Fabrication of Microfluidic Devices using SU-8 for Detection and Analysis of Viruses

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Abstract
A passive microfluidic device using SU-8 photoresist has been developed for detection and analysis of viruses. Due to its high aspect ratio with almost vertical sidewalls and its chemical stability, SU-8 offers a wide variety of applications in the fabrication of microfluidic systems. This photoresist is transparent allowing optical detection and thus, identification of biomolecules. Additionally, SU-8 shows a good biocompatibility offering the possibility to develop assay on the surface that allows the specific binding of biomolecules for analytical applications. In this work, the performance of the microfluidic device has been successfully demonstrated by detection of specific contaminants in test solutions. The microfluidic chip is composed of SU-8 microstructures which have been fabricated by means of photolithography, using glass and silicon as substrate materials. Beside the fabrication of microchannels by means of SU-8, we have also used SU-8 as adhesive layer.

Introduction
Microfluidic chips are nowadays a concept widely used in different fields like biology, pharmacy, medicine and other biotechnologies. Microfluidic or lab-on-a-chip technology promises significant advantages for carrying out rapid, efficient and specific analysis of microorganisms, which could be found in chemical substances. The cost and reagent consumption required for analysis will be reduced by using microfluidic devices due to their small size that is very useful for µTAS (Micro Total Analysis Systems) [1]. Furthermore, analysis time is reduced diffusion paths are shorter and reactions occur with higher efficiency. Depending on the design and complexity of the microstructures, such fluidic systems can provide all necessary functions as well as to identify or quantify specific pathogens. These functions include cell handling, sample mixing [2], sorting [3], transport and measurement of biomolecules [4].

IMSAS develops multichannel microfluidic systems (see figure 1) by using the negative photoresist SU-8. With this photoresist, microstructures up to 2mm of thickness and aspect ratios up to 20 can be constructed [5]. Besides, the SU-8 is a favourable material for fabrication of microchannels because of its excellent chemical stability against several acids and bases and its thermal stability. These properties make SU-8 a very attractive material for a wide range of applications like micro optics, micro machining, packaging and analytical microfluidic applications, as described in this paper.

Figure 1: Picture showing one of several microfluidic devices which have been developed and fabricated at IMSAS. It is a microchannel with integrated columns.

This paper presents a method for the fabrication of microchannels, which are composed completely of SU-8 for biological applications. The microstructures were fabricated by using a soft lithography process, which involves the negative resist and utilizes SU-8 itself as adhesive bonding, under low temperatures.
Borosilicate glass and silicon were selected as substrate material, because processing techniques of the silicon are well developed and SU-8 provides good adhesion to both materials.

**Work principle**

Immunoassay represents a powerful tool for bio-analytical systems that permit specific, rapid and sensible detection of the antibody-antigen interaction. Besides the excellent properties of the SU-8 to fabricate microfluidic systems, this photopolymer is compatible to bio-sample [6] allowing the development of immunoassay systems directly onto its surface. It supports the possibility to immobilize microorganisms on a microfluidic device, in which the microchannels are composed of SU-8. Several strategies have been studied and performed successfully, to modify a surface and use it to fix biomolecules directly on the channel walls of a microfluidic chip. Interesting results have been obtained by methods like physical adsorption, entrapment and covalent binding or silanization as described in [7]. Based on this concept, bioparticles (specific antibodies) could be fixed on the SU-8-based microstructures. The required reagents have been introduced by way of pressure driven flow into the channel. When a contaminated fluid has been introduced through same procedure, viruses that are flowing through the microfluidic system bind with the immobilized biomolecules onto the SU-8 surface. Figure 2 shows a schematic concept of the analytic technique. This surface provides an innovative tool for detecting the antibody-antigen reaction, without requiring complicated and sophisticated instruments and several hours for sample preparation before the users can visualise the results. Passive microfluidic devices have been fabricated by using SU-8 as the unique component of the microstructures, which offers the advantage of being able to active biologically all inner surfaces. Consequently, a significant number of biomolecules-antibodies can be immobilized on all sides of the microchannels.

**Fabrication process**

We used two masks to produce the microfluidic devices. The first mask defines inlet and outlet and the second mask defines the geometry of the microstructures that involves the width and length of the microchannels. Silicon, glass wafers and the epoxy polymer SU-8 2 and SU-8 3050 acquired from Microchem Corporation have been used for this purpose. According to the second mask, microchannels with integrated columns of about 50µm in diameter have been fabricated as shown in figure 3.

![Microchannel schematic](image)

**Figure 2**: Schematic representation of the developed Microchannel work principle.

![Microfluidic device](image)

**Figure 3**: Conceptualized graphic of a microfluidic device by using SU-8 as the unique component of the channels. This is a small channel with columns to increase surface area for binding viruses.
Fabrication procedures for bottom wafer (silicon) and top wafer (glass or pyrex) are explained step by step in the figure 4 and 6, respectively.

The process sketched in figure 4 describes the fabrication steps of the SU-8 microstructures, which were implemented on the glass wafer. Adhesion of SU-8 layer to the wafers is an important requirement for our fabrication process. Therefore, to improve it, we have prepared the glass wafer immediately to prior SU-8 processing. For this propose, we have used Caro’s acid cleaning to remove organic materials from glass substrate. Then, it was heated at 200°C for at least 45 minutes to an oven to remove water from its surface. Afterwards, a thin film by using SU-8 2 photoresist was applied and coated for 30 sec at 2000 rpm on the glass wafer, resulting in a film thickness of about 3µm. This procedure was followed by a prebake for one minute at 65°C and another three minutes at 95°C on a hotplate to evaporate solvent contained in the SU-8. The wafer was unstructured exposed (flood-exposure) for 30 sec and a post expose bake has been performed at 65°C for 1min and 95°C for 1min also.

We have experimented that a thin film of SU-8 is recommended, before a thick SU-8 layer is deposited. It enhances sufficient adhesion between the SU-8 layer channels and the substrate. Additionally, our fabrication process offers microfluidic devices where bottom, ceiling and walls of the microchannels are made out of SU-8 photoresist completely, ensuring a higher surface area to immobilize antibodies. Consequently, a large amount of viruses could be collected and then analyzed through the microfluidic device. Because of this, the deposition of a thin film prior to a think layer of SU-8 in this approach is required.

The height of the structures depends on the dispensed volume, viscosity and SU-8 coating process. SU-8 polymer allows the fabrication of structures with thickness that can be varied from 1µm until 2mm. Microchem Corporation provides information about parameters such as viscosity, spin speed, recommended times and temperature for prebake and postbake steps and exposed time. Figure 5 displays film thickness vs. spin speed curve for the SU-8 3000 photoresist provided by Microchem© [8].

For first experiments, SU-8 3050 has been coated to obtain a thickness of 100µm. According to the figure 5, we have spun the wafer at 1000rpm. However, we measured the resulting layer with a thickness variation of about ±30µm. More exactly, the film at the centre was thinner than that at the wafer edge.

Previously published results [9] and our experimental results demonstrate that by thick layers of SU-8 (up 100µm), the thickness varies significantly. This non-uniformity of the layer leads to an inappropriate contact between silicon and the glass wafers in bonding process. This variation must be controlled in our fabrication process to avoid not seal microfluidic systems. For this reason, we have coated two layers of SU-8 3050 obtaining less thickness variation and good uniformity in the wafer edge region. We could even achieve higher microstructures than 100µm [10].
SU-8 3050 was spin coated on an exposed SU-8 2 glass wafer at 2300rmp for 30 sec, followed by soft-bake at 65°C and 95°C, for 5 and 15 minutes, respectively. When the substrate was cooling down, the second layer of SU-8 3050 could be coated at the same spin speed as the first film. After this, the baking process proceeded at 95°C for 2 hours within a slow ramping to reduce the internal stress of the thick SU-8 layer. The coated SU-8 films onto the glass wafer have been exposed to UV light for 120 sec, making the exposed areas less soluble. In the postbake step, the wafer was heated for 20 minutes at 95°C using a slow ramping. Development was performed by immersion in SU-8 developer during seven minutes.

With this multilayer technique described above, we could implement microstructures of approximately 130 µm height with a thickness variation of ±10 µm.

As mentioned earlier, the first mask is used to define the inlet and outlet of the microfluidic device, which were made on a silicon wafer through Deep Reactive Ion Etching (DRIE) process (figure 6). Aluminum acts here as a stop layer during etching. Circular holes in diameter 1.5mm are formed accurately and simultaneously in the silicon wafer.

![Figure 6: Schematic illustration of the fabrication process of the inlet and outlet, which were made on a silicon wafer.](image)

(a) Silicon wafer is used as carrier substrate
(b) Etch stop layer is deposited (Aluminum)
(c) Deep Reactive Ion Etching of inlet and outlet
(d) Removal of the etch layer stop
(e) Thermal oxidation
(f) Deposition of bonding layer (unexposed SU-8 2)

After removing etch layer, the silicon wafer is oxidized through thermal oxidation process to get a thin and adherent layer. Next, on the same silicon substrate, a bonding layer has been applied using the photoresist SU-8 2 of about 10µm of thickness. It was done by means of airbrush coating techniques. Subsequently, a prebake step has been applied at 65°C for two minutes.

This coated and unexposed silicon wafer and the glass wafer with SU-8 microstructures were ready for bonding process to integrate the microfluidic device as shown in figure 7. Bonding parameters like temperature and time have been optimized to avoid channel filling [11]. In our experiments, where we deposited a bond layer ~ 10µm of thickness, we have experimented with different temperatures and times.

![Figure 7: Wafer bonding process using unexposed SU-8](image)

We have found that the bonding temperature at 60°C during one hour showed good results. However, the temperature depends directly on the bond layer thickness. Finally, adhesive SU-8 2 layer was exposed through the glass wafer during 40sec and post exposure bake was done at 95°C within 20 minutes on a hotplate under a slow temperature ramping.
Results
The main problem usually appeared in the fabrication process was the non-uniformity of the SU-8 layers. Due to the thickness variation, some structures were not entirely in close contact during bonding process, resulting in voids of various sizes. Consequently microchannels were not sealed, which is crucial in microfluidic systems for practical applications. This problem has been also solved when SU-8 multilayer technology has been applied. We have used this multilayer technique, obtaining uniform SU-8 films and good adhesive properties. Fabricated microstructures could be observed through figure 8 that illustrates a cross section of a microchannel with integrated small columns. We could fabricate successfully microchannels with dimensions of around 130µm in height and 500µm in width, using four SU-8 levels, as given in this figure. Microfluidic devices were interfaced through a Teflon® holder which includes two 1/4-inch side ports. These ports were connected through male barbed adaptors, acquired from Omnifit®. After establishing connections, fluids could be pumped with standard fittings and driven by pressure through a peristaltic pump. During tests, air bubbles were generated into the microchannels because of the SU-8 hydrophobic surfaces [12]. Therefore, we recommend rinsing inner sides of the fluidic chip with propanol to facilitate easier wetting between fluids and SU-8 surfaces. Afterwards, we have tested microfluidic devices with DI water. It was easily pumped away from channels, showing excellent results. Additionally, experimental tests for detection and analysis of specific viruses in test solutions was satisfactory realized, pointing out the importance of having microchannels, in which all inner surfaces to be made on the biocompatible material.

Conclusion
In this paper, we designed and fabricated different microchannels based on photoresist SU-8 using a multilayer method. SU-8 could be used not only to define the microstructures for microfluidic systems, but for bonding layer as well. Based on this, we could fabricate microstructures by using SU-8 only, offering the advantage of being able to active biologically on all inner surfaces. The technology described in this work shows a high potential for microfluidic devices, which can be transferred to innumerable applications for monitoring of contaminants in liquid process streams.

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Literature