A novel dry method for surface modification of SU-8 for immobilization of biomolecules in Bio-MEMS

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Abstract

SU-8 has been primarily used for structural elements and microfludics components in MEMS. Microsystems for biological applications require immobilization of biomolecules on the MEMS structures. In order to functionalize SU-8 for such purposes, the surface needs to be modified. In this paper, we report a novel dry method of surface modification of SU-8 which is compatible with standard microfabrication techniques. The surface obtained by spin coating SU-8 (2002) on silicon wafer was modified by grafting amine groups using pyrolytic dissociation of ammonia in a hotwire CVD setup. To demonstrate the presence of amine groups on modified SU-8 surface, the surface characteristic after modification was assessed using Fourier transform infrared spectroscopy. The change in SU-8 surface morphology before and after surface modification was investigated using atomic force microscopy. To show the utility of this process for application in Bio-MEMS, SU-8 microcantilevers were fabricated and subjected to the same surface modification protocol. Following this, the cantilevers were incubated first in a suspension of human immunoglobulin (HIgG) and then in FITC tagged goat anti-human IgG in order to demonstrate the utility of the surface modification performed. The efficacy of the process was assessed by observing the cantilevers under a fluorescence microscope.

Keywords: Bio-MEMS; Hot wire CVD; Microcantilevers; SU-8; Surface modification

1. Introduction

Microfabricated materials such as silicon dioxide, silicon nitride, gold, etc., are commonly used in the fabrication of microsystems for biological applications. Such microsystems require immobilization of biomolecules on the sensor element or the surface of the microsystem. Immobilization of biomolecules using organosilane functionalization of silicon dioxide and silicon nitride surface is well studied (Lin et al., 1998; Tlili et al., 2005). Also, immobilization of biomolecules on gold surface using thiol derivatization has been demonstrated (Nakata et al., 1996; Jin et al., 1999; Prats-Alfonso et al., 2006). Over the past decade various polymers have emerged as possible materials for MEMS structures. As a result, the need to immobilize biomolecules on these polymers has assumed importance. Immobilization of biomolecules on polymer surfaces requires modification of the surface. For covalent immobilization of biomolecules, a polymer surface needs to be modified so as to have at least one functional group, such as CHO, NH_2, SH, etc., which binds to biological molecules. Grafting of amine group (NH_2) on polymer-based sensor surface can be achieved using wet chemical surface modification (Park et al., 2002). However, wet chemical methods use strong oxidizing/hydrolyzing agents (acids/bases), which may damage the surfaces adjacent to the sensor while modifying the area of interest. Most of these processes also require multiple steps and as a result, are time consuming. Material handling in wet phase (repetitive immersion, washing and drying) can also cause structural damage to microsensors especially micro-cantilevers or other suspended structures. In wet surface modification techniques, process parameters like pH, concentration, temperature, etc., need to be carefully controlled, which add to the complexity of operations.

Immobilization of biomolecules can also be achieved by suitably modifying polymer surfaces using dry surface modification techniques. Hydroxyl or amine groups may be grafted on polymer surfaces using oxygen or ammonia plasma treatment, respectively (Meyer-Plath et al., 2003). However, plasma treatment may damage microstructure surfaces. Suspended polymer

microstructures such as SU-8 cantilevers suffer from the damages caused by plasma pressure (Kaixi and Ping, 1997). Amine groups can also be grafted on polymer surfaces by exposure to UV-light (typical wavelength 200–400 nm) in NH_3 atmosphere (Amos et al., 1995). In this method the time required for polymer surface modification is large (10–12 h) and the UV light may modify the bulk properties of the structure (Svorcik et al., 2004).

