Protein patterning using germanium as a sacrificial layer *

Bochao Lu and Michel M. Maharbiz, Member, IEEE

Abstract—With the rise of microfluidic diagnostics, there is a need for more efficient methods of patterning surface-attached moieties, including proteins like antibodies, onto microchannel surfaces. This arises because almost all of the solvents and processes used for surface-attachment chemistries (or their payloads) are incompatible with sacrificial layers usually photoprotect during microfabrication, rendering it difficult to easily pattern active chemistry onto a surface in manufacture scale. We present a simple method, based on thin film germanium dissolution, which is compatible with both modern nanolithographic techniques and surface chemistries. Simply, because germanium thin films dissolve readily, controllably and rapidly in water (but not organic solvents), these films can be used to mask and protect areas of the substrate during the attachment of surface chemistries. We demonstrate the process and results using microscale patterns. The resolution and alignment of this method depends on the photolithography tool used; nanoscale patterning is not difficult to achieve. In addition, we show that with non-conformal germanium deposition (e.g. e-beam evaporation), the conjugation of surface chemistry on vertical side walls can be manipulated by controlling the thickness of the deposited germanium layer, opening another dimension for microfluidic devices and cell manipulation research.

I. INTRODUCTION

High-resolution protein patterning is in high demand for miniaturized biomedical devices for either clinical use or fundamental research, with examples in biosensors [1], diagnostics [2-4] and microscale bioreactors [5-6]. The most popular protein patterning methods include gasket-based patterning [2-7], microcontact printing [3], [8-10] and dip pen lithography [11]. Gasket-based patterning is one of the easiest and cheapest approaches for protein coatings on biosensors. Resolution, however, is limited and alignment is performed manually, so this method is suitable for fundamental research with low throughput. Microcontact printing and dip pen lithography (DPL) are both contact based approaches. DPL can obtain nanoscale resolution due to the use of atomic force microscopy (AFM) tips in the process [12-14] but is a comparatively difficult manufacturing process. Microcontact printing is additionally limited by difficulty in obtaining good alignment between the desired pattern and existing (or to-be-processed) features.

To obtain micron-scale or better resolution and precise alignment of surface chemistry patterns, it would be desirable to develop a process which makes use of accessible thin film and photolithography to fabricate the protein patterns. A common technique used in other areas is to deposit and pattern a sacrificial layer and lift off excessive protein molecules after immobilization; for example, in the common “lift-off” techniques used for patterning thin films, the sacrificial layer is photoresist [6], [15-16]. Attempting to use photoresist as a sacrificial layer for surface attachment applications is problematic because photoresist dissolves in organic solvent (e.g. acetone, isopropyl alcohol (IPA), ethanol and dimethyl sulfoxide or DMSO). Biomolecule and surface functionalization chemistries are almost always sensitive to organic solvents, however. For example, the use of silane / polyethylene glycol (PEG) chemistries to functionalize molecules onto silica surfaces requires preventing the hydrolysis of silane groups in water; to prevent this, organic solvent is used to dissolve the adhesion molecules and maintain activity during deposition. Problems may arise when attempting to use dissolvable metals as a sacrificial mask. Yin et al. [17] demonstrated several water soluble metals that could be potentially used as a lift off material for this purpose. However, most dissolvable metals are flammable, hard to deposit, dissolve slowly at neutral pH (i.e. usually require acids to dissolve) and are expensive.

In this paper, we report a simple and inexpensive technique that employs the commonly deposited semiconductor germanium (Ge) as a sacrificial layer for any biomolecule, including DNA, RNA and proteins, with high resolution and precise alignment. Germanium is known for its solubility in water and has been studied extensively as a sacrificial layer in the fabrication of microelectromechanical system (MEMS) [18-19]. Pure germanium does not dissolve or react with water directly. While in contact with water, germanium is oxidized into germanium dioxide by dissolved oxygen molecules (or hydrogen peroxide, if present). Germanium dioxide dissolves in water spontaneously leaving germanium underneath to be exposed for further oxidation and dissolution [18-19]. Since germanium is commonly used in MEMS fabrication, there are well-developed deposition (both chemical vapor deposition (CVD) and physical vapor deposition (PVD) methods are characterized), etch and patterning processes developed for this material, enabling ease-of-use, high throughput, high resolution (depending on patterning method used) and minimal misalignment. The biocompatibility of Ge and its product after dissolution enables the use of Ge for biomolecule patterning in both in-vitro and in-vivo devices [20].

