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Design and Fabrication of Microchannel for Microfluidic in Biosynthesis

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ABSTRACT

A low cost fluid handling system with limited sample availability has been demonstrated. The device was analyzed using COMSOL 3.5 Multiphysics software and then fabricated using very simple and low cost lithography method. The design is based on differential pressure drop flow using capillary effect concept which has facilitated a number of interesting flow phenomena in micro-domains. For an average pressure drop of about 100/m in the setup, flow rates of about 0.5 to 1 µl/s were obtained. The components consists of microchannel and microchamber which were fabricated using very simple and clear soft-lithography using Polydimethylsiloxane (PDMS) as structural material. The system was designed and fabricated for continuous flow in micromolecular manipulation where low residence time due to fast reaction/diffusion rates are required.

Key words: COMSOL Multi-physic, Capillary effect, Microfluidic, lab on chip, SU-8, Polydimethylsiloxane (PDMS), fluidic system

Introduction

Microfluidic technology has become mature technology. Application of microfluidic technology in various fields has increased such as macromolecule separations, enzymatic assays and cell-based assays. Various testing and analysis of fluids are enabled in micro scale through a device called lab on chip (Kuo-Kang Liu et al., 2010). The lab on chip is the microfluidic device which is capable of undergoing laboratory functions and biomedical analysis, in a manner competitive to bench-top instruments. Lab on chip system is developed to serve the purpose of accelerating and automation of the diagnosis process like sample holding, staining, destaining, separation, detection, sizing and quantification. Lab-on-chip is a microfluidic device in which various laboratory functions are possible to be minimized and integrated onto a single chip which is only a few square millimeters in size. This application has been contributed in some chemical analytical process, for instance electrochemical, mass spectrometry, thermal detection, capillary electrophoresis and electrophromatography (Schumacher et al., 2008). Lab on chip involved the field of engineering, physics, chemistry, microtechnology and biotechnology. By pumping the few drops of testing fluids, lab on chip is capable of handling this small amount of fluids and is able to automate and perform the chemical analysis alone. It is a combination of MEMS device which consists of microfluidics and mechanical flow control devices, such as micropump, microchamber, microvalve, micromixer and separators. Lab on chip is normally related to Micro Total Analysis Systems (µTAS) which is the integration of the total sequence of lab processes in order to perform chemical analysis (West et al., 2008). The integration of microfluidics with nano-electronic sensor for the implementation of complex reaction protocols Need Capillary that has no moving parts, is simple and easy to implement and thus is very attractive for low-cost Microfluidic devices is very important and There is a wide spread interest in micron-scale integrated bio-molecule analysis or synthesis systems which is referred to as lab-on-a-chip. A critical to this is the ability to drive a sample through the device without both moving parts and an external actuation since at the microscale level moving parts in an active mixer are very fragile. Capillarity is a force that results from the interaction of cohesion of molecules of a liquid to each other and adhesion of these molecules to the surrounding material, Lab on chip technology possesses several advantages, such as programmability and straight forward process integration. Lab on chip device is perfectly sealed, and hence the chance of contamination of samples is reduced. It involved very low fluid volumes consumption, in other words, less waste, lower reagents costs and less sample volumes for diagnostics. Lab on chip is also advanced in its compactness as the integration of much functionality. The analysis is accelerated and response times is reduced because short diffusion distances, fast heating, high surface to volume ratios, small heat capacities. Lab on chip device has better process control and able to provide precise measurements since the system can response faster (Sato et al., 2008; Park et al., 2009) The material to built lab on chip is chemically inert, so the device can be cleaned easily. It is also a safe platform to run chemical, radioactive or biological studies because of integration of functionality, smaller fluid volumes

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and stored energies. The fabrication cost of lab on chip device is lower, which makes it a cost-effective disposable chips and able to be fabricated in mass production. It is said that the improvement in lab on chip technology is analogous with the microelectronic (Adam et al., 2012; T. M. Squires, and S. R. Quake, 2005)

Materials And Method

Device Design:

The design approach presented in this paper uses a passive microfluidic capillary-driven microchannel that exploits the capillary forces to allow fluid flows: The resulting flow is driven by a combination of forces due to gravity and capillarity, the pressure and the corresponding velocity of this flow were computed using the Navier-Stokes equations for the detailed fluid motion through the device. Due to hydrostatic forces, the pressure at the inlet is given by $P = \rho g h$, where $P$ is the pressure, $\rho$ is the density of the liquid (roughly 1000 kg/m³), $g$ is the gravitational constant (9.8 m/s), and $h$ is the height of the liquid, for water and similar substances, $P \sim 10^4 h$, with $P$ in Pascal and $h$ in mm. At atmospheric pressure, $P \sim 10^5$ Pa, so $H = 10$ mm; If we replace the water with real sample, the effective pressure drop can be simulated since the volume of liquid that enters the channel is a negligible fraction of droplet, so that $H$ is constant. In general, the pressure due to capillarity in a vertical cylindrical tube of radius $r$ can be described by (equ.1):

$$P = \frac{2 \gamma \cos \theta}{r}$$  (1)

Where $\gamma$ is the surface tension between the liquid and air, $\theta$ is the contact angle (in radians, measured from the downward vertical between the liquid and wall, and $r$ is the radius of the inlet channel. The fluid flow tend to encounter resist due to the friction between co-molecular force and adhesive force.

$$\frac{\partial P}{\partial x} = \mu \frac{\partial^2 u}{\partial y^2}$$  (2)

Where $P$ is the applied pressure, $x$ is the dimension along the length of the channel, $\mu$ is the fluid viscosity, $u$ is the fluid particle velocity (as distinguished from the volume velocity), and $y$ is the dimension across the channel. With no-slip boundary conditions where $\frac{\partial u}{\partial y}$ is finite so $u(0) = u(h) = 0$, it is easy to show that $u(y) = cy(h - y)$, where $c$ is a constant. The peak velocity $u_{\text{max}} = u(h/2) = ch^2/4$, so $c = 4u_{\text{max}}/h^2$

Throughout the fabrication of lab on chip device, there are several chemical material involved, such as acetone, SU-8 photoresist, PDMS, curing agent for PDMS and Isopropanol (IPA) and Glass. Acetone and IPA serve in substrate cleaning to remove the contaminants and particles.

Device Fabrication:

Initially the design of the device is run on simulations for numerous times to obtain the optimized performance. The data and observations are analyzed to investigate the relationship between parameters and performance. By using the computer simulation, designs and operations for lab on chip can be studied and optimized.

To manufacture the lab on chip device, there are a few steps involved:

i. Cleaning
ii. Photolithography
iii. Soft lithography
iv. Bonding

Cleaning:

The wafer is scribed into segment sized 4cm x 1.5cm. The wafer pieces are cleaned with acetone by using ultrasonic cleaner at high frequency for 10 minutes. This process is essential to remove any particles or contaminants on the surface of wafer pieces. If the substrate is not free of contaminants, the adhesion of the photoresist will be decreased. SU-8 photoresist: The photoresist selected to create patterns on substrate is SU8. SU-8 is a typical used epoxy-based negative photoresist which is used to create patterns with high aspect ratio structures. Su-8 is a very viscouspolymer that can be spunor spread over a thickness. SU8 are available to develop vertical sidewalls of micrometer height on glass or silicon wafers. During exposure, the molecular chains of SU-8 are cross-linkedand hardened. The developer used for SU-8 is 1-Methoxy-2-propanol acetate.SU-8 was used once as a high-resolution mask in fabrication process. But it is mostly used in the fabrication of microfluidicsdevice and MEMS parts. SU-8 has high transparency in the ultraviolet region, which
allows the fabrication of thick structures with nearly vertical side walls. After exposure and developing, the high cross-linked structure has strong immunity to chemicals and radiation damage.

**Polydimethylsiloxane (PDMS):**

is the polymeric organosilicon material. It is usually used as silicon-based organic polymer for its extraordinary performance in rheology. The transparency of PDMS is high and it is chemically inert, non-flammable and non-toxic. PDMS is mostly used as the stamp resin in soft lithography, which is a process of transferring patterns onto the polymers or silicon. After producing the mold for stamping, PDMS is used to build the devices with the shape that is in contrary with the mold. In Bio-MEMS, soft lithography is used to mass produce the bio chips. Patterns such as channels and chambers are created on the silicon substrate, and PDMS is poured over the substrate and cured. When the layer is hardened, the pattern on substrate is imprinted onto the PDMS.

**Photolithography:**

Photolithography process of microfabrication is performed to create patterns on the substrate. The process steps involved are spin coating, soft baking, exposure, hard bake and development. The photoresist used is SU-8 which is a negative photoresist. Initially, very small amount of SU-8 is dropped at the centre of the silicon wafer. The speed is set to 800rpm for 10s. This process step is purposed to spread the thin SU-8 layer all over the surface of the substrate and improve the adhesion of the whole SU-8 layer on the silicon segment. After that, about 3ml of SU-8 is dropped at the centre of the wafer, and undergoes the second spin coating. The spin speed is set to 2000 rpm for 20s with the ramp up speed at 800rpm for 20s. After spin coating with SU-8, the wafer is soft baked at temperature of 65ºC for 10 minutes by using hot plate. After that, the wafer is baked at the temperature of 95ºC for 20 minutes. This soft baking process is to produce the high aspect ratio imaging. During the soft baking process, the solvent level of SU-8 layer is reduced, and hence decreases the risk of exposed resist loss, swelling and the adhesion defects. The wafer is then left on a cold plate to cool down for 30 minutes.Upon cool down, the wafer is exposed to UV light by using mask aligner through the designed mask. Since negative photoresist is used in our cases, the exposed region will remain after development. The exposure process goes on for 55s. After exposure, the wafer is transferred to hot plate to hard bake at the temperature of 95ºC for 20 minutes. Shapes of patterns can be observed clearly on the SU-8 layer of wafer after the post exposure baking.

The wafer is then proceeding to development. The substrate is immersed into 1-Methoxy-2-propanol acetate, which is the developer for SU-8 photoresist. The exposed region will be hardened while the unexposed region will be removed by developer. The development time is approximately 8-10 minutes. If white stain of unfinished development is observed on the surface of the substrate, the wafer is developed for another 5 minutes. The photolithography process ends by spinning the wafer on spin coater at high speed to remove the liquid on the substrate as shown figure 1.

**Soft Lithography:**

The process that comes after photolithography is soft lithography. In this process, the lab on chip device is created by using PDMS. 25g of PDMS and 2.5g of curing agent for PDMS are prepared by using the electronic weight scale. After the preparation, these two solutions are mixed and stirred for about 10 minutes. The stirring will create bubbles in the mixture. The wafer with SU-8 mold is placed in petri dish before the well mixed of PDMS and curing agent is poured into the petri dish. It is advisable to wrap the petri dish with aluminum paper before inserting the wafer pieces and pouring the mixture into the dish. This is to ease the lifting off of patterned PDMS elastomer layer from the petri dish afterwards. There are two methods that can be used to cure the PDMS. Upon pouring the mixture into the petri dish figure 2, the dish is left on a flat surface for 24 hours. Another alternative involve the implementation of vacuum chamber. The petri dish is inserted into the vacuum chamber for vacuuming to remove the bubbles inside the mixture. After vacuuming, the temperature of the vacuum chamber is increased to 75ºC and the substrates are left inside the chamber for 1 hour. After the soft lithography, the PDMS elastomer layer is carefully lifted off from the petri dish and cut into segments. Isopropanol (IPA) is used to soften the PDMS layer to ease the lifting of the layer.
During the stirring of PDMS and curing agent, the PDMS is cured through organometallic crosslinking process. The curing agent for PDMS contains a proprietary platinum-based catalyst that activates the addition of the SiH bond across the vinyl groups in order to form the Si-CH$_2$-CH$_2$-Si linkages. The PDMS base oligomers which contain vinyl groups will have at least 3 silicon hydride bonds each after curing process. The advantage of curing process is that there will be no byproducts such as gas or water after reaction. In other words, there will be no waste generated. It is recommended to set the ratio of PDMS and curing agent at 10:1. Addition of the excess curing agent will cause the PDMS elastomer to be harder and poor elasticity.

**Results And Discussion**

A COMSOL Multiphysics software was used for Capillary flow experiments in a microchannel, the channel was formed by two parallel plates separated by distance H. The plate separation (H) is assumed to be precisely maintained throughout the length of the channel covering a range of 8 mm to 10 mm as shown in figure 1c. Experiments have been conducted using Newtonian liquids (Navier-Stokes) for model flow. Figure 1a Shows the variation of the velocity with length of the channel, the inertia point where the first time when the fluid is injected of typical runs with unstable fluid dynamics. At the inlet, are dual forces associated in driving the fluid, a gravitation force since the inlet is vertical (ρgH) and the forces due capillary, in the figure 3a, where the first point marked with pink arrow indicate the flow orientation is vertical where the velocity increases rapidly. The point shown with yellow arrow in figure 1a showing distinct point where the fluid entered the channel and stabilizes for the flow, the fronts rises and stabilize and reaches the equilibrium rise height (H) at 1.28 mm/s.
most interesting point is where a constant velocity is maintained throughout the channel length with this velocity. A sudden upshot is experienced due to the fluid leaving the channel indicated by convective flux and at 1.3 mm/s. Which is a very interesting result, to explain this, the difference in velocity between the stable state to convective influx is 0.02 mm/s at least 4% higher. This indicates that all the fluids entered the channel leave the channel at the outlet without any disturbance hence, the channel will work perfectly without any clog of sample.

![Graph showing a Constant velocity maintained across the microchannel](image1.png)

![Showing an average pressure drop across the microchannel](image2.png)

![Showing 70µm width microchannel model designed with Comsol Multiphysics software](image3.png)

**Fig. 3:** (a) Graph showing a Constant velocity maintained across the microchannel (b) Showing an average pressure drop across the microchannel (c) showing 70µm width microchannel model designed with Comsol Multiphysics software

Figure 3b: shows the pressure drop across the length of channel which fluid flow, this due to capillary forces. And Capillary forces result from the interaction of liquid, gas and solid surfaces, at the interface between them. In the liquid phase, molecules are held together by cohesive forces. In the bulk of the liquid, the cohesive forces between one molecule and the surrounding molecules are balanced. However, for the same molecule at the edge of the liquid, the cohesive forces with other liquid molecules are larger than the interaction with air molecules. As a result, the liquid molecules at the interface are pulled together towards the liquid, here in this experiments, and the effect of the pressure could be explained in three major points within the microchannel. At the inlet where the pressure must be greater than every point before subsequent point ahead for the fluid to start flow and in this case is greater than unity but here, the value is chosen for the purpose of explanation since the pressure used here is atmospheric pressure and this will save the purpose. The yellow arrow indicates the point at which the pressure the fluid started flowing 1.1x10^5 (Pa) and from there the pressure gradually drops to zero with constant value as the fluid flows.

Upon completion of photolithography, surface of the wafer substrate is analyzed. Testing such as leakage testing is run after completion of the fabrication process. In Figure 5, the device being characterized using (HMP). Throughout the project, silicon wafers are used and scribed. In order to further save the fabrication cost, we tried to use the glass slide for the substrate to replace the silicon wafer in photolithography. After cleaning the glass slides with ultrasonic in acetone, SU-8 photoresist layer is spin coated on it. Similar to the silicon wafer, the SU-8 layer adhere quite well with the glass slide. The implementation of the glass slide in the fabrication process possesses several advantages, such as the cheap cost and the ease of observations from the back side. However, the patterns created on the glass slides cannot withstand the soft lithography process in long term. The patterns will be torn off after undergoing several times of soft lithography. The adhesion of SU8 photoresist to glass slides degrades with time. Hence, silicon wafer is used as the substrate for creating the mold.

During the photolithography process, in order to create patterns with high similarity to the mask, the gap between the mask and substrate with SU-8 layer has to be very small. SU-8 layer is thicker and stickier compare to other photoresist. Any contact of the layer with other surface will leave the vestige of the surface. To overcome the problem, the soft bake and cool down duration has to be increased to 30 minutes and 15 minutes respectively. Upon the increased time of soft bake, the SU-8 layer is hardened and the gap between mask and
substrate can be minimized without worrying the sticking problem. As the hardness of the SU-8 layer increasing, the difficulty of create patterns increase in proportionality. The development time has to be increased as well.

**Fig. 4:** shows concentration profile

After the software design, we further design the device using AutoCAD to create transparent mask for Photolithography process, various designs of micromixer are created and patterned onto the wafer. Micromixer is a device used to mix the fluids through diffusion as shown in figure4.

**Fig. 5:** show design of microchannels and chamber for mixing device

**Bonding:**

The patterned PDMS is bonded with glass in this process steps. Both the glass and the patterned PDMS pieces are inserted into plasma preen system and exposed to oxide plasma for 45 seconds. Upon the exposure, a piece of glass slide is attached onto the patterned side of PDMS. The glass slide will be bonded tightly to the PDMS and create permanent sealing figure5.
During the soft lithography process, in order to produce the optimized quality of elastomer, the ratio of PDMS and curing agent is 10:1. Varying the ratio of mixture for PDMS and curing agent will affect the quality of the hardened elastomer. If curing process is undergone with the ratio of curing agent to PDMS less than 1:10, the PDMS cannot be fully cured and the elastomer formed is soft or liquefied if worse. If the ratio of curing agent to PDMS is increased to more than 1:10, a harder and more crosslinking elastomer is formed. Simply increasing the ratio after 1:10 will cause degradation to the elasticity of elastomer.

The surface of the PDMS elastomer is studied by using surface profilometer. In this surface studying, the roughness of the surface is measured through the light reflected from the surface and taken without causing any contact with the surface. The resolution size ranged from a few microns to sub microns.
**Fig. 7:** shows the microfluidic under test

**Fig. 6:** shows the results obtained in surface study through the surface profilometer. (a) Observation through the microscope on the microchamber. (b) 2D analysis for the surface study on microchamber. (c) 3D analysis for the surface study on microchamber pattern and (d) is showing inlet opening with microchannel extended.
Fig. 7: shows the results obtained in surface study through the surface profilometer. (a) Observation through the microscope on the microchamber. (b) 2D analysis for the surface study on microchamber. (c) 3D analysis for the surface study on microchamber pattern.

Two areas on SU-8 molded wafer pieces is studied, that is microchannel and microchamber. Surface analyze are taken and generated for the areas and the measurements are analyzed in 3D form. The results of analysis is as shown in figure 6 and 7. In Figure 6, the 3D surface analysis is performed in 0.31nm x 0.44nm area on the microchamber. From the observation and analysis in the result, we can conclude that the average height of the microchamber is above 100 micron. The surface roughness is due to the transparency of SU-8 mold on the wafer surface. In overall, the surface of the mold is flat and is capable to produce good patterns of microchamber on PDMS elastomer. From the study of the results obtained in Figure 7, we can observe that the mold has a smooth channel on the wafer substrate. The average height of the microchamber is above 150 microns.

Conclusion:

Capillary effect for driving a fluid within microchannel have been demonstrated. Both the numerical analysis and the experimental analysis were performed, each provided an insight into the flow dynamics of sample in a microchannel due, when 70µm channel was design and fabricated, a uniform pressure drops and constant velocity were observed, this demonstrated the potential of using this phenomena to employ for passive fluid control within microchannel which a fluid could flow passively without any external force and in the second of this work is the fabrication process of the microfluidic lab on chip. The fabrication process involves the photolithography and soft lithography. This microfluidic device possesses the characteristic of MEMS and microelectronic, that is miniaturization, microelectronic integration and mass production. With the miniaturization of the device, it performs faster and consumes less reagent volume.

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Reference


