A Simple Process for Selective Bio-functionalization of SU-8 surface for Lab-on-a-Chip applications

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ABSTRACT

This paper presents a simple process of bio-functionalizing the surface of UV crosslinked SU-8 surfaces. SU-8 is a popular photo-patternable epoxy based polymer used extensively in Lab-on-a-Chip and MEMS applications. In this work we realized a SU-8 surface with Hydroxyl (–OH) groups by adding a copolymer Glycidol. Biotin Hydrazide is immobilized over this surface after APTES and Glutaraldehyde treatment. FTIR spectroscopy analyses were performed on the samples at various stages of bio-functionalization process.

Keywords: su-8, bio-functionalization, glycidol, ftir, biotin Hydrazide.

1 INTRODUCTION

SU-8 is a popular epoxy based negative photoresist developed by IBM. It has been widely used as structural material in Bio-MEMS and Lab-on-a-Chip (LoC) devices due to its bio-compatible nature, ease of fabrication and inertness to most chemicals (and bio-fluids).

Various procedures were proposed earlier for bio-functionalizing SU-8 surfaces for Biosensing applications. Often SU-8 surface has to be made hydrophilic for microfluidic transport and bio-functionalization purposes. Plasma treatment, the most frequently used technique is neither selective nor it can retain the hydrophilicity over an extended period of time. Selective functionalization and permanent hydrophilicity are most desired for LoC application and increased shelf life. If this could be achieved using a procedure which involves low temperature and less corrosive chemicals, it would enable fabrication of structures on Back End of Line (BEOL) layers of Integrated Circuit by CMOS post processing. Several processes have been demonstrated to achieve selective and stable hydrophilic SU-8 surface using low temperature methods [1] [2].

Photo patterned hydrophilic surfaces can be realized using a mix of SU-8 photoresist and Glycidol, a hydrophilic copolymer [1]. The resulting hydrophilicity is due to hydroxyl groups (-OH) present in Glycidol. The SU-8 and Glycidol mix (here onwards referred to as SU-8 + Glycidol) can be patterned using conventional SU-8 Photolithography process and hence involves lower temperatures only. It was proven that the hydrophilicity remains stable for a long duration when compared to plasma treatment process by following [1] and [2].

In this work, we extend the techniques proposed by [1] and [2] and bio-functionalize the SU-8 surface in order to develop a generic LoC platform using hydrophilic SU-8 based micro-channels. As a proof of concept, immobilization of Biotin Hydrazide molecules is demonstrated. For achieving this APTES molecules were covalently attached to the –OH groups on the SU-8+Glycidol surface. Then the Biotin Hydrazide molecules were immobilized over this layer by using Glutaraldehyde molecules as crosslinkers. The immobilized Biotin can be used to detect Avidin or Streptavidin molecules.

2 EXPERIMENTAL

2.1 Chemicals and Molecules

The process was carried out on a Silicon wafer. The following were the chemicals used in this process. Microchem SU-8 2002, SU-8 developer (PGMEA), Sigma Aldrich Glycidol 96% (G5809), Biotin Hydrazide (B7639), Phospate Buffer Saline (PBS) tablets (P4417), Technic France high purity Dimethyl Sulfoxide (DMSO), Alfa APTES((3-Aminopropyl)triethoxysilane) 98% (Alfa A10668), Glutaraldehyde 25% aq. Soln (A17876), Toluene (high purity without moisture), Tween 20 and Isopropyl Alchohol (IPA).

2.2 Procedure

Initially a 4 inch Piranha cleaned Silicon wafer was diced into small pieces of about 2 cm^2 for using as substrate. These substrates were baked at 200°C on hot plate for 1 hour for completely removing moisture.

Step 1: Each sample was spin coated with SU-8 2002 and crosslinked using UV lithography. The procedure is as follows:

Spin coating rate: Step1- 500 rpm, 10 sec with acceleration of 300 rpm/sec and Step2- 3000 rpm, 30 sec with acceleration of 300 rpm/sec.
Soft bake: 65°C for 3 minutes and 95°C for 6 minutes.
Exposure: Flood exposure with UV (365nm) for 80 sec (80mJ/ cm^2) using Suss MicroTec MA6/BA6 aligner system.
Post Exposure Bake (PEB): 65°C for 3 minutes and 95°C for 6 minutes. The samples were cooled down to room temperature gradually in a time of about 1 hour to avoid internal stresses in the film.

Then the SU-8 coated samples were rinsed in IPA for dissolving uncrosslinked residues and dried using purified compressed air (CDA). This first layer of SU-8 was used as a base for SU-8+Glycidol layer which would be functionalized.