SU-8, an epoxy-based photosensitive polymer, is used as a structural material for MEMS structures due to its attractive mechanical properties like low Young's modulus and chemical properties like inertness to various chemicals used in microfabrication. As a result, immobilization of substances, especially bio-molecules, on SU-8 surfaces is of interest because of their application in Bio-MEMS, assays using immobilization of biomolecules, biosensors, membrane bioreactors, etc. The field of possible applications is equally vast, viz. clinical diagnostics, molecular biology, agricultural and environmental science, etc. However, bare SU-8 patterned with conventional photolithography techniques does not allow covalent immobilization of biomolecules on its surface. The surface, therefore, needs to be functionalized in order to immobilize biomolecules on it. Antibody immobilization on polymerized SU-8 surface can be achieved via grafting of amine groups on SU-8 surface (Joshi et al., 2006). It has also been demonstrated that DNA may be immobilized on SU-8 surfaces (Marie et al., 2006). Both of these methods uses wet processes of surface modification followed by immobilization of biomolecules.

In this paper, we report a novel dry method of surface modification of SU-8 which makes it amenable for immobilization of biomolecules on it. SU-8 surface was grafted with amine groups using pyrolytic dissociation of ammonia in a hot wire chemical vapor deposition (HWCVD) setup. This was followed by the immobilization of biomolecules on the modified surface. The nature and efficacy of the surface modification process was studied using Fourier transform infrared spectroscopy (FTIR) and atomic force microscopy (AFM). The compatibility of the surface modification process with standard microfabrication techniques is demonstrated by applying it on SU-8 microcantilevers followed by immobilization of antibodies on the cantilevers.

2. Materials and methods

The objective of this study was to develop and demonstrate a process for functionalization of SU-8 surfaces, which will affect the surface of this polymer only, to the exclusion of silicon (or its derivatives) and gold surfaces. SU-8 (2002) was obtained from MicroChem, USA, human immunoglobulin (HIgG) and FITC tagged goat anti-human immunoglobulin (Ga-HIgG) from Bangalore Genei, India. All other chemicals, e.g. glutaraldehyde, etc., were obtained from SD Fine Chem Ltd., India.

2.1. Fabrication of SU-8 cantilevers

In order to demonstrate the applicability of this process in the development of bio-MEMS, it was decided that the process will be demonstrated on SU-8 cantilevers as well. The microfabrication process steps to create SU-8 cantilevers are shown in Fig. 1. The dimensions of cantilever fabricated for this purpose were; length = 200 μm, width = 40 μm and thickness = 1.8 μm.

Silicon wafers were oxidized at 1100 °C to get a sacrificial oxide layer of 1 μm thickness (step I), which was measured using spectroscopic ellipsometry. Even though the final release of the cantilevers was done using bulk etching of silicon, a sacrificial silicon dioxide layer promotes adhesion of SU-8 film to the substrate thereby eliminating problems of peel-off during the bulk etching process. Structural layer of the cantilever (1.8 μm thick) was obtained by spin coating SU-8 (2002) at

