Fabrication of shallow microchannels for highly uniform blood smear preparation

Jeethu Raveendran a, John Stanley b, T. G. Satheesh Babu b*

a Department of ECE, Amrita School of Engineering, Coimbatore, Amritanagar, India.
b Department of Sciences, Amrita School of Engineering, Coimbatore, Amritanagar, India.
*Corresponding Author E-mail address: tgsatheesh@gmail.com (T. G. Satheesh Babu)

Abstract. Polydimethylsiloxane (PDMS) based microfluidic channels for blood cell analysis were fabricated using etched glass as the master for soft lithography. The design consisted of shallow microchannels with uniformly spaced micropillars that aid in the formation of thin blood films (smear) through capillary filling of the microchannels. The concentration of hydrofluoric acid (HF) and the time duration of etching were varied and conditions optimized for fabrication of microstructures of different depths. Morphological analysis revealed the structure and dimension of the microstructures to be highly consistent. It was also noted that the micropillars formed during soft lithography prevented the roof of the PDMS microchannel from collapsing, a common phenomena observed while using shallow microfluidic channels. The fabricated prototype was used for blood cell analysis and the blood smear formed due to capillary flow was found to eliminate the drawbacks associated with manual smear preparation. Thus, a novel cost effective microfluidic device for cell analysis using glass etching was successfully developed and tested.

1. Introduction

Miniaturization using micro and nanofabrication technologies has resulted in the development of portable handheld devices that require minimal sample and reagents [1]. This advantage has made the technology an integral part of many biomedical devices and are commonly used in cell biology, molecular biology, biochemistry and in biosensors [2]. Lithography, deposition and etching are the three techniques used for microfabrication [3]. A newer approach involves the use of glass etching as an alternative for the fabrication of microstructures [4-6]. Selective etching of glass can be carried out using wet chemicals [7] or dry etching [8] methods using materials like Cr, Au, silicon and photoresists [9-12] as mask. By varying the concentration of etchant and duration of etching, micropatterns of different dimensions and shapes can be successfully fabricated.

Fabrication of shallow microchannels is necessary for many applications such as separation of blood plasma from whole blood, biosensors and microchannels for cell analysis [13, 14]. Microchannel for cell analysis is good alternatives for traditional cell analysis techniques like cell counters and blood smear preparation on glass slides. The traditional blood smear preparation by wedge technique requires expertise and poor smear preparation is still common [15]. A poor blood smear can occur due to insufficient or excess blood, failure to keep and push the spreader slide properly [16] and leads to errors in diagnostics. The problems associated with traditional smearing
technique can be overcome by allowing automatic capillary filling of blood in shallow microchannels resulting in the formation of a liquid film with uniform distribution. Also, fractionation of blood can be achieved by changing the depth of microchannels. This helps to filter blood cells based on their size and also remove unwanted cells from the smear.

The present work deals with the fabrication of shallow microchannels in PDMS via glass etching technique. The fabricated microchannels were used for blood smear preparation and analysis. Automatic filling of microchannels by capillarity was used for smear formation. This resulted in the removal of errors occurring due to the manual smear preparation.

2. Experimental

2.1 Materials and reagents

Positive photoresist Shipley 1813 was purchased from Shipley (MA). PDMS (184 silicon elastomer) prepolymer and curing agent were obtained from Dow Corning (Midland, USA). Microscopic glass slides (HV Slides-75x26x1.2 mm) were purchased from Hover Labs. Piranha solution was prepared by mixing concentrated H$_2$SO$_4$ and H$_2$O$_2$ (30%) in 3:1 ratio. All other chemicals were of analytical grade and used as received.

2.2 Instrumentation

Maskless photolithography unit (μPG-101, Heidelberg instruments, Germany) was used for transferring the desired pattern on the positive photoresist. Surface profile of micropatterns was studied using NanoMap-PS surface profilometer (aep Technology, USA). Corona treatment of PDMS and glass samples was carried out using BD-20ACV (Electro-Technic Products, USA).

2.3 Fabrication of prototype

The microfluidic channels used in this study were designed using CleWin software and the proposed design is shown in Fig. 1. The design is divided into 3 zones, an inlet for sample loading, microchannels for smearing and an outlet. The inlet and outlet diameters were 4 mm and 1 mm respectively. Three microchannels of 400 µm width were used for connecting the inlet and outlet. Micropillars of 100 µm height were distributed uniformly throughout the length. The microchannels were converged at the outlet.

Fig. 1. Design of the microfluidic system with inlet, microchannels and outlet
2.4 Glass patterning by photolithography and glass etching

The patterning of the photoresist on the glass surface for selective etching was carried out using photolithography. The schematic of the steps involved is shown in Fig. 2. Briefly, the glass substrate was thoroughly cleaned with piranha solution and rinsed with millipore water and baked at 130 °C for 30 min (Fig.2A). The photoresist was spin coated onto the substrate at 1000 rpm for 30 s (Fig.2B) and the pattern of interest was transferred using direct write laser lithography (Fig.2C). The exposed areas were selectively removed in 0.2 M NaOH solution (Fig.2D) and the patterned glass plate was gently agitated in HF solution. The areas of the glass plate that do not have the photoresist were selectively etched (Fig.2E). After etching, the photoresist was removed using acetone (Fig.2F). Glass etching was studied with HF concentrations of 20 %, 10 % and 5 % up to 240 s. Efficiency of etching was studied by analysing the depth of micropatterns using surface profilometer. Glass microstructures developed served as negatives of the patterns (Master) for the development of microchannels (Stamp) by soft lithography.

The PDMS stamps were developed by thoroughly mixing the base and curing agent of SYLGARD 184 in the ratio 10:1 and dispensing them on the master placed in a petridish and air bubbles were removed using vacuum desiccators. After degassing, the PDMS was cured at 100 °C for 30 min and stamp was peeled off from the master. The PDMS stamps were oxidized using corona treatment before use. For flow visualization studies, corona treated PDMS stamps were tightly pressed over glass slide and colored solutions were introduced into the inlet reservoirs. Blood smearing studies were performed by placing blood in the inlet of microchannels.

3. Results and Discussion

3.1 Effect of concentration of HF and duration of etching on depth of microchannels

Mechanism of glass etching by HF is well known [17]. Briefly, etching occurs by the reaction of HF with silicon dioxide and forms gaseous or water-soluble silicon fluorides (Eqn. 3.1 and 3.2).
SiO$_2$ + 4 HF $\rightarrow$ SiF$_4$(g) + 2 H$_2$O  \hspace{1cm} (3.1)
SiO$_2$ + 6 HF $\rightarrow$ H$_2$SiF$_6$ + 2 H$_2$O \hspace{1cm} (3.2)

Effect of HF concentration and time of etching on the thickness of micropatterns developed was studied. For this glass was etched with different concentrations of HF solution for different time durations. From the results obtained a graph was plotted with the time on the X-axis and depth of the channel obtained on the Y-axis (Fig. 3A). From the graph it is found that the height of the microstructures increased linearly with time and the regression constant was calculated to be 0.990, 0.997 and 0.988 for 20 %, 10% and 5% of HF respectively. The reproducibility of microfabrication process was studied by etching different glass plates in 20 % HF for 30 s. The etch depth was found to be uniform in all trials with a relative standard deviation of 5.5 % (Fig. 3B). These studies prove that the glass etching technique can be easily used for the fabrication of microchannels with a high degree of accuracy.

Micropatterns for this study were fabricated by etching glass in 20 % HF for 30 s. The magnified optical image of etched patterns is shown in Fig. 3C. It was found that the micropores (negatives for micropillers in soft lithography) had a constant depth of 5 µm throughout the length of the microchannels. It was also observed that the edges of the glass structures were not a straight cut, an indication that HF had induced isotropic etching.

3.2 Fabrication and characterization of PDMS microchannels

A photograph of PDMS stamp developed by using the etched glass plate as the master is shown in Fig. 4A. PDMS in its native form is hydrophobic and to enhance the fluid flow through the
PDMS microchannels corona oxidation was performed. During corona oxidation, polar groups are introduced onto the surface of the PDMS microchannels which in turn makes the surface highly hydrophilic [18]. The hydrophilicity of the PDMS surface together with the small dimension of the microchannel (0.02-2 mm) enables fluid flow by capillary effect. Fig. 4B shows the capillary filling of colored solutions inside the microchannels at various time intervals. From the Fig. 4B it s obvious that capillary movement causes uniform filling throughout the length of microchannels without any voids. Thus the technique is adequate for the automatic preparation of smears.

![Fig. 4. Photographs of stamp fabricated by soft lithography (A) and capillary filling of microchannels at different time intervals (0 s (a), 15 s (b), 45 s (c) and 60 s (d))](B)

The fabrication of shallow microchannels is difficult as the roof tends to collapse due to the elasticity and deformations in PDMS, resulting in complete blockage of the microchannels [19]. In this study no roof collapse was observed as the micropillars acted as support for the entire length of the channel and fluid filling of the microchannels was complete. To verify this claim, microchannels of 100 µm width were fabricated without micropillars and fluid flow studies were conducted. No fluid flow occurred through the channel due to roof collapse. From this it was clear that the micropillars prevent the roof of the microchannels from collapsing.

### 3.4 Blood flow analysis

A drop of blood was placed on the inlet and allowed to fill the microchannels through capillarity. The fluid flow was observed under 10 X magnification of the optical microscope. Well distributed spherical red blood cells were seen inside the microchannels (Fig. 5). This indicates smearing or thin blood film formation inside the microchannels. The shallow microchannels allowed formation of very thin film flow through the channels. Smearing inside the microchannels removed manual variations associated with traditional smearing and ensured preparation of similar smear in every repeats. Thus capillary filling of shallow microchannels overcome the disadvantage of non uniform distribution of blood smears.
4. Conclusion

A prototype for cell analysis was fabricated using shallow microfluidic channels of 5 µm depth. Micropatterns were fabricated by etching glass in 20 % HF for 30 s. Soft lithography using PDMS was performed for stamp development. The problem of roof collapse in shallow microchannels was overcome by placing micropillars within the flow path. Uniform cell distribution was seen inside the microchannels as fluid flow occurred due to capillarity. The fabrication process was found highly reproducible and removed the disadvantages associated with manual smearing. Thus the developed technology is a good tool that can be used in laboratories for cell morphology analysis and removes the need for skilled personnel.

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