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Silanization and antibody immobilization on SU-8

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Abstract

SU-8, an epoxy based negative photoresist, has emerged as a structural material for microfabricated sensors due to its attractive mechanical properties like low Young's modulus and chemical properties like inertness to various chemicals used in microfabrication. It can be used to fabricate MEMS structures of high aspect ratio. However, the use of SU-8 in BioMEMS application has been limited by the fact that immobilization of biomolecules on SU-8 surfaces has not been reported. In this study, the epoxy groups on the SU-8 surface were hydrolyzed in the presence of sulphochromic solution. Following this, the surface was treated with [3-(2-aminoethyl) aminopropyl]-trimethoxysilane (AEAPS). The silanized SU-8 surface was used to incubate human immunoglobulin (HIgG). The immobilization of HIgG was proved by allowing FITC tagged goat anti-human IgG to react with HIgG. This process of antibody immobilization was used to immobilize HIgG on microfabricated SU-8 cantilevers.

Keywords: SU-8; AEAPS; Silanization; HIgG; Antibody immobilization

1. Introduction

Miniaturized biosensors are fabricated using microfabrication techniques. Materials used for fabrication of such sensors are silicon, silicon dioxide, silicon nitride, gold, etc. Such materials are achieved using standard microfabrication techniques, such as oxidation, chemical vapor deposition, physical vapor deposition, etc. Patterning these materials requires processes like lithography and etching, which further add to complexity, cost and production time of sensor fabrication.

SU-8 (glycidyl ether of bisphenol A) polymer is a negative photoresist and has emerged as a structural material for biosensors. There are different methods for immobilization of biomolecules on to a polymer surface, e.g. entrapment, encapsulation, adsorption, covalent binding, etc. Covalent immobilization is often necessary for binding molecules that do not adsorb, adsorb very weakly or adsorb with improper orientation and conformation to polymer surfaces [1–3]. This may result in better biomolecule activity, reduced non-specific adsorption and greater stability.

Covalent immobilization can be achieved on the polymer surface by modifying it to have at least one functional group, such as CHO, NH_2, SH, etc. which can be used to bind biologically active molecules.

One of the preferred methods of creating amino groups on the surface of substrates is by treatment with aminosilanes. In this paper, we describe a process to immobilize antibodies on SU-8 surfaces using silanization. The C–O bonds (~99 kcal/mol) in the epoxy group on SU-8 surface are cleaved using sulphochromic solution, resulting grafting of hydroxyl groups on it. Such a modified SU-8 surface is treated with aminosilane followed by antibody immobilization on it.

2. Materials and methods

SU-8 2000 was obtained from MicroChem USA, [3-(2-aminoethyl) aminopropyl]-trimethoxysilane (AEAPS) was obtained from Sigma–Aldrich USA and HIgG/FITC tagged goat anti-human IgG from Bangalore Genei, India. All other chemicals were obtained from SD FineChem India Ltd.

2.1. Sample preparation

SU-8 was patterned on silicon wafer using standard photolithography techniques. The mask used for photolithography

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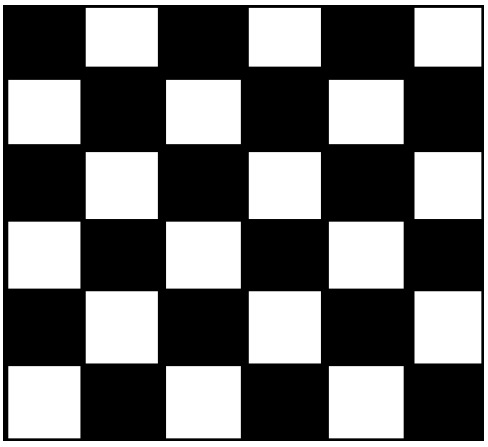


Fig. 1. Prototype of mask used for photolithography. Each window in the mask is of (2 mm × 2 mm) size.

