Micromachining of non-fouling coatings for bio-MEMS applications

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Abstract

Standard photolithography is used to pattern a poly (ethylene glycol) (PEG)-like polymer onto silicon substrates. The coating has excellent non-fouling properties and good adhesion to various substrate materials, such as silicon, oxide, nitride, gold, and platinum. This method allows precise control of the shape, size and alignment of the polymer, thus providing a reliable tool to pattern protein sheets as well as cell cultures. This method also enables the incorporation of patterned cell cultures with various predefined elements such as electrodes, channels, and sensors. To demonstrate the properties of our technique, we apply it to build cell cultures and to protect metallic electrodes from protein and cell adhesion. We show that the thin coatings provide excellent protection without compromising the conductivity of the electrodes. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

The rapid growth of micro electro mechanical systems (MEMS) research in the last few decades has given rise to a wide variety of novel applications. Among the most notable are small, portable systems for medical and biological applications (bio-MEMS). These devices are currently developed with the vision of building miniaturized, fast, and portable analytical instrumentation for drug delivery, electrochemical monitoring [1] and DNA analysis [2]. These devices would speed the analysis time for researchers in the lab, for medical staff at hospitals or might be used as carry-on devices by chronically ill patients.

The interaction of a device with a biological environment leads to various challenges that have to be taken into account in order to allow its proper operation. A major issue is bio-fouling, the strong tendency of proteins and organisms to physically adsorb to synthetic surfaces [3]. The adsorbed protein layer tends to create undesired perturbations to the operation of devices such as pH [4] and glucose [5] sensors. In addition, the protein layer can mediate various biological responses such as cell attachment and activation, which may interfere with the optimal operation of the device by, for

example, reducing its life span or increasing its power consumption [6].

A convenient approach to control protein adsorption is by surface modification [7]. This approach is particularly convenient for bio-MEMS development since it allows the use of commonly used substrates and materials (as long as they are non-toxic), and requires only a final coating procedure. Many common non-fouling coating techniques are based on poly (ethylene glycol) (PEG) [8,9]. PEG is a hydrated [10], non-toxic coating and FDA-approved for use in bio-technology and consumer applications. In recent years, various methods have been adopted to coat surfaces with PEG, including silicon surfaces [11]. In our study, we use glow discharge plasma polymerization of tetraglyme $(CH_3-O-(CH_2-CH_2-O)_4-CH_3)$ to obtain a PEG-like material. Plasma polymerization is a gas-phase, thin-film deposition process. This process is capable of producing thin coatings (of the order of 10 nm) with superb surface conformality [10] which is an extremely important factor in determining the success of the PEG coating [12]. The excellent surface coverage of the plasma polymerization process also contributes to its excellent non-fouling properties.

In previous studies, it was demonstrated that plasma polymerized tetraglyme (pp4G) coated surfaces show good resistance to protein adsorption [10]. The focus of our current study is to examine the compatibility between the pp4G coatings and photolithography and micro-machining

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processes in order to allow pp4G patterning as a standard MEMS process. In this paper, we focus on devices with a topography suitable for photolithography. Such conditions are common for many Bio-MEMS devices which consist of two bonded elements with at least one suitable for photo-lithography. Patterning the pp4G directly on high aspect ratio structures is limited by the ability to achieve uniform photoresist coatings on these structures. However, such coatings may be achieved by using very thick photo-resist or a special application procedure [16].

We describe below the integration of pp4G coatings into microfabrication processes for bio-MEMS applications. We characterized the coatings using environmental scanning electron microscopy (ESEM) which we used to verify the pattern definition. Electron spectroscopy for chemical analysis (ESCA), a surface sensitive analytical technique, was also utilized to analyze the coating surface chemistry after the photoresist lift-off. We found that the patterning process did not affect the pp4G coating.

Finally, we discuss experiments which demonstrate the capabilities of our technique: A protein adsorption experiment demonstrates the spatial control of these interactions on patterned pp4G surfaces. We use the patterned pp4G coatings to confine cell cultures to specific regions on the substrate. The advantages over contemporary methods that consist of soft lithography [13] are discussed. The pp4G was also tested as a protection layer for electrodes in electrochemical sensing.