In addition to basic patterning, we demonstrate that non-conformal germanium deposition (e.g.E-beam evaporation) onto RIE etched channels enables the functionalization of side walls with resolution independent from the horizontal surface patterning. Not only does this provide a method to functionalize vertical structures, a topic of recent interest [5] but it provides a mechanism to generate nanometer thick vertical functionalization geometries without

*Research supported by Berkeley Sensor and Actuator Center (BSAC) funds and DARPA Advanced Study.

B. Lu is with the UC Berkeley/UCSF Joint Bioengineering PhD. Program, Berkeley, CA 94720 USA (corresponding author: 510-857-4453 email: steven.lu@berkeley.edu).

M.M. Maharbiz is with the Electrical Engineering and Computer Sciences and Bioengineering Department, UC Berkeley, Berkeley, CA, 94720 USA (email: maharbiz@eecs.berkeley.edu).
the use of e-beam lithography. This study, to our knowledge, is the first demonstration of using germanium as a sacrificial layer for surface functionalization on both horizontal and vertical walls.

II. Fabrication

Germanium was patterned using a variant of the classic ‘lift-off’ process (Fig. 1). First, I-line photoresist was spun at 4100 rpm (1.2 µm), exposed in Karl Suss MA6 and developed to expose areas where germanium was desired. Germanium was then deposited in CHA E-beam evaporator at 10⁻⁶ Torr. This was followed by removal of the photoresist using 1-methyl-2-pyrrolidone (NMP). Alternatively, the germanium can be deposited first (e.g. if using LPCVD) and then etched (using Lam TCP 9400SE Etcher). All Ge films were patterned with a ‘lift-off’ process. Following germanium processing, the substrates were functionalized with biomolecules. In this paper, we functionalized the surface with biotin. Substrates were then immersed in 25mg/ml silane-PEG-biotin (Nanocs, New York, NY) dissolved in ethanol. This was followed by immersion in deionized (DI) water to remove the germanium. Alternatively, the substrates were immersed in DI water with 0.35% w/v hydrogen peroxide, leading to more rapid removal (see Results in Fig. 2). After dissolution of germanium, substrates were rinsed with DI water and blown dry with N₂. In order to show the density and activity of the patterned biotin molecules, after 30 min blocking in 1% bovine serum albumin (BSA) to prevent unspecific binding, we brought biotin patterned substrates in contact with 3.8 nM streptavidin conjugated with Alexa-Fluor 647 (Strep-647) in 1x PBS for 15 min, followed by washing with DI water and blown dry with N₂. Then these chips were ready to be used and tested.

III. Experimental methods

A. Germanium dissolution tests

Germanium films of 2 nm thickness were E-beam evaporated (as per Fabrication) on silica chips. Kapton tape was used to cover some chip area (leaving the center exposed), in order to provide enough contrast between Ge and non-Ge areas. Four circles were drilled around Ge deposited area, so that we could identify the Ge area even after complete dissolution. Ge deposited silica chips were then immersed into pure DI water, DI water with 0.35% w/v hydrogen peroxide, NMP, IPA, acetone, DMSO and ethanol for 10 min, 30 min, and overnight to test the dissolution rate in different solutions.

B. Surface functionalization in microchannels

Multiple 30 µm channels were etched with reactive ion etching (RIE; Surface Technology Systems Advanced Planar Source oxide etch system) on a silica surface. Each channel was 300 nm deep in silica. The germanium process was used to functionalize with biotin two 20 x 20 µm squares in each 30 µm wide and 300 nm deep channel using 50 nm thick Ge and silane-PEG linkers (as per Fabrication). Then each chip was incubated with Strep-647 to label immobilized biotin molecules after blocking in 1% BSA for 1 hour. Fluorescent images were taken after washing chips with 1x PBS for 3 times and blowing dry with N₂ gas.