Step 2: Next a polymer mix of SU-8 2002 and Glycidol was made with a ratio of 1:0.3 (by wt. %) using micro-balance. This mixture was thoroughly probe sonicated in an ice bath for 30 minutes to avoid lumps and ensure uniformity. This mix was used to form patterned layer of SU-8+Glycidol over the pure SU-8 layer. The following steps were followed in fabricating this layer.
Spin coating rate: Step1- 1000 rpm, 40 sec with acceleration of 300 rpm/sec. This parameters helps in getting uniform film of SU-8+Glycidol polymer.
Soft bake: 65°C for 3 minutes and 75°C for 6 minutes. The flash point of Glycidol is 81°C and hence the highest temperature used was 75°C.
Exposure: Exposed using mask with square patterns (1cm^2) with UV (365nm) for 200 sec using Suss MicroTec MA6/BA6 aligner system. This dosage was chosen as the film thickness is larger than pure SU-8 2002.
Post Exposure Bake (PEB): 65°C for 3 minutes and gradually increased to 95°C in 15 minutes and holded at the same temperature for 6 minutes. The samples were cooled down to room temperature gradually in a time of about 1 hour to avoid internal stresses in the film. In PEB, 95°C was used as it is necessary for the SU-8 monomers to crosslink. However, gradual temperature raise is important, so that the SU-8 and Glycidol's epoxy structures open up and corsslinks to form a stable polymer matrix below the flash point of Glycidol.

Later the samples were developed using SU-8 developer (PGMEA), vigorously washed in IPA and dried using CDA. Contact angle measurement was done using goniometer and a lower angle (~ 65°) was observed on this layer than a pure SU-8 2002 (~78°) layer due to the presence of Hydroxyl groups on the sufrace [1].

Step 3: APTES solution was prepared using Toluene as solvent with a concentration of 2.0% (v/v). Toluene was chosen as it supports in forming covalent bonds between APTES and –OH groups on the SU-8+Glycidol surface [7]. The samples coated with SU-8+Glycidol layer and one sample with only pure SU-8 layer were incubated in this solution for 2 hours at room temperature. Then the samples were thoroughly washed in pure Toluene to detach the unbonded APTES and dried with CDA.

Step 4: Next the samples were smeared with Glutaraldehyde solution and left for 2 hours at room temperature. Later the samples were washed using copious amout of DI water and dried using CDA.

Step 5: Further step is to immobililze Biotin Hydrazide over the surface. Biotin Hydrazide powder is dissolved in DMSO(1mL)+PBS(9mL) buffer (pH ~ 7.4) solution with a concentration of 0.5mg/mL. The samples were incubated in this Biotin Hydrazide solution for 12 hours at room temperature. Then the samples were washed thoroughly in PBS+Tween 20 solution to remove excess and non-specifically adsorbed Biotin Hydrazide molecules. The procedure for preparing the PBS+Tween 20 solution is as follows, 1 PBS tablet was dissolved in 200mL DI water to obtain a solution of pH 7.4 which is used for Biotin Hydrazide solution. Tween 20 (0.5% v/v) was mixed in this PBS solution to use for washing purpose.

