BIOMOLECULE IMMOBILIZATION ON AU SURFACES WITHIN A SEALED PDMS MICROSYSTEM

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Abstract

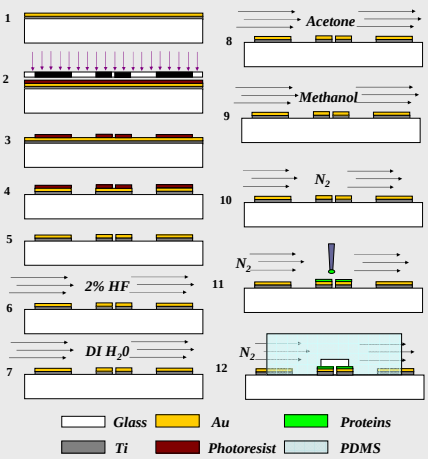
A novel chemical surface treatment has been developed to immobilize proteins on Au while activating the surface for an irreversible PDMS bond. This novel process has applications that extends to advanced proteomics, immunoassays, early biowarfare detection, and DNA and cell manipulation. The surface 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 O_2 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 O_2 plasma they would be destroyed once O_2 plasma is applied to the surface. However, protein immobilization after O_2 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 O_2 plasma and causes protein filled solutions to spread to the surrounding area making the glass substrate unbondable 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

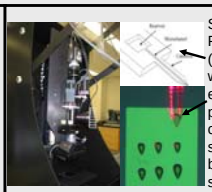


1) 100nm of Au is deposited on an adhesion layer of 20nm of Ti.
2) 10μm of Shipley 1827 photoresist is coated on the device and is UV exposed to form Au pads.
3) Resist is developed using Microposit MF 319.
4) The Au and Ti are etched using the chemicals Transene Gold Etchant GE-8148 and Transene Ti Etchant TFT.
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 1min followed by drying using a N_2 stream.
8) The device is then rinsed with acetone for 1min.
9) Following the acetone rinse it is rinsed with methanol for 30sec followed by drying using a N_2 stream.
10) The device is stored in a N_2 enclosure.
11) Protein is deposited using Nano eNabler SPT tip (Au surface is touched with tip – humidity kept at 15-25%)
12) PDMS is placed in O_2 plasma for 2min at 10W at 200mTorr and placed in contact with glass surface to form bond.

Reagent Delivery Protocol

Whatman Blocking Buffer	10μl/min for 12min
Primary Antibody - VIG 1:1000	2μl/min for 20min
Whatman Washing Buffer-10x	20μl/min for 10min
Secondary Antibody – Cy3-goat antihuman IgA+IgG+IgM	2μl/min for 20min
Whatman Washing Buffer-10x	20μl/min for 10min
DI	Rinse

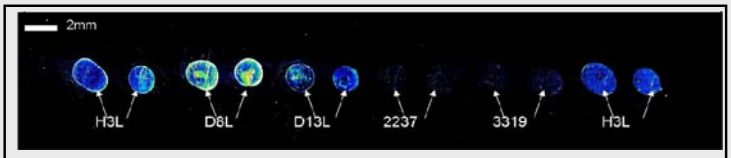
Reagent delivery protocol used to determine antigen-antibody binding on Au surface. All reagents were delivered through microfluidic channel with PicoPlus Syringe Pump.



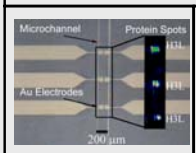
Surface Printing Tips (SPTs) used with the Nano eNabler are placed in contact with surface leaving behind protein spots.

Nano eNabler Molecular Printing System from BioForce Nanosciences.

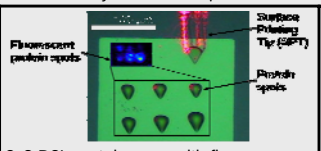
Results and Discussions



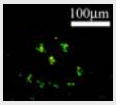
The proteins used three 'specific proteins' (H3L, D8L, D13L) belonging to the Vaccinia family and two 'non-specific proteins' (2237, 3319) belonging to the Burkholderia Pseudomallei family. Vaccinia Immunoglobulin, VIG, was used as the anti-H3L antibodies for the experiments in 1:1000 dilution with blocking buffer [2]. Fluorescently tagged Cy3-conjugated goat antihuman IgA + 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 5μm resolution.



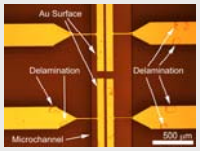
H3L proteins spotted on 100μm x 100μm electrodes within sealed PDMS microchannel. Fluorescence signifies active proteins after immobilization and PDMS bonding. All reagents were delivered via microchannel using syringe pump. Scan was taken with Perkin-Elmer microarray scanner at 5μm resolution.



2x3 D8L protein array with fluorescence verification of binding on Au surface. Surface printing tip is shown next to protein spots of approximately 20μm in diameter.



Protein array in the shape of a 'smiley face' on Au surface sealed within a PDMS microchannel. H3L proteins are spotted using the Nano eNabler with spots sizes approximately 20μm in diameter. All reagents were delivered via microfluidic channel.



Bonding characteristics of the PDMS to glass substrate was determined by applying nitrogen to the system at increasing pressures until delamination occurred between the glass and PDMS. The PDMS/glass bond withstood approximately 40psi which is comparable to that of literature [1].

Protein immobilization is through non-specific adsorption with a strong hydrophobic-hydrophobic bond however it is known that this will not be suitable for all proteins. SAMs have been used on the Au surfaces with the use of micro-stamping to enhance protein binding. Glass will remain active for a PDMS bond if micro-stamping is done in a N_2 chamber. This allows for a covalently bonded protein layer on Au while allowing a glass/PDMS bond.

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

1. McDonald J C *et al, Fabrication of Microfluidic Systems in Poly(dimethylsiloxane).* 2000 *Electrophoresis* 21 27–4.
2. D. Huw Davies, Megan M. McCausland, Conrad Valdez, Devan Huynh, et al., *Vaccinia Virus H3L Envelope Protein Is a Major Target of Neutralizing Antibodies in Humans and Elicits Protection against Lethal Challenge in Mice.* Journal of Virology, Sept. 2005. Pg. 11724-11733.

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