM surface.
5. CONCLUSION

We have successfully demonstrated a high performance, high throughput, low cost, and simple technique for large area patterning of self-assembled monolayer (SAM) of PEG molecules on SiO$_2$ substrate for selective protein and cell adhesion using capillary force lithography. The patterned SAM surface can significantly reduce more than 99% of nonspecific protein adsorption compared to bare SiO$_2$ substrate. This method also alleviates most of the intricacies in conventional SAM patterning technique, including soft lithography, imprint lithography, and photolithography. This high feature fidelity and high protein selective patterning technique can substantially improve the conventional platforms for low density or single mammalian cell and bacteria studies.

6. ACKNOWLEDGEMENT

This work is supported by NASA Institute of Cell Mimetic Space Exploration (CMISE, NCC2-13364) and NIH National Institute of Dental and Craniofacial Research (NIDCR) (U01DE15018).

REFERENCE