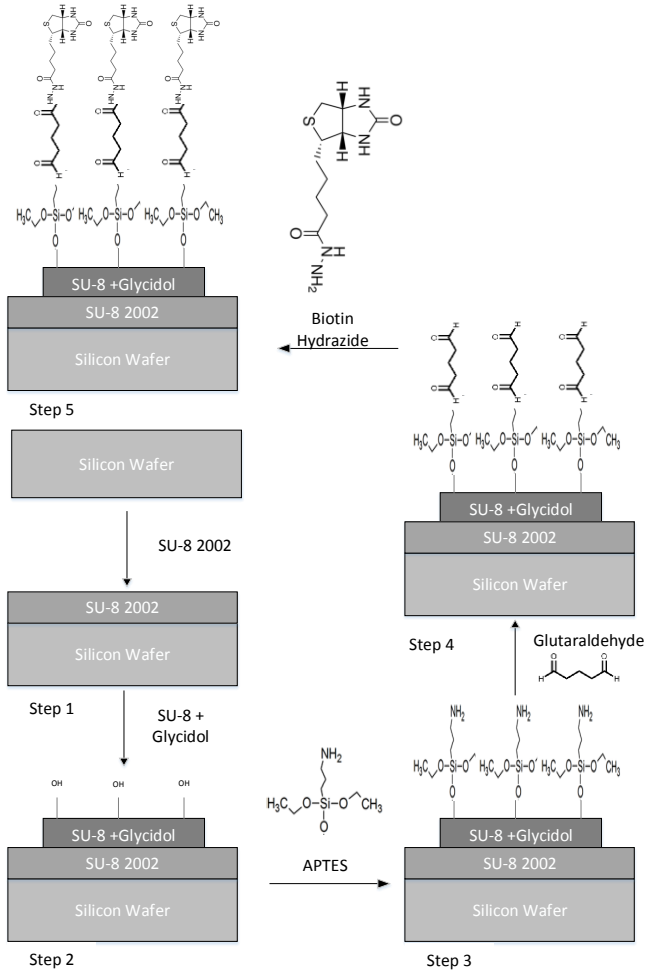

Figure 1: Schematic illustration of the SU-8 Bio- functionalization process

3 RESULTS AND DISCUSSION

A qualitative Fourier Transform Infra Red (FTIR) spectroscopy analysis to study the process was carried out using Bruker TENSOR37 system. FTIR Transmittance spectra characteristics were studied on SU-8+Glycidol film, Glutaraldehyde treated surface and Biotin Hydrazide immobilized surface.

A typical SU-8 film's FTIR transmittance spectra has epoxy-group and solvent signatures between 700 to 1800 cm^{-1} [8]. The Figure 2 is the spectra of the SU-8+Glycidol film which shows the presence of Hydroxyl groups by decreased transmittance around 3500 cm^{-1}. These Hydroxyl groups are part of Glycidol and acts as binding sites for the APTES molecules in the crosslinked layer.

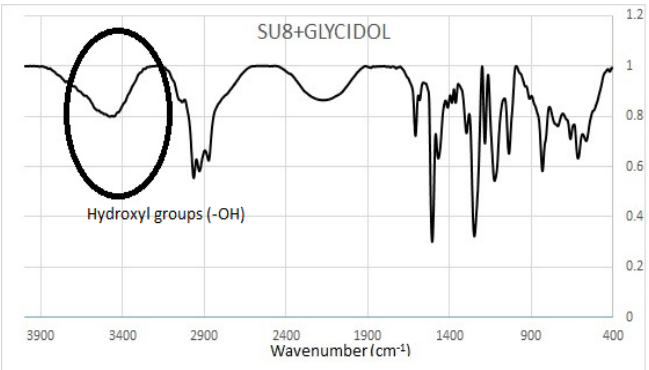

Figure 2: FTIR Transmittance spectra of SU-8+Glycidol film

Figure 3 indicates the presence of Aldehyde groups (2830–2695 cm^{-1}) after treating the SU-8+Glycidol surface with APTES and Glutaraldehyde solutions (GA curve). One of the Aldehyde groups in the Glutaraldehyde reacts with the Amine group (-NH_2) in APTES to form a bond. The other Aldehyde group of Glutaraldehyde is available for immobilizing molecules with -NH_2 at the surface.

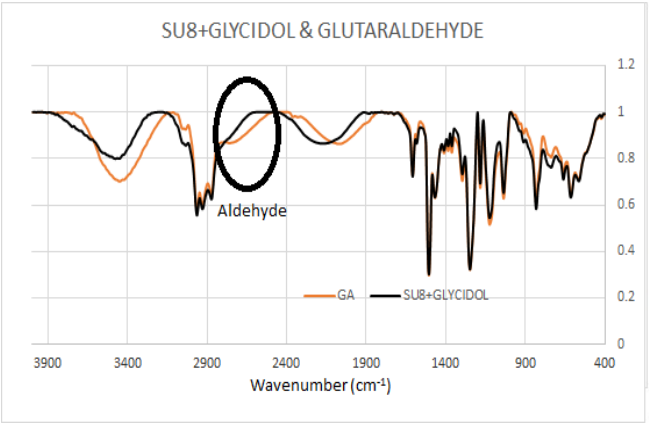

Figure 3: FTIR Transmittance spectrums of SU-8+Glycidol film and APTES+Glycidol (GA) treated films

The trasmittance at Aldehyde region of the spectrum has increased after immobilizing Biotin Hydrazide molecules. This can be observed in Figure 4.

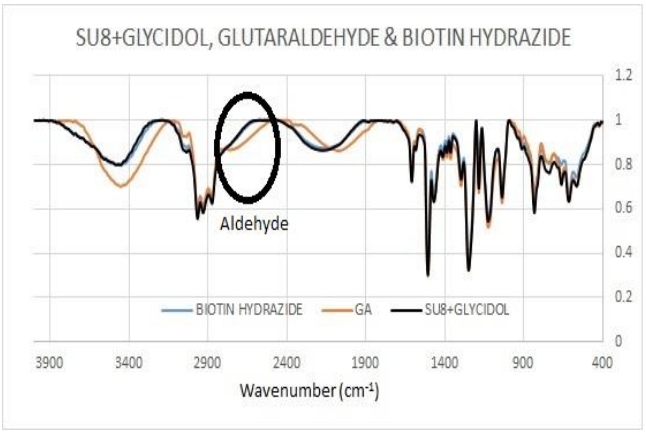

Figure 4: FTIR Transmittance spectrums of SU-8+Glycidol, GA and Biotin Hydrazide immobilized films

Figure 5 is the magnification of Aldehyde region of the FTIR spectrum. It can be observed that the Aldehyde region transmittance has increased to the level of normal SU-8+Glycidol film after Biotin Hydrazide immobilization.

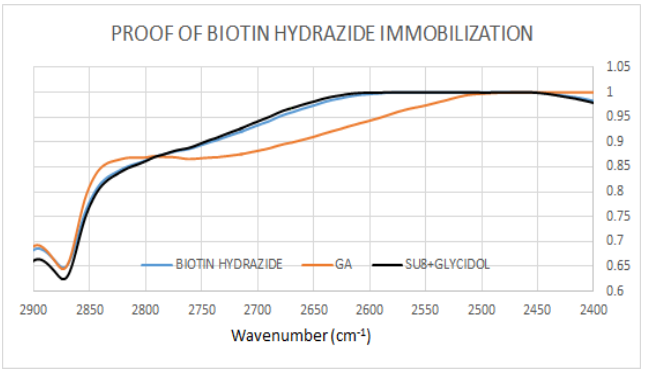

Figure 5: Magnified view of Aldehyde region of FTIR spectrum of SU-8+Glycidol, GA and Biotin Hydrazide

This is due to the well known fact that Aldehyde fuctional group in the Glutaraldehyde linker reacts with the Amine in the Hydrazide part of Biotin Hydrazide (in pH 6.5-7.5) to form Hydrazone bond [10]. This results in the reduction of Aldehyde signature in the FTIR spectrum. Hence we could achieve a stable Biotin functionalized SU-8 surface could be used to further immobilize other bio-molecules or sense Avidin and Streptavidin molecules.

It was also observed that the Aldehyde region transmittance has reduced only in SU-8+Glycidol regions of the sample and not in the background layer made up of pure SU-8. This is due to the lack of binding spots (-OH groups) for APTES molecules over the pure SU-8 surface. Hence selectivity in bio-functionlization has been achieved.

4 CONCLUSION

Selective bio-functionalization of SU-8 surface was performed successfully using a simple, low temperature chemical process. We believe that this procedure has tremendous potential in fabrication of Lab-on-a-Chip and Biosensor devices. Further research on using this process in fabricating Bio-MEMS structures and immobilizing various other molecules are on going in our group.

REFERENCES

[1] Chun-Lung Wu, Ming-Hung Chen, and Fan-Gang Tseng, "SU-8 Hydrophilic Modification by Forming Copolymer with Hydrophilic Epoxy Molecule," Proceedings of μTAS 2003, pp. 1117-1120, October 5 – 9, 2003.

[2] Maria Nordstrom, Rodolphe Marie, Montserrat Calleja and Anja Boisen, "Rendering SU-8 hydrophilic to facilitate use in micro channel fabrication," J. Micromech. Microeng. 14 (2004) 1614–1617.

[3] Vincent T. Moy, Ernst-Ludwig Florin and Hermann E. Gaub, "Intermolecular Forces and Energies Between Ligands and Receptors," Science, New Series, Vol. 266, No. 5183 (Oct. 14, 1994), pp. 257-259

[4] Manoj Joshi, Richard Pinto, V. Ramgopal Rao, Soumyo Mukherji, "Silanization and antibody immobilization on SU-8," Applied Surface Science 253 (2007) 127–3132.

[5] Z. Wang, M. Stangegaard, M. Dufva, J.P. Kutter, and A. Wolff, "A simple hydrophilic treatment of SU-8 Surfaces for cell culturing and cell patterning," Proceedings of μTAS 2005, pp. 1117-1120, October 5 – 9, 2003.

[6] Peng Xue, Jingnan Bao, Yon Jin Chuah, Nishanth V. Menon, Yilei Zhang, and Yuejun Kang, "Protein Covalently Conjugated SU−8 Surface for the Enhancement of Mesenchymal Stem Cell Adhesion and Proliferation," dx.doi.org/10.1021/la500048z | Langmuir 2014, 30, 3110−3117

[7] Joonyeong Kim," Investigation of the Formation and Structure of APTES Films on Silicon Substrates," www.piketech.com

[8] Stephan Keller, Gabriela Blagoi, Michael Lillemose, Daniel Haefliger and Anja Boisen, "Processing of thin SU-8 films," J. Micromech. Microeng. 18 (2008) 125020 (10pp)

[9] V.S. Goudar, S. Suran, M.M. Varma, "Photoresist functionalisation method for high-density protein microarrays using Photolithography," Micro & Nano Letters, 2012, Vol. 7, Iss. 6, pp. 549–553

[10] Greg T. Hermanson,"Bioconjugate Techniques, 2nd Edition," Academic Press, pp. 506-545