2. Fabrication

We used silicon wafers as substrates for the pp4G coatings. The wafers were sputtered with silicon nitride or oxidized for electrical passivation. Nitride is better suited for our experiments because it has better corrosion resistance in aqueous environments than oxide [14]. To achieve the pp4G patterning, photoresist (Clariant AZ 1512, 1.5 μm) was deposited, and then exposed and developed in order to create openings in the resist for the later pp4G lift-off. After a plasma oxygen descum etch of 15 s in an RIE chamber (Trion Technology), the wafers were introduced to the plasma reactor for the pp4G deposition. The substrates were briefly cleaned in an argon plasma, the tetraglyme vapor was introduced to the chamber and the samples were coated. The details of the monomer preparation and the deposition process will be discussed elsewhere [15]. Following the plasma deposition process the wafers were soaked in acetone an ultrasonic bath for approximately 20 s. Finally, the wafers were rinsed with methanol and dried with nitrogen. The microfabrication processes were done at the Washington Technology Center (WTC) and at the Electrical Engineering Micro-fabrication Labs at the University of Washington. The pp4G deposition was done at the University of Washington Engineered Biomaterials (UWEB) labs.

Integrating the lift-off and the plasma deposition processes was rather straightforward. However, special attention had to be directed to the location of the wafer in the plasma chamber. We placed the wafers in low monomer condensation regions to prevent tetraglyme vapor from dissolving the photoresist. After the plasma deposition, the wafers were rinsed in acetone (in an ultrasonic bath) to remove resist leftovers.

2.1. Lift-off results

To discuss the quality of our results we begin with a visual examination of the pp4G coating after lift-off. In Fig. 1a and b, we show ESEM images of patterned pp4G coatings on thermally oxidized substrates. Here, arrays of Au squares
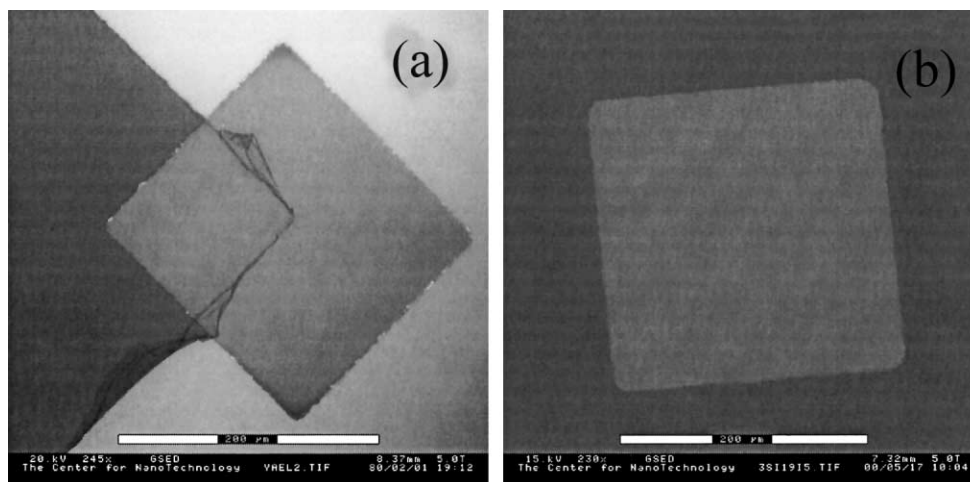

Fig. 1. ESEM images of pp4G features after lift-off. The scale bar is 200 μm for both images. (a) The pp4G structure (left) aligned to predefined CrAu structure. Lift-off without ultrasonic power. (b) Exposed SiO₂ substrate (bright square) with pp4G background. Lift-off is done in an ultrasonic bath. Similar results were obtained for structures as small as 10 μm. While current results were produced with low-cost printed masks (features greater than 10 μm), minimum feature size and alignment precision for these protein-resistant coatings is limited only by the lithographic resolution. We recently achieved similar results with 2 μm resolution.

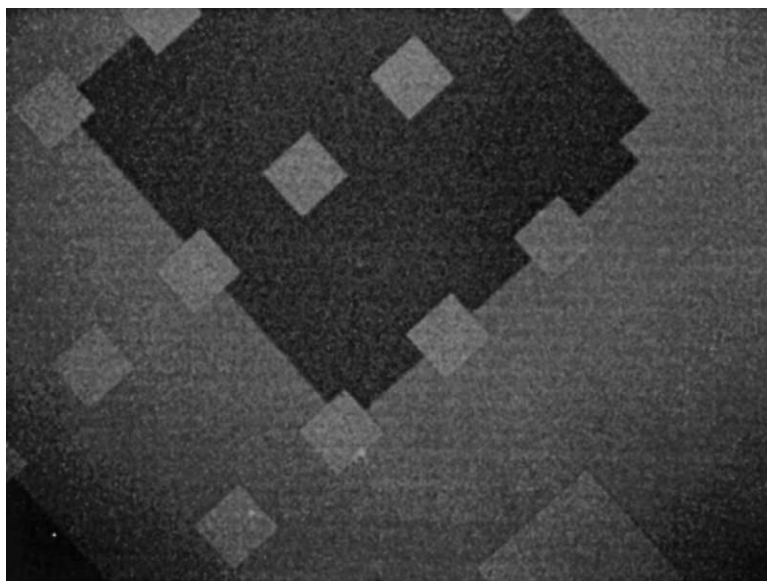Fig. 2. A fluorescence microscope image of a patterned protein-resistant pp4G pad (1500 μm × 1500 μm) with Au pads (200 μm × 200 μm) on SiO$_2$ substrate after incubation in a solution with fluorescently labeled proteins. The pp4G pad appears dark as the adsorbed proteins cover exposed SiO$_2$ and Au areas while pp4G coated areas resist protein adsorption.

were first patterned using the lift-off technique. Then the pp4G was deposited and patterned using lift-off. Using a stylus stepper (Alpha-Step), the pp4G coating was measured to be approximately 20 nm. In Fig. 1a the pp4G pad is shown with a predefined chrome–gold (CrAu) feature. To perform the pp4G lift-off, the sample in Fig. 1a was dipped in acetone for several minutes and then rinsed in methanol and dried. Note the pp4G leftovers at the edges of the pattern. This is a result of the conformal nature of the deposition process. It is important to note that conformality may be useful for channel coating purposes and for various three-dimensional structures where shadowing is a major challenge.

To improve the edge definition of the patterned pp4G we used an ultrasonic bath during the lift-off process (see Fig. 1b). As can be clearly seen in Fig. 1b, the edge definition is significantly improved compared to the sample in Fig. 1a. In addition, the adhesion of the coating is good enough to keep the coating intact despite the aggressive ultrasonic treatment. High resolution ESEM imaging and the coating performances (discussed later in the text) demonstrate the viability of the coating despite the ultrasonic treatment. We can now summarize the advantages of our process: the coating has good adhesion to the substrate and to the metal, it is conformal, it can be reliably patterned and can be incorporated onto complex structures using standard lithography and lift-off processes.

2.2. Surface chemistry

The next step in characterizing the patterned pp4G coatings is to study their surface chemistry. The surface chemistry of pp4G on SiO$_2$ and on metal layers was examined using ESCA. The ESCA characterization demonstrated that the patterning process does not affect the chemistry of the pp4G coating and that there are no organic residues left on the exposed or coated surfaces. The ESCA data are discussed in detail elsewhere [15].

To assess the effectiveness of the coating, we performed protein adsorption tests. For this experiment we used samples similar to the samples in Fig. 1. In Fig. 2, we show fluorescence microscopy image of adsorbed fluorescently labeled (Oregon Green, Molecular Probes) bovine serum albumin (BSA) on a patterned surface. The sample consist of an array of small, 200 μm × 200 μm, Au pads (bright squares) and big, 1500 μm × 1500 μm pp4G pads (dark areas) on a SiO$_2$ substrate. The adsorption experiment was carried out at room temperature in citrated phosphate buffer solution at pH 7.4. After incubation (for 1.5 h) the surface was thoroughly rinsed in a buffer solution. It is readily seen, in Fig. 2, that the proteins non-specifically adsorbed only to exposed SiO$_2$ areas (bright areas) whereas the pp4G coated regions appear dark in the absence of adsorbed proteins.

3. Applications

Micro-machined non-fouling coatings can be useful for various applications, in particular when incorporated into complex MEMS structures. Here, we choose to focus on two issues: patterned cell cultures and improved biocompatibility for electrochemical electrodes.

3.1. Integrated micro-patterned cell cultures

In Section 2, we demonstrated our ability to pattern protein layers. An additional, related application is micro-

patterning of cell cultures. It is widely known that proteins mediate cell adhesion. There are two general classes of cell adhesion mechanisms: non-specific attachment (due to dispersion forces and the hydrophobic effect) and specific protein-cell interactions. We use the selectivity of our patterned surface to protein adsorption to enable selective cell adhesion.

Micro-patterned cell culture devices are useful for the study of cellular activity and development. Such devices allow careful monitoring in a well defined environment by controlling the size of cell-adhesive islands [17] or the configuration of neural networks [18]. Micro-patterned cell cultures could also be useful when incorporated into more complex structures such as micro-electrode arrays [19], micro-channels or micro-analyzers. Contemporary methods to realize patterned cell surfaces consist of soft lithography [13]. While this technique offers the advantage of simplicity and the ability to pattern unusual materials over large areas, it is mainly limited to single-layer structures due to limited alignment capabilities. Our technique to incorporate cell domains into MEMS devices using standard photolithography is demonstrated next.

For the cell culture experiment we used line shaped openings in pp4G on SiO_2. The bovine aortic endothelial cells (BAECs) we used in the experimet were cultured in Iscoves Modified Dulbeccos Medium supplemented with 10% fetal bovine serum, 1% 100 mM MEM sodium pyruvate solution, 1% 10 mM MEM non-essential amino acids solution, 100 units/ml penicillin and 100 mg/ml streptomycin. BAECs were cultured on 75 cm^2 tissue culture flasks inside a humidified incubator at 37 °C with 5% CO_2 and 9% air. The cells were cultured on the patterned pp4G surfaces for 4 days. After 4 days in culture, the cell cultures were fixed and stained. Fig. 3 shows lines of cell cultures defined by the patterned pp4G. The cell cytoskeleton, f-actin, stained with rhodamine phalloidin is false-colored red (appearing bright). DNA inside the cell nucleus, stained with DAPI, is false-colored blue (appearing dark). Detailed information on the cell experiments is discussed elsewhere [15].

3.2. Protection layer for electrochemical electrodes

The hydrated nature of the pp4G coating and its thickness suggest its potential as an inert protection layer for electrochemical electrodes. Good ionic conductivity is important for various electro-chemical sensing applications such as micro-electrodes for neural recording [20]. We verify the conductivity properties of pp4G by comparing electroplated electrodes with and without pp4G coatings. The pp4G coating was integrated into the following process: a CrAu seed layer for an electroplating step was sputtered on a nitride layer. Photoresist was then patterned and the wafers were electroplated with platinum. The electroplating process was carried out in ultrasonic bath in chloroplatinic acid solution (1 g of $PtCl_6H_2$ to 100 ml H_2O) with Pt wire as a counter electrode. The dc current density was fixed at approximately 5 $nA/\mu m^2$ for 1 min. Next, the wafers were rinsed in acetone to remove the photoresist. A second layer of photoresist was applied and patterned and the seed layer was etched in gold etchant. A third lithography process defined the pp4G patterns.

Our devices consist of two sets of six square electrodes with different sizes (250–2250 μm side lengths). One set is coated (Fig. 4a) while the second is bare. To demonstrate the effectiveness of the coating we show images of the electrodes with and without pp4G (Fig. 4b and c, respectively) after 24 h in a solution with BAECs. By slightly shaking the dish, the suspended cells from the coated areas are removed and
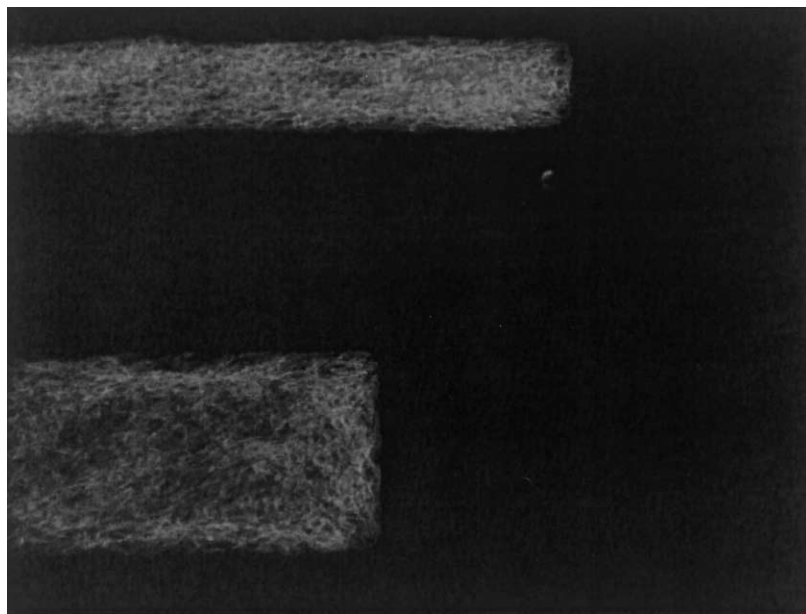

Fig. 3. Cell patterns achieved by culturing BAECs on line-shaped oxide domains with a pp4G background. The thinner line is 100 μm thick. Despite the multilayer formation of these cells on the substrate, they remain outside the pp4G boundary.
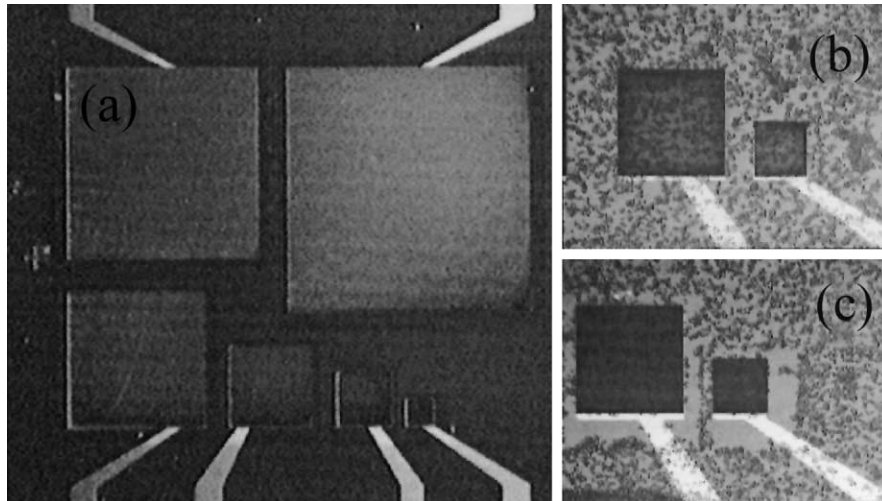
Fig. 4. (a) Electroplated platinum electrodes (dark squares) with pp4G coating on silicon nitride surface. The two smallest electrodes ((b) bare and (c) coated) after 24 h in a medium solution with BAECs. The pp4G coating (in c) prevents cells from adhering to the electrode surface.

the electrodes are exposed (seen in Fig. 4c). In comparison, without the protection of the pp4G coating (Fig. 4b) the entire surface is covered with cells. Since no special effort was made to rinse the access cells form the coating, some cells remain on the coated area and are still attached to the cells that adhered to the edges of the pp4G coating.

The ac and dc resistivity of various bare and coated samples was measured. In Fig. 5, we show a typical ac (10 Hz) conductance versus size plot of a coated electrode set. The derived resistivity is $5 \times 10^9 \, \Omega \, \mu m^2$. The resistivity for the bare set was found to be $8 \times 10^9 \, \Omega \, \mu m^2$. In other tests, including measurements in clean medium solutions and in solutions with proteins, similar results were obtained. The slight consistent difference in favor of the electrodes with coating can be attributed to some fouling of the bare electrodes either during the processing or during the measurement.

The results discussed above demonstrate the ability of the pp4G to protect the electrodes from protein and cell adhesion while maintaining the electrode conductivity properties. The effects of protein adsorption and cell adhesion on electrode performance are known to occur during periods of hours [4], days [5] or even months. These effects may vary for different applications and biological models. Further in vivo experimental study with coated and bare electrodes could clarify the significance of the coatings to issues such as tissue irritation, signal to noise ratio, signal stability, and signal distortion for various applications.

4. Conclusions

In summary, using standard photolithography and a plasma polymerization process we were able to realize patterns of non-fouling coatings on silicon substrates. Key features of this non-fouling technology include excellent coating quality, good biocompatibility, and ease of incorporation into MEMS fabrication. Because pp4G is patterned by a photoresist lift-off process, high alignment precision and good spatial resolution are attainable. This enabling technique, thus, offers precise spatial control of protein adsorption and cell attachment with good alignment to underlying features. Such precision can be a great advantage in engineering the surface chemistry of bio-MEMS, such as biosensors and microfluidic systems, to better interface devices with the biological environment. Here, we demonstrated the usefulness of our technique to build domains of proteins, as well as domains of cell cultures. We also demonstrated the effectiveness of the coating in protecting electro-chemical electrodes.
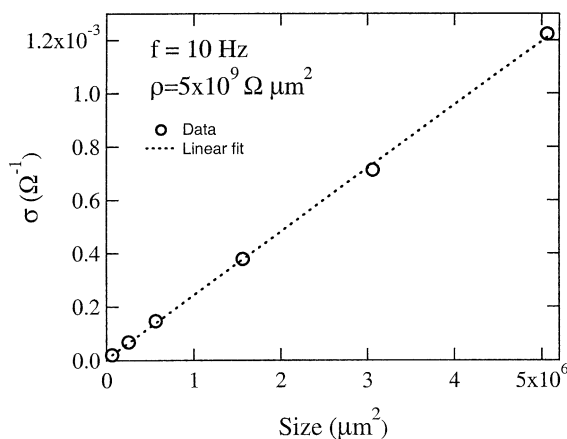

Fig. 5. Conductance data at 10 Hz plotted vs. electrode size for a set of pp4G coated electroplated electrodes in a medium solution with cells. Similar results were obtained for bare electrodes.

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