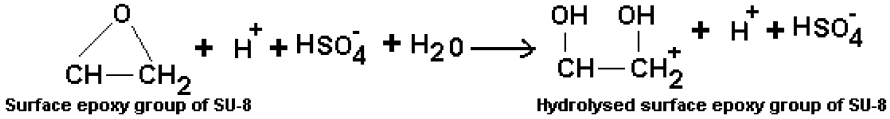
had a chequer-board pattern with alternate windows for silicon and SU-8 under study (Fig. 1). This would subsequently help to prove the selectivity of the immobilization process towards SU-8 over silicon. The parameters used to obtain the SU-8 surface were: prebake temperature 70 °C (5 min), UV exposure of 6 s, post-bake temperature 95 °C (5 min). Silicon surfaces completely covered with SU-8 were also prepared for FTIR and AFM studies. The process parameters for creating the SU-8 film was the same as mentioned earlier. The surface modification and antibody immobilization processes after creation of the SU-8 film were identical for both types (patterned and solid) of samples.

2.2. Silanization and antibody immobilization

Native oxide from the silicon squares on the patterned samples was removed by dipping the surfaces in 2% HF for 30 s. All samples were subjected to sulphochromic solution treatment for 10 min followed by DI water rinse. The chemical bond structure of SU-8, before and after sulphochromic solution treatment is as shown in Fig. 2. In sulphochromic solution, $K_2Cr_2O_7$ is used as a catalyst and H_2SO_4 in the ionic state is given by,

$$H_2SO_4 \Leftrightarrow H^+ + HSO_4^- \quad (1)$$

The chemical reaction associated with the hydrolysis of surface epoxy group of SU-8 is given by Eq. (2)



(2)

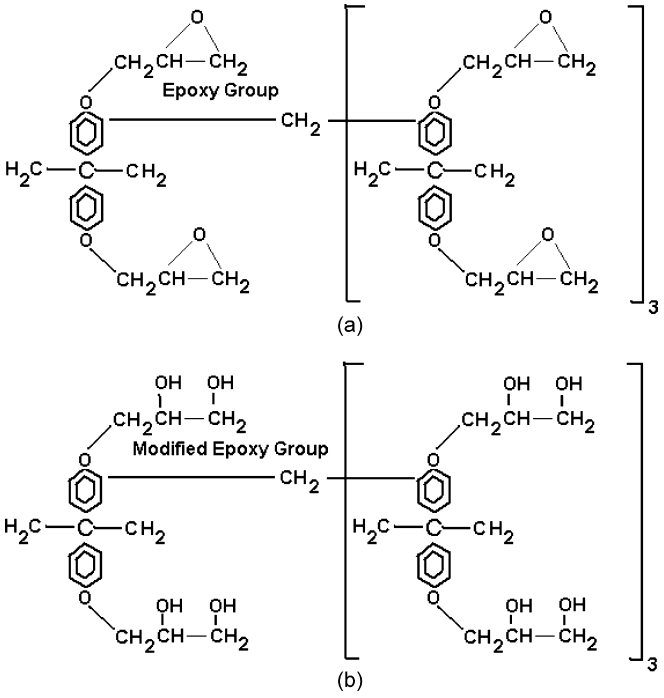


Fig. 2. Chemical bond structure of SU-8 surface: (a) before sulphochromic solution treatment and (b) after sulphochromic solution treatment.

Surface adsorbed water was removed by heating the samples at 110 °C for 2 h under vacuum. Two percent AEAPS solution in ethanol was prepared in argon ambient [4,5]. To maintain orientation of NH_2 group of AEAPS away from the surface, the pH of the silane solution was optimized to 3.7 by adding acetic acid. The samples were kept in the silane solution for 7 min. The excess amount of silane on the SU-8 surface was removed by rinsing in ethanol. This was followed by condensation at 110 °C in argon ambient for 10 min. The silanized samples were dipped in 1% aqueous solution of glutaraldehyde (homo-bifunctional cross linker) for 30 min. They were then ready for antibody immobilization.

The samples were incubated in HIgG (0.5 ml/ml in phosphate buffer saline) suspension for 1 h. Loosely adsorbed antibodies were removed by rinsing the samples in PBS solution three times. The unsaturated aldehyde sites and non-specific adsorption sites on the antibody immobilized surfaces were blocked by dipping the samples in 2 mg/ml solution of BSA in PBS at room temperature for 1 h, followed by rinsing in PBS for three times [6]. To identify the grafted antibody layer, FITC tagged goat anti-human HIgG (0.5 ml/ml in PBS) was incubated at room