C. Vertical sidewall functionalization

For vertical sidewall conjugation experiments, a 300 nm deep, 30 µm wide channel was etched into silica substrates with RIE. Either 50 nm or 300 nm thick germanium was then deposited onto the surface using E-beam evaporation with the same setting as above. Chips were then conjugated with biotin and labeled by strep-647 as described in Fabrication. In this

![Figure 1. Surface functionalization with germanium based lift-off. 50nm or 300nm Ge is patterned and the chips are then brought in contact with functionalization solution. After washing away excessive surface conjugating molecules with organic solvent, Ge is dissolved in water or H₂O₂ solution, leaving patterned bio-functional groups on the surface.](image)

![Figure 2. Germanium dissolution test. Silica chips with 2nm thick Ge layer were incubated in different solutions at room temperature for 10min, 30min and overnight.](image)
experiment, we didn’t pattern squares of biotin in channels, since we were characterizing the vertical side wall conjugations.

IV. RESULTS & DISCUSSION

A. Germanium dissolves in both DI water and DI water with hydrogen peroxide but not in organic solvents

Germanium dissolves easily in aqueous solution but not organic solutions. As shown in Fig. 2, brown areas indicated by black arrows are areas deposited with 2nm Ge, which dissolved completely in pure DI water and water with 0.35% w/v H₂O₂ in 30 min and 10 min respectively, but did not dissolve in any organic solvent after an overnight incubation at room temperature. Note, the Ge dissolution rate in 0.35% H₂O₂ is higher than 2nm per 10min. This experiment was to demonstrate the different dissolution rates of Ge in different solvent solutions qualitatively and to prove the feasibility of using Ge as a sacrificial layer for protein ‘lift-off’.

B. The germanium process is compatible with surface functionalization in microchannels

Biotin can be accurately conjugated into each 30um channel with little background noise or unspecific binding. Fig 3A shows a column of biotin conjugated squares labeled with Strep-647 aligned precisely in each trench. A closer look of functionalized area is shown in Fig. 3B. All the squares are bright and aligned in a 30 µm wide channel with straight and clean edges. No unspecific binding or background signal is apparent out of the binding area; this demonstrates that our 50 nm Ge films are sufficiently pinhole free as to be good hard masks. One thing to note is that trench side walls (red arrows in Fig. 3B) are also conjugated with biotin (see next section).

C. Thin germanium layer enables conjugation of biomolecules on vertical features

Thicker Ge layers result in less vertical sidewall conjugation. For the same deposition setting, there is much less bound fluorophore (and thus, less biotin) on the sidewalls of the channels deposited with 300 nm germanium (Fig. 4A) as compared to 50 nm germanium (Fig. 4B). This arises because for very non-conformal deposition (like evaporation), very thin films (e.g. 50 nm) only deposit on the horizontal surfaces (i.e. top and bottom of channel) and not on the vertical sidewalls; this leaves the vertical sidewalls as the only exposed features for functionalization. For thicker films (e.g. 300 nm), enough germanium is deposited that the sidewalls are coated, resulting in less conjugation.

V. CONCLUSIONS

We have developed a simple, thin film microfabrication compatible, high-resolution, precise alignment, and inexpensive method to functionalize both horizontal and vertical surfaces. Since this method is fully compatible with conventional photolithography processes, the resolution and alignment completely depends on the capability of the lithography tool employed. For example, with a deep ultraviolet (DUV) lithography tool we have been able to
perform this process with 250 nm resolution and comparable alignment accuracy (unpublished). Furthermore, using non-conformal Ge deposition, vertical features can be exposed for conjugation.

The method is demonstrably extensible to other common chemistries. For example, if crosslinking with carboxyl or amine groups on the opposite side of silane group, we can easily conjugate peptides (e.g. antibodies) or extracellular matrix (ECM) proteins onto the surface through peptide bond formation chemistry. Carboxyl groups are, in fact, more robust to hydrogen peroxide attack. This method provides a simple way to conjugate water hydrolysable crosslinking molecules like silane groups as well as to immobilize hydrophobic molecules like transmembrane proteins that cannot be dissolved in aqueous solutions. In addition to the capability of side wall functionalization, this method can open another door for fundamental research in cell-cell or cell-ECM interaction.

ACKNOWLEDGMENT

This work was funded by Berkeley Sensor and Actuator Center (BSAC) funds and DARPA Advanced Study HR0011-16-C-0023.

REFERENCES