Rapid prototyping of PDMS microchannels for animal and plant cells using cutting plotter and double casting

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Abstract
The facilitation of access to rapid and low-cost microfabrication methods is essential for improving the development cycles of testing new chip designs and research ideas. A simple fabrication method of biocompatible microchannels with a height of 0.3 mm is significant for biological scientists to study animal and plant cells (~0.1 mm). Microfabrication using a cutting plotter and a knife blade provides a rapid and low-cost technology. In a previous study, designed cut pieces were used as a channel mold, but they were easy to deform during handling, and therefore the large-scale production of channels was difficult. Moreover, it is required to characterize the cutting process of 0.3-mm-thick sheets with a low-cost plotter. Here, we report the development of an easy fabrication method of 0.3-mm-high polydimethylsiloxane (PDMS) microchannels based on a desktop cutting plotter (300 USD) and an engraved sheet as follows. 1) A 0.3-mm thick sheet of cast-coated paper or silicone rubber was used as a mold material and patterns were created with a cutting plotter. 2) It was pasted to a double-sided tape to make a master mold of a flow channel. 3) The mold was replicated in PDMS to make a negative mold. 4) This PDMS mold was again transferred to a final PDMS chip. 5) It was bonded to a glass substrate to form a PDMS flow channel. We characterized the pattern transition of rectangular shapes from an engraved sheet mold to a final PDMS channel, that is essential for the design of a channel using this method. I- and Y-shaped microchannels (height: 0.3 mm) were fabricated and fluorescent particles and 0.2-mm diameter Volvox were introduced into the channels to show their performances.

Keywords: Xurography, Cutting plotter, Microfluidics, Low-cost microfabrication, Molding

1. Introduction

Microfluidic devices with a hydraulic diameter of 1 to 1000 µm have become increasingly popular for applications in chemistry, biology, and medicine. To take advantage of microfluidics, researchers in the fields of animal and plant cells (~0.1 mm) need a simple fabrication method of biocompatible microchannels with a height of 0.3 mm. Soft lithography based on a thick photoresist and flat substrate is a common fabrication method for making single- and multi-layer microchannels (McDonald, et al., 2000, Nagai, et al., 2013a, Unger, et al., 2000). After the photolithography process, a photoresist serves as a mold. This photolithographic production of molds usually requires a clean-room environment, photomasks, and exposure apparatuses (Qin, et al., 2010), which slows down device development in research institutions without such facilities. For improvement of the development cycles of testing new chip designs and research ideas, it is essential to facilitate access to rapid and low-cost microfabrication methods.

Non-lithographic low-cost techniques have increased the interest of the industrial and research communities to develop simple, rapid and low-cost microfluidic structures (Bhattacharjee, et al., 2016, Faustino, et al., 2016, Thomas, et al., 2010). These alternative techniques include laserjet (Bao, et al., 2005, Vullev, et al., 2006) and wax (Kaigala, et al., 2007) printers, 3D printers, cutting plotters, and laser cutters (Ziya, et al., 2016), which use equipment available in many laboratories. Xurography is based on a cutting plotter machine and adhesive polymer films to fabricate micropatterns. Among the non-lithographic techniques, Xurography is advantageous because of its fast fabrication time and low-cost of equipment and materials.
A fine flow channel was produced by laminating multiple polymer films engraved with a cutting plotter (Bartholomeusz, et al., 2005, Do, et al., 2011, Yuen and Goral, 2010). A double-sided tape was sandwiched between glass slides to make a channel (Jenny, et al., 2007). Since films were used directly as a flow channel material, the methods were not suitable for introducing and observing a living sample from the viewpoints of low oxygen permeability and poor biocompatibility. This problem can be solved by using polydimethylsiloxane (PDMS) as a channel material. Thin PDMS membranes on flexible plastic foil were cut into user-defined shapes and assembled into a microdevice via plasma bonding (Cosson, et al., 2015). This technique required a spin-coater to obtain thin films which increases the overall cost. A small vinyl cut piece was placed and molded in PDMS without employing a spin coater (Bartholomeusz, et al., 2005, Pinto, et al., 2015). However, these studies had difficulties in preventing the deformation of small pieces and placing in large quantities, thus decreased the productivity. In addition, a low-end desktop plotter is preferable to improve availability for biological scientists, but it has a smaller cutting force than high-end cutting plotters, and it is challenging to cut relatively thick samples (~0.3 mm). Cutting of 0.3-mm-thick sheets with a low-cost plotter has not yet been fully characterized, and there is a need for characterizing and developing such a cutting method.

To avoid handling these pieces, this paper uses an engraved sheet and double casting for the fabrication of a PDMS microchannel. An engraved sheet creates new advantages of easier handling than small pieces and large-scale production. The first pattern is created in 0.3-mm thick sheets with a low-cost desktop cutting plotter and transferred twice by molding. We characterize the transition of rectangular shapes from an engraved sheet mold to a final PDMS channel, which is useful for designing a channel with target dimensions. I- and Y-shaped PDMS microchannels (height 0.3 mm) are fabricated to develop a fabrication method and the channels are evaluated by flowing solutions containing particles or 0.2-mm diameter microorganisms through them.

2. Experimental Methods

2.1 Cutting conditions and pattern

We made a mold with a commercially available desktop cutting plotter (silhouette CAMEO, GRAPHTEC) having a mechanical step resolution of 0.05 mm and program step resolution of 0.025 mm. This plotter costs 300 USD and is affordable for many laboratories. An accompanying blade (SILH-BLADE -3J, GRAPHTEC) was used with a blade depth of 4 (about 0.4 mm). A blade was observed with a microscope for further study of cutting conditions. Pattern drawings were created in AutoCAD and loaded to software, silhouette Studio. The following major conditions were input into silhouette Studio: (1) thickness 33 (highest force, about 2.1 N), (2) speed 1 cm/s (slowest), (3) double cut ON, and (4) overcut 0.1mm (starting- and end-points).

We formed rectangles as well as I-shaped and Y-shaped flow channels (Fig. 1). 1:10 ratio rectangles were prepared for evaluating the machining accuracy to compare with designed values. The total of 18 rectangular patterns had different widths from 50 to 600 µm (50 µm increment) and from 600 to 1200 µm (100 µm increment). The I-shaped and Y-shaped flow channels served for evaluation of a function as a flow channel.

![Fig. 1 Cut patterns in a mold with a cutting plotter. (a) Rectangle (I-shaped channel). W is the width and the length of a rectangle is 10 W. (b) Y-shaped channel. X and Y are the widