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FABRICATION OF AN INTEGRATED MICROFLUIDIC PERFUSION SYSTEM FOR MIXING DIFFERENT SOLUTIONS

ALIREZA BAHADORIMEHR, AZRUL AZLAN HAMZAH, BURHANUDDIN YEOP MAJLIS

Institute of Microengineering and Nanoelectronics (IMEN), Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia. Email:burhan@ukm.my

ABSTRACT

We present a simple and low cost fabrication of Microfluidic mixer using in different applications in Micro Total Analysis Systems especially for automated perfusion of multiple solutions to a single cell culture chamber. This device consists of 6 inlets and 2 outlets with additional 3 inlet/outlet channels for flow rate alteration and other mixing purposes such as electrophoresis activation, which is capable of producing mixtures of the various solutions. The volume of the microchamber is 600 pm^3. All the inlets are connected to Polytetrafluoroethylene (PTFE) and silicone tubings using special Polydimethylsiloxane (PDMS) fittings technique for fluid leakage avoidance. The inputs are controlled by a programmable micro-syringe pump in order to activate each input in any sequence of time. Typical microscopic glass slides are utilized as a substrate for fabrication of microchannels and chamber which has the minimum effect on most of biology samples. The same un-etched glass is used as cover glass for bonding purposes. Using photoresist as an etch mask instead of some deposition methods makes the procedure more convenient and cost effective compared to other expensive techniques such as Deep reactive-ion etching (DRIE), laser, etc. The baking time of photoresist is a critical factor for longer resistance against etchant solution which is optimized by this method. In addition the etchant concentration in wet etching process is discussed to achieve a decent surface and wall characteristics for bio-applications. A smooth channel surface with acceptable sharp wall edges makes this procedure suitable for many applications which vertical walls are not crucial. Different dye samples are tested inside the chamber and promising results attained. The mixing validation is performed using image processing technique.

Keywords: Micromixer, glass, Photoresist, Wet etching

INTRODUCTION

Microfluidic Lab-On-a-Chip (LOC) technology has emerged over the last decade to miniaturize and make some procedures faster and more convenient. It has been demonstrated that microfluidic systems have the capability to provide a reliable platform for biological assays such as DNA sequencing, polymerase chain reaction (PCR), electrophoresis, DNA separation and mixing different liquids together in micro/nano liters.

Microfluidic mixers play an important role in biological analysis systems. Mixing of different fluids strongly depends on turbulence and inter-diffusion of them. Magnetic stirring is one of the conventional methods to achieve a homogenized mixing on the order of liters. With the development of molecular biology, tube vibration systems become the tools for mixing volumes on the order of milliliters[1]. By emerging microfluidic devices mixing in micro and nano liters is applicable.

Generally, micromixers are divided to passive and active types. An active type uses external energy to improve mixing procedure and decrease time of mixing like ultrasonic activation, while a passive micromixer uses special geometries of channels for making fluids mix together[2]. In passive mixers, when two channels of fluid flow into a single channel, the fluid mixing inside the merged channel is insignificant because of the low diffusion rate, comparing to the flow rate. In addition the low Reynolds number in micro-fluidic devices usually produces laminar flow.

Lamination is a most known technique which is used for agitation. In lamination mixers, the fluid streams are splitted into several small streams, which are later joined in a mixing channel[3-4]. In this technique, mixing of the two solutions occurs by simple diffusion of molecular species from one side of the channel to the other. Complete mixing by simple diffusion along a microchannel with a width of 50 µm to 100 µm would be prohibitively slow requiring either low flow rates (on the order of mm/s or less) or unreasonably long microchannels (on the order of centimeters to meters depending on the molecule)[5].

In this paper we present a simple and inexpensive method to fabricate micro-mixer on microscopic glass slide in order to achieve mixing of fluids in a smooth and deep micro-chamber using in Micro Total Analysis Systems with nano-liter capacity. This method uses typical microscopic glass slides as a substrate for fabrication of micro-channels and chamber of micromixer. Using photo-resist as a mask makes the procedure more convenient and cost effective compared to other techniques. A smooth channel surface with acceptable wall edges can be achieved using this method. A UV glue bonding method is introduced for glass to glass bonding. This device consists of 6 inlets and 2 outlets with additional 3 inlet/outlet channels for flow rate alteration and other mixing purposes such as electrophoresis activation, which is capable of producing mixtures of the various solutions. The surface area of the microchamber is 2mm by 3mm and the depth of the studied microfluidic device is 100µm. Therefore, the volume of the microchamber is 600pm^3. With this configuration the liquids in the order of nano-liters can be mixed together easily. For validation of fluids mixing, image processing technique is used in MATLAB software. This device has the advantage of faster mixing time compared to other passive micromixers as a result of fractal structure.

EXPERIMENTAL

Commercially available microscopic glass slides (75mm×25mm×1mm) were utilized as substrate for etching micro-channels. The photo-masks were designed using Corel-Draw software. Photoresist (PR) AZ4620 was used to transfer the pattern on the glass after lithography.

The fabrication process was started by cleaning the glass substrates in acetone and methanol in 10 minutes separately and boiling in piranha solution for 15 minutes. Immersing in DI water and drying with nitrogen gas was the next step. Removing residual humidity on glass substrate can be achieved by putting them in an oven with 100ºC for 15 minutes. Using spin coater to coat the glass with Photoresist (PR) was the next step. After coating the pre-baking procedure for de-bubbling and removing humidity was applied to avoid sticking the PR to the transparent photo-mask under the UV exposure. Hard baking step was applied next. Etching process was performed in a magnetic stirring buffered oxide etch, BOE, (6:1) bath.

For bonding two microscopic glasses together, the same cleaning procedure was applied. The method involves UV curable glue that can

be used for glass microfluidic chips bonding at room temperature. The use of UV-curable glue was found to be a quick, easy, and inexpensive method for attachment of glass substrates together. The glue with low viscosity which ensures formation of a thin layer after spinning was selected. A thin layer of UV glue was applied on the plain slide by spinning it at 4000 rpm for 20s. The etched glass slide containing the microchannels was then brought in contact with the glue surface of the plain slide with applying a low pressure on it.

In order to complete the entire device the tubing connection using Poly-Dimethyl-Siloxane (PDMS) was applied.

Mixing 25 g of PDMS with 2.5 g (10%) hardening agent and pour it into the dish was the first step. Next the petri dish was placed on a hot plate at 100ºC and cure for 1h. For making holes through the PDMS, we used the typical needle and then cut the squares around each hole using a blade. The silicone glass sealant was used in order to adhere each square piece PDMS on glass substrate.

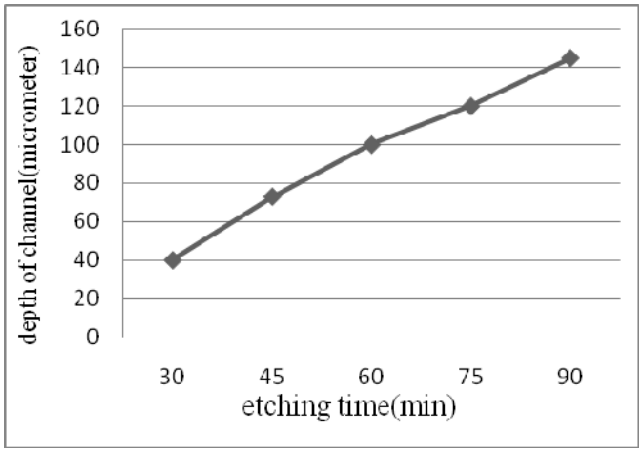

Fig. 1: Depth of channel vs. etching time.

RESULTS AND DISCUSSION

As illustrated in Fig. 1, the glass can be etched up to 150 µm after 90 min of wet etching. The etching rate is highly sensitive to concentration of diluted BOE. It shows that the photoresist resistivity time against etchant attacks is around 90 min which is enough for appropriate etching. The linear slope of this graph is the proof of constant etch rate during entire process. This approximately constant slope is achieved by addition of hydrochloric acid (HCL) in etchant concentrations in the magnetic stirring bath.

The adherence of photoresist to glass substrate is an important factor in order to eliminate under etching defect. After etching, the SEM photo (Fig. 2) shows that the sharp edges of photoresist still have remained unchanged and the cross section view in edges illustrates extremely high adhesion after removal of photoresist (Fig. 3).

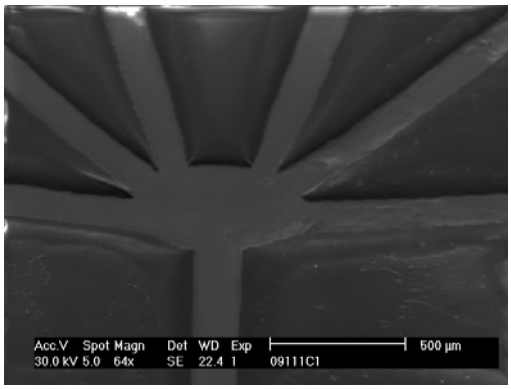

Fig. 2: Sharp edges of photoresist after etching.

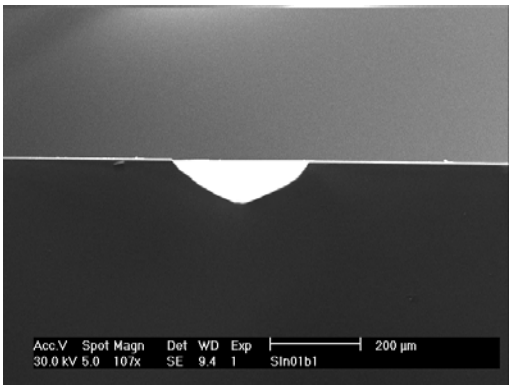

Fig. 3: SEM cross section view after photoresist removal.

Fig. 4 shows the overall view of the micromixer design. It shows the inputs and outputs channels have filled with dye water and the tubings are connected to the inlets and outlets without any leakage inside the channels and also via the connections. Therefore, it is clear that the UV glue has not clogged the micro-channels.

In order to justify the mixing of liquids inside the microchamber, typical dye water is used. The dyes are injected in to the channels via silicone tubings and inlet connections using programmable syringe pump and after mixing inside the chamber can be collected from the outlets. For validation of mixing fluids versus time, photos were captured in different intervals. The results of analysis of mixing colors using image processing technique in MATLAB software is shown in Fig. 5a and Fig. 5b . The results illustrates that after 7 min of holding, the fluids inside the chamber are homogenous in terms of color distribution. As the depth of the chamber is much less than the surface area of the chamber, the results can expand with very acceptable approximation to whole the liquid inside microchamber.

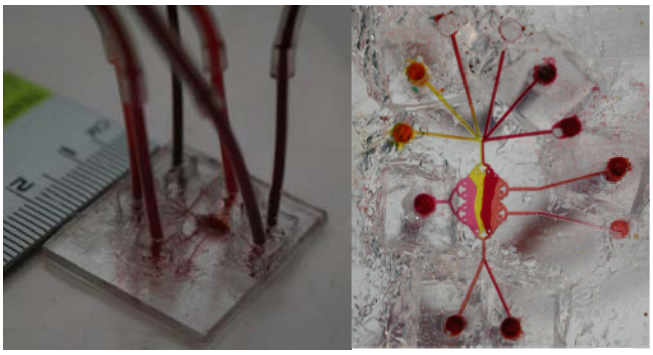

Fig. 4: Photograph of micromixer with tubings.

a

b

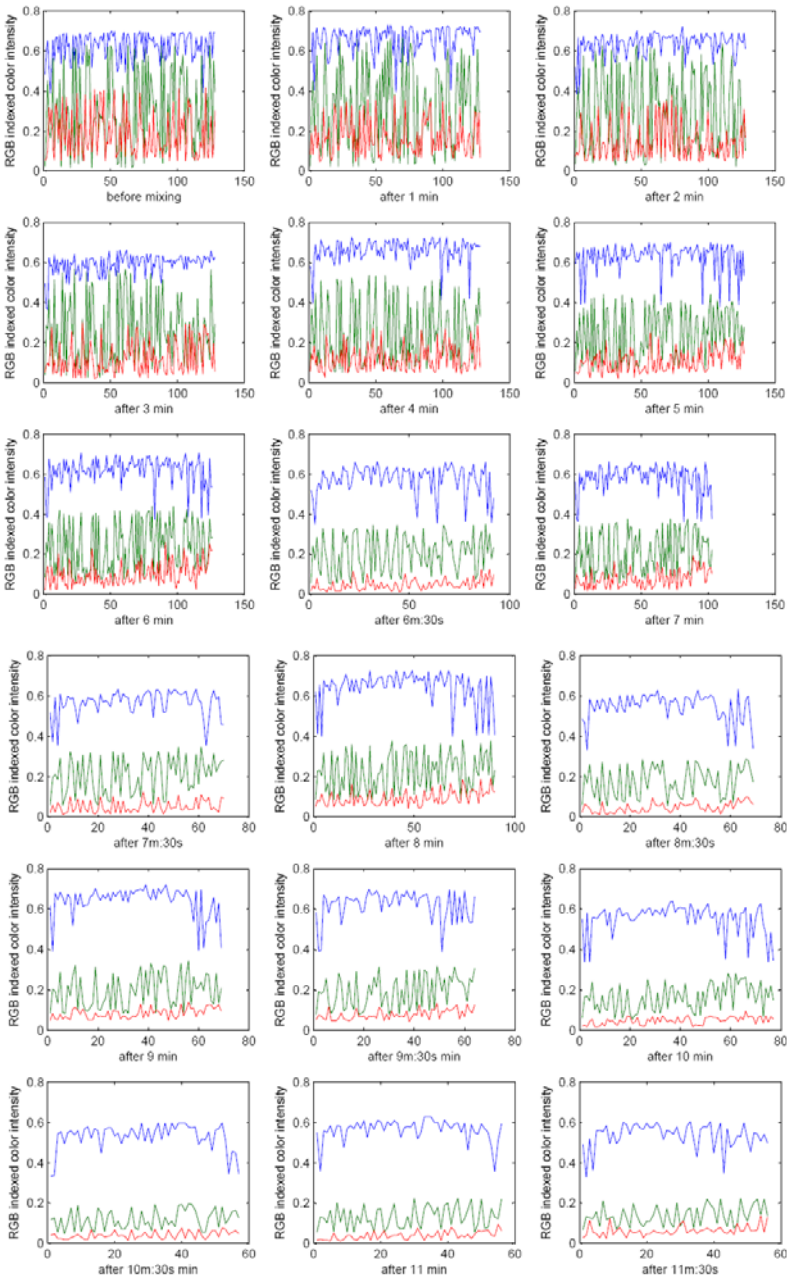

Fig. 5: Image processing results (a) from 0 to 7 min and (b) from 7min:30sec to 11min:30sec.

An RGB image, sometimes referred to as a true-color image, is stored in MATLAB as an m-by-n-by-3 data array that defines red, green, and blue color components for each individual pixel. RGB images do not use a palette. The color of each pixel is determined by the combination of the red, green, and blue intensities stored in each color plane at the pixel's location. Graphics file formats store RGB images as 24-bit images, where the red, green, and blue components are 8 bits each. This yields a potential of 16 million colors. We use this capability of MATLAB to analyze the mixing of different dyes versus time. As shown in Fig. 5, at the very beginning of the experiment the graph is very complicated and all the colors have high density. However, after some time intervals the separation of red, green and blue components is a proof of mixing. After 7 minutes, the mixing is not very changeable which can conclude that the mixing has been reached to a saturation level.

CONCLUSION

In this paper, we present a simple and cost effective fabrication method for making microchannels and chambers using in microfluidic devices. This method uses typical microscopic glass slides as a substrate for fabrication of micro-structures. Using photo-resist as a mask led to gain precise results. A smooth channel surface with approximately vertical wall edges is achieved. In addition, the UV glue glass to glass bonding technique is used in order to bond glass slides together. The results show no penetration of liquid outside of the microchannels. PDMS is used for tubing connections of microfluidic device to the outside world using an especial technique that let to make the connections without any leaking. Image processing is used as a validation method for mixing using MATLAB. The results of the mixing show that the mixing occurs in first 7 minutes of process in fractal structure chamber which is faster compared to other designs.

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