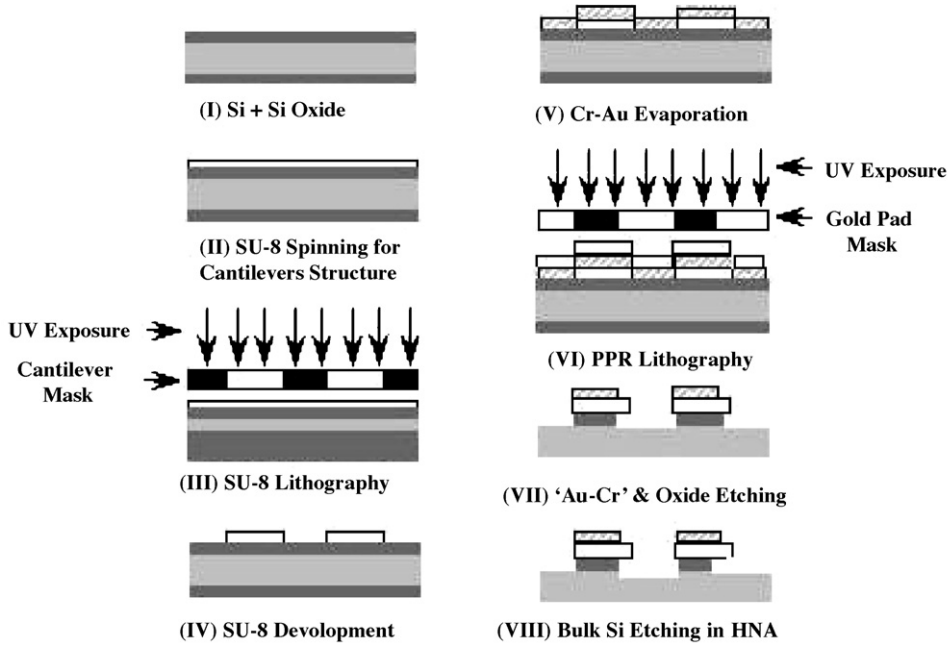

Fig. 1. Microfabrication steps for SU-8 cantilevers.

3000 RPM for 60 s followed by prebake at 70 °C for 5 min and 95 °C for 10 min (step II). Cantilever structures with pads (1.5 mm × 3.5 mm) were patterned using standard photolithography technique with UV exposure of 6 s followed by post-bake at 95 °C for 5 min (step III). The unexposed SU-8 was developed and removed (step IV). It was also decided to pattern gold on the pads of SU-8 cantilevers, considering that the pads might be there in a SU-8/polysilicon piezoresistive cantilever. This would subsequently help to prove the selectivity of the surface modification and biomolecule immobilization process towards SU-8 over silicon and gold. In order to obtain the gold pads, Cr–Au layer of ∼10 nm was sputtered on the SU-8 patterned substrate (step V). Gold pads were patterned near fixed end of cantilevers using standard PPR photolithography (step VI). Unwanted gold was etched in gold etchant (KI (5 g) + I_2 (10 g) + H_2O (200 ml)) for 15 s followed by striping of PPR using acetone. Following this, the chrome was etched in chrome etchant (NaOH (10 g) + $K_3Fe(CN)_6$ (10 g) + H_2O (200 ml)) for 30 s at 70 °C. The sacrificial oxide layer was etched in buffered oxide etch (BHF) (5:1) for 20 min (step VII). The silicon underneath the SU-8 cantilever was etched using bulk isotropic etchant. HNA (HF-3 ml + HNO_3-64 ml + CH_3COOH-30 ml) was used for bulk silicon etching (step VIII). The etch rate of silicon in HNA solution at room temperature was ∼2 μm/min. The free cantilevers were finally rinsed using *iso*-propanol alcohol (IPA) and allowed to dry.

Silicon surfaces completely covered with SU-8 were also prepared for FTIR and AFM studies. The process parameters for creating the SU-8 film were same as mentioned earlier. Such obtained SU-8 surfaces and cantilevers were subjected to surface modification in the HWCVD chamber.

2.2. SU-8 surface modification

Hot wire chemical vapor deposition (HWCVD) is a well known technique used for deposition of thin amorphous, polycrystalline and epitaxial films. The prototype of HWCVD setup is demonstrated in (Matsumura and Tachibana, 1985; Patil et al., 2003). It involves thermal decomposition of source gases at the surface of resistively heated filament (usually tungsten and tantalum) within the chamber at pressure near 10^{-6} mBar. In the SU-8 surface modification process demonstrated in this paper, pyrolytic dissociation of ammonia gas near the filament generates amine groups and reactive hydrogen. The energy associated with reactive hydrogen species during pyrolytic dissociation of ammonia was used to cleave the C–O (99 kcal/mol) bonds in the epoxy group of SU-8 on the surface followed by the formation of C–NH_2 bond on its surface. Fig. 2 shows the chemical structure at the SU-8 surface before and the probable structure after surface modification.

The SU-8 surfaces spin coated on silicon wafer and the SU-8 microcantilevers before dicing the silicon wafer were loaded on the substrate within the HWCVD chamber. For the present study of surface modification, the distance between the filament and the substrate was kept at 5.5 cm and the substrate was held at room temperature. The chamber was evacuated up to 10^{-6} mBar. Ammonia gas was introduced into the chamber at a flow rate

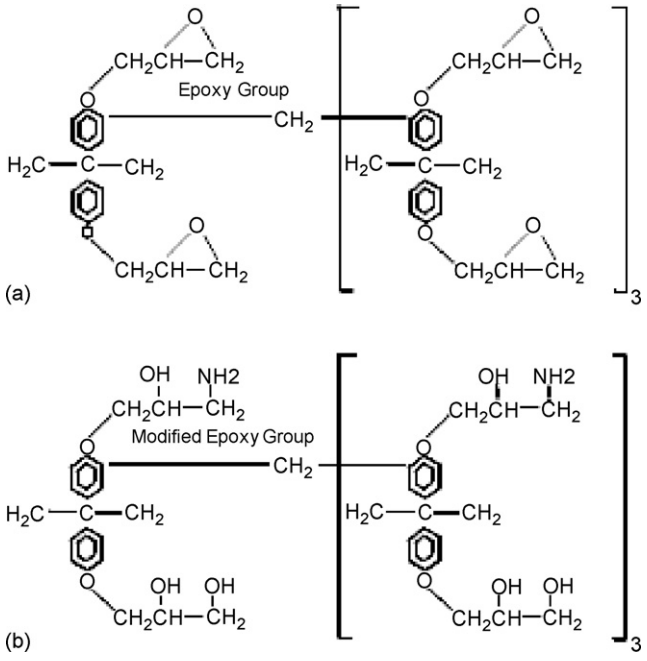

Fig. 2. Chemical structure of SU-8 monomer on the surface (a) before and (b) after pyrolytic dissociation of ammonia on its surface.

of 20 sccm. The gas pressure within the chamber during the pyrolytic dissociation of ammonia was 500 mBar. Ammonia gas is known to dissociate above 1100 °C at atmospheric pressure (Zheng and Reddy, 2003). However, at low chamber pressure and filament temperatures (∼1100 °C) the density of amine groups grafted on SU-8 surface was negligible (as was evident in FTIR studies discussed later) and at higher temperatures suspended structures (e.g. microcantilevers) get damaged due to rise in temperature of the SU-8 surface. Therefore, a filament temperature of 1500 °C and surface treatment time of 10 min was used for all but the preliminary studies. The modified SU-8 surfaces and cantilevers were subjected to antibody immobilization.

2.3. Antibody immobilization

Homo-bifunctional linkers may act as spacers between the target surface of immobilization and the biomolecules and may prevent denaturing of biomolecules causing increase in shelf life of biosensors. Hence, the modified SU-8 surface and SU-8 cantilevers were treated with 1% aqueous solution of glutaraldehyde (25%, w/w), for 30 min. Following this, the surface was incubated with HIgG (0.1 mg/mL in PBS constituted with 15 mM phosphate buffer, 138 mM NaCl, 2.7 mM KCl added in 1 l DI water giving pH approximately 7.4 at 25 °C) for 1 h at room temperature. In order to remove loosely adsorbed biomolecules, the surfaces were washed with a detergent solution consisting of 0.1% (w/v) aqueous solution of Tween-20. The unsaturated aldehyde sites and non-specific adsorption sites on the antibody immobilized surface were blocked using bovine serum albumin (BSA) by dipping the samples for 1 h at room temperature in 2 mg/ml solution of BSA in PBS (pH 7.4 at 25 °C). Such antibody immobilized

SU-8 surfaces and cantilevers were washed with PBS solution after each step of immobilization and stored at 4 °C.

In order to identify and qualitatively assess the density and uniformity of the grafted layer of HIgG, FITC tagged goat anti-HIgG (0.1 ml/ml in PBS) was allowed to react with the HIgG immobilized surface.

3. Results and discussion

The SU-8 surface at various stages of surface modification and antibody immobilization was studied using different characterization tools. The presence of chemical bonds on the SU-8 surface was investigated using Fourier transform infrared spectroscopy (FTIR). Tapping mode AFM was used to study the SU-8 surface morphology. Fluorescence microscopy was used to investigate the selectivity of immobilization and qualitatively assess the grafted layer of biomolecules on SU-8 cantilever surface.

3.1. Fourier transform infrared spectroscopy (FTIR)

The SU-8 surface before and after modification was studied using FTIR in order to investigate the nature of chemical bonds present on the surface. A Nicolet Magna-IR spectrometer-550 in the grazing angle mode was used for this study. Polarized infrared light was used to scan the SU-8 surface. In these experiments, maximum instrument sensitivity was achieved at an angle of incidence of 86°. The wave number associated with the R–NH_2 group is in the range of 1560 cm^{-1} to 1640 cm^{-1} (Nakanishi, 1962). The R–NH_2 peak is absent in the grazing angle FTIR of unmodified SU-8 surface (Fig. 3a). However, grazing angle FTIR of modified SU-8 surface (Fig. 3b) clearly shows the presence of peak of R–NH_2 group at 1607 cm^{-1}. This may be taken as evidence that amine groups have been grafted on the surface.

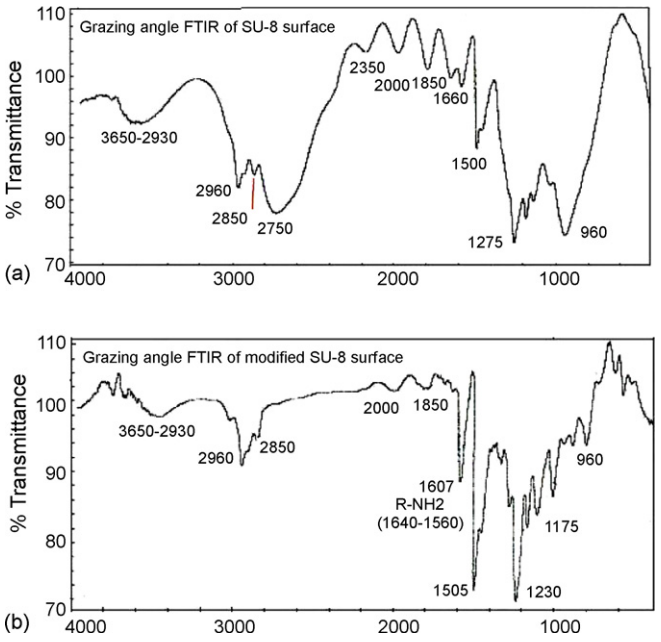

Fig. 3. Grazing angle FTIR of SU-8 surface (a) before and (b) after surface modification showing additional R–NH_2 group at 1607 cm^{-1}. The other peaks are—960–1275 cm^{-1}: phenyl in plane bending; 1500–1600 cm^{-1}: phenyl nucleus; 1660–2000 cm^{-1}: aromatic overtone of dCH; 2350 cm^{-1}: CO_2; 2750 cm^{-1}: chelation intermolecular bond with C═O; 2850 cm^{-1}: –CH_2–; 2960 cm^{-1}: –CH_3; 2930–3650 cm^{-1}: water.

3.2. Atomic force microscopy (AFM)

Digital instruments nanoscope III AFM system with high aspect ratio silicon cantilevers were used for these studies. In order to obtain high resolution images of antibody immobilized SU-8 surface using contact imaging mode in AFM, molecules need to be firmly attached to the solid support so that they resist the contact force exerted by the scanning tip (Wei et al., 2000; Tatte et al., 2001).

However, there is a possibility of loosely adsorbed proteins being present on the surface, which may contaminate the scanning tip, in spite of all precautions. This may give rise to an increase of interaction between the tip and surface proteins, thereby affecting adversely the resolution of the AFM images (You and Lowe, 1996). Hence, tapping mode AFM was used to investigate the SU-8 surface at various stages of experimentation. In order to avoid the cross-contamination of AFM tip, a new AFM cantilever was used to obtain each one of the images shown in Fig. 4. Before scanning the surface in tapping mode, the AFM cantilever was tuned to its resonant frequency and its phase was corrected to zero. Since the excitation voltage to the AMF cantilever decides its oscillation amplitude, all the images were taken at an amplitude set point of 1 V and picture quality was maximized using proportional and integral gains.

The RMS roughness of unmodified SU-8 surface was 2.3 nm (Fig. 4a). Surface roughness of SU-8 increases after the process of surface modification. RMS roughness of the modified SU-8 surface was found to be closely dependent on the surface treatment time during the pyrolytic dissociation of ammonia gas and filament temperature within the HWCVD chamber. At a filament temperature of 1500 °C and surface treatment time of 10 min, the RMS roughness of modified SU-8 surface increased up to 5.23 nm (Fig. 4b). However, it was also found that the surface roughness reduces to 1.88 nm with the steps involved in the antibody immobilization (Fig. 4c). The flattening noticed in Fig. 4b to c takes place due to antibody immobilization and the BSA treatment that precedes the reaction of HIgG and FITC tagged Goat-anti-HIgG. Stearic interaction between these large flexible molecules may lead to filling up of the valleys left after CVD treatment.

3.3. Fluorescence microscopy

The antibody immobilization on SU-8 surface before and after surface modification was investigated using fluorescence microscopy. SU-8 surface with and without ammonia treatment was incubated in HIgG. In order to assess whether an antibody layer has been grafted onto the surface or not, FITC tagged goat anti-HIgG was incubated on these surfaces followed by observation under fluorescence microscope. We used a Zeiss

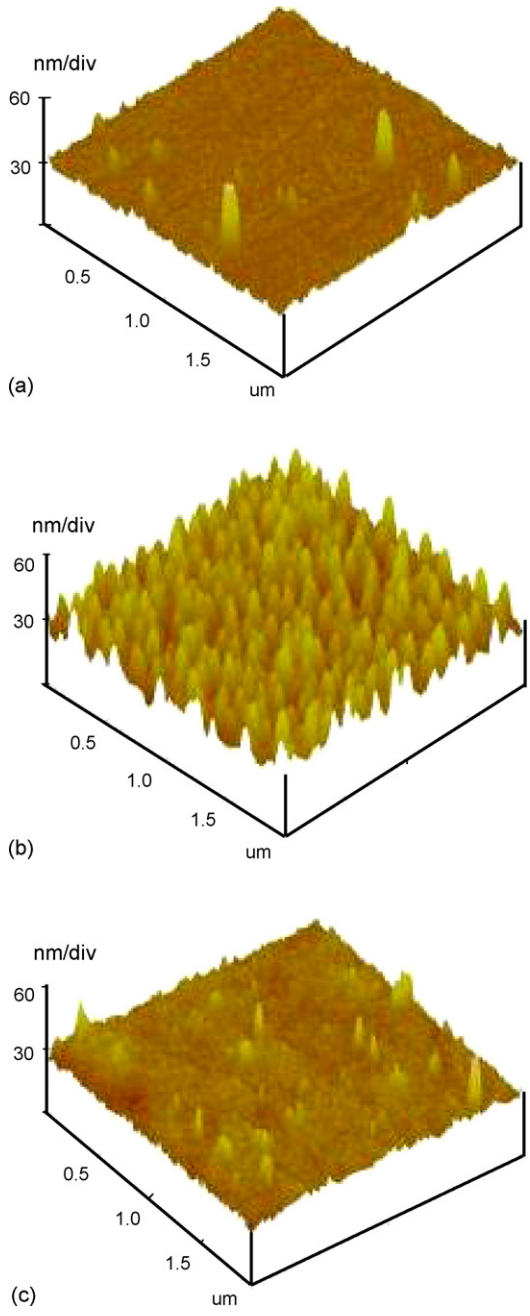

Fig. 4. AFM pictures of SU-8 surface (a) before surface modification, (b) after surface modification and (c) after antibody immobilization.

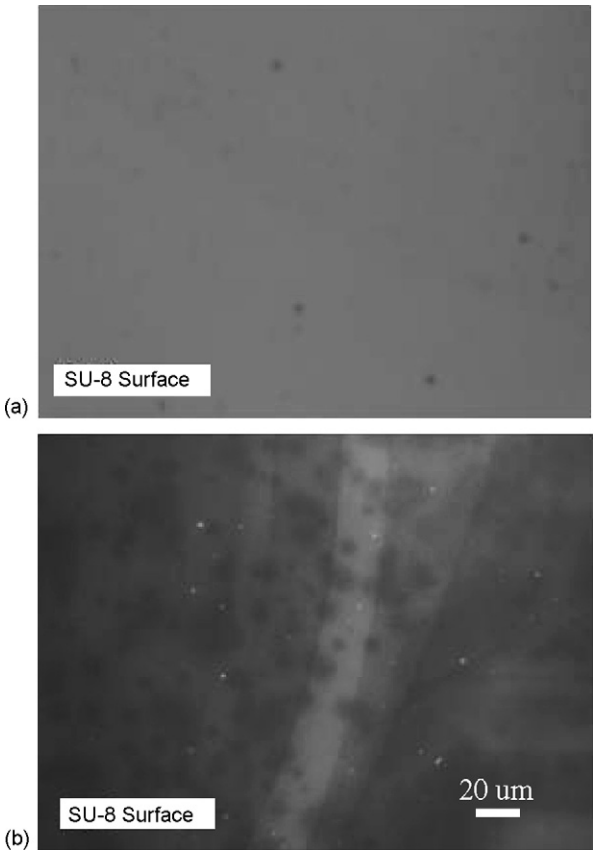

Fig. 5. Micrograph of unmodified SU-8 surface treated with HIgG followed by FITC tagged goat anti-HIgG observed under (a) optical microscope and (b) fluorescent microscope.

Axioskope-2 MAT microscope with fluorescence attachments for excitation wavelength in the range of 450–490 nm and emission detection around 520 nm.

The images obtained using normal optical microscopy was used for preliminary identification of surface features. Following this, fluorescence micrographs of the sample surfaces at the same spot were obtained. As observed from micrographs shown in Fig. 5b, weak and random fluorescence is detectable on parts of the surface, although the complete sample surface was incubated with HIgG and the drop of FITC tagged goat anti-HIgG was administered. This may be due to the random and scattered adsorption of HIgG or FITC tagged goat anti-HIgG on the SU-8 surface. At this level of immobilization it may be difficult to use this surface for sensing purposes.

However, the surface of SU-8 cantilevers having gold pads treated with pyrolytic dissociation of ammonia in HWCVD chamber and on being subjected to the same antibody immobilization protocol shows much brighter and diffused fluorescence (Fig. 6a and b). This demonstrates that the SU-8 surface treated with hotwire induced pyrolytic dissociation of ammonia has made it amenable to the immobilization of biomolecules. It may also be noticed that, there is negligible aggregation of biomolecules on the plane surface area of SU-8 cantilever as compared to the edges of the cantilever. This aggregation may be explained by the limitation on resolution of custom-made photolithography masks. Due to this limitation, the SU-8 cantilevers had rounded edges (instead of sharp edges) giving rise to a comparatively larger number of binding sites for the biomolecules on the edges only. This may be overcome by using better quality lithography masks. The minor aggregation on the planar areas on the surface of the cantilevers may be minimized in the various steps of immobilization by increasing concentration and/or time of detergents washing and/or adding more rinsing steps. Forced rinsing, as done in immobilization assays on solid surfaces, is however not an option in case of sensitive structures like microcantilevers since these may break during the rinsing process.

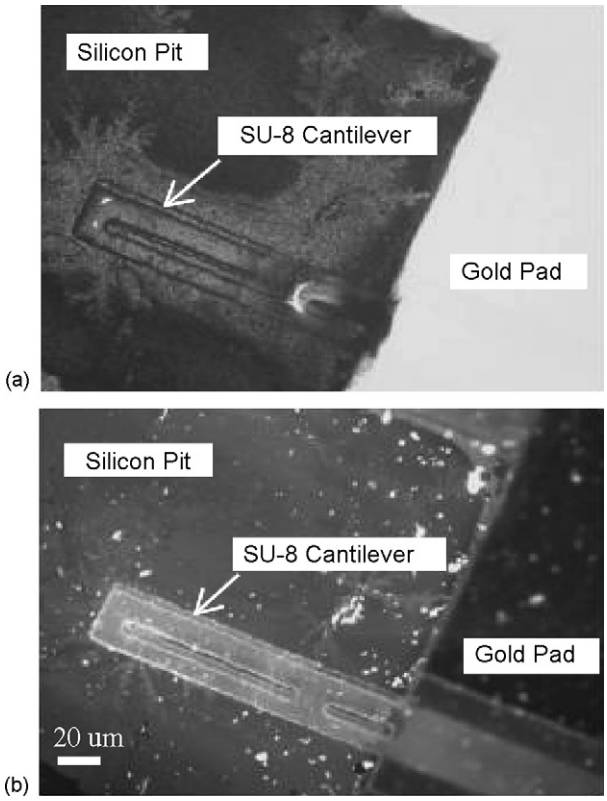

Fig. 6. Micrograph of SU-8 cantilevers with gold pads treated with pyrolytic dissociation of ammonia using hot wire CVD setup followed by incubation of HIgG and FITC tagged goat anti-HIgG and observed under (a) optical microscope and (b) fluorescent microscope.

Some of the established surface modification processes may be selective towards SU-8 over gold, but frequently the treatment also makes silicon and silicon dioxide surfaces amenable towards immobilization as well (David et al., 2003). This may raise confounding issues in creating sensors and micro-reactors. Our process of dry surface modification demonstrated in this paper; selectively modifies only the SU-8 polymer surface as opposed to silicon and gold. Fig. 6b shows the selective immobilization of biomolecules only on SU-8 covered areas to the exclusion of areas covered by gold and silicon. In the microfabrication process, release of the cantilever is achieved by bulk etching of silicon underneath, which gives rise to a rough silicon surface in the pit. Such rough surfaces may exhibit a certain degree of adsorption as seen in Fig. 6b. This weak fluorescence in the silicon pit, also, could not be totally eliminated in these experiments due to the limitation of rinsing force. However, selectivity (in immobilization) studies made on chequer-board patterns (2 mm × 2 mm) of gold, SU-8 and silicon, where forced rinsing could be performed, demonstrated virtually no biomolecular attachments to the gold and SiO_2.

As shown in Fig. 6, it was found that, there is no damage to the surface of the SU-8 cantilever structure. This demonstrates that, the process described in this paper is compatible with microfabrication technology and useful in Bio-MEMS applications. As the temperature of the cantilever surface during the modification and subsequent processing is maintained near room temperature, thermal damages to the surface of the cantilever and adjoining surfaces are unlikely. Further, since the surface modification takes place within few minutes (5–30 min), a high throughput can be achieved during the sensor microfabrication process.

4. Conclusion

The process described in this article grafts NH_2 (amine) groups on the surface of SU-8 by using hotwire induced pyrolytic dissociation of ammonia. Since there is neither significant increase of substrate temperature during this surface modification process, nor is it subjected to harsh chemicals, this process is ideally suited for the use on SU-8 microstructures commonly found in MEMS applications. It may be noted that since the process relies on modification of the epoxy groups on the surface, all polymers containing such groups may be treated in the same fashion, to obtain similar results. Further, many different kinds of biomolecules (antigen, antibodies, proteins, DNA, RNA, etc.), which have either amine or aldehyde groups on their surfaces may be immobilized without or with the homo bifunctional linker such as glutaraldehyde.

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