Biomolecule immobilization on Au surfaces within a sealed PDMS microsystem

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Abstract

A novel chemical surface treatment has been developed to immobilize proteins on Au while activating the surface for an irreversible PDMS bond. This novel process has applications that extends to advanced proteomics, immunoassays, early biowarfare detection, and DNA and cell manipulation. The surface treatment of the glass substrate allows the Au to remain hydrophobic and the glass to maintain an active state that is required for PDMS bonding. This allows for a completely irreversible bond of the PDMS to the glass without inhibiting the functionality of the proteins that reside within the microchannel.

Introduction

Protein immobilization within a PDMS microchannel is a critical aspect of creating a truly portable microfluidic biomolecule sensing platform. Current PDMS microfluidic applications extending to proteomics and immunoassays require an irreversible PDMS and substrate seal for biomolecule protection and precise flow control. Standard PDMS microfluidic fabrication processes require that both the glass (substrate) and PDMS be placed within O2 plasma for a specified amount of time to form an irreversible seal [1]. If the proteins are immobilized on the Au surface prior to being subjected to O2 plasma, they would be destroyed once O2 plasma is applied to the surface. However, protein immobilization after O2 plasma is almost impossible due to the hydrophilic behavior of the Au surface following the exposure. The Au surface becomes hydrophilic due to a temporary charge caused by the O2 plasma and causes protein filled solutions to spread to the surrounding area making the glass substrate un-bondable with a PDMS substrate. This standard bonding process makes protein or biomolecule immobilization within a PDMS microfluidic system very difficult to accomplish when using glass as the main substrate. A process was developed to immobilize biomolecules on a patterned Au surface on glass while activating and maintaining the glass surface for an irreversible PDMS bond. The immobilization of the proteins within a microfluidic channel has eliminated the need for more sophisticated delivery techniques within the channel.

Materials and Methods

Fabrication Process

1. 100nm of Au is deposited on an adhesion layer of 20nm of Ti.
2. 10gm of Shipley 1827 photoresist is coated on the device and is UV exposed to form Au pads.
3. Resist is developed using Microphos MF 310.
4. The Au and Ti are etched using the chemicals Transene Gold Etchant GE-8148 and Transene Ti Etchant TPT.
5. The resist is then removed using an acetone bath and methanol rinse.
6. The device is placed into a 2% HF bath for approximately 10 sec.
7. The device is rinsed with DI water for 1 min followed by drying using a N2 stream.
8. The device is then rinsed with acetone for 1 min.
9. Following the acetone rinse it is rinsed with methanol for 30sec followed by drying using a N2 stream.
10. The device is stored in a N2 enclosure.
11. Protein is deposited using Nano eNabler SPT tips (Au surface is touched with tips – humidity kept at 15-25%)
12. PDMS is placed on O2 plasma for 2 min at 10W at 200mTorr and placed in contact with glass surface to form bond.

Reagent Delivery Protocol

Whitman Blocking Buffer: 20µl/min for 12min
Primary Antibody: 250µl at 1:1000
Secondary Antibody – Cy5 goat antihuman IgG: 1µM
Whatman Washing Buffer: 10x
Nano eNabler Molecular Printing System from BioForce Nanosciences.

Results and Discussions

The proteins used three ‘specific proteins’ (H3L, D8L, D13L) belonging to the Vaccinia family and two ‘non-specific proteins’ (2237, 3319) belonging to the Burkholderia Pseudomallei family. Vaccinia Immunoglobulin, VIG, was used to coat the anti-H3L antibodies for the experiments in 1:1000 dilution with blocking buffer [2]. Fluorescently tagged Cy5 conjugated goat antihuman IgG + IgG + IgM secondary antibodies in 1:200 dilution with blocking buffer were used to confirm antigen/antibody binding within the microchannel using fluorescent detection [2]. The fluorescent detection was achieved using a Perkin-Elmer microarray scanner at 5um resolution.

Conclusion

A novel process to immobilize biomolecules on a patterned Au surface on glass while activating and maintaining the glass surface for an irreversible PDMS bond has been developed. Using the Nano eNabler molecular printing system, specific protein patterns have been demonstrated on Au surfaces with precise control and repeatability. This novel process has applications that extends to advanced proteomics, immunoassays, early biowarfare detection, and DNA and cell manipulation.

Works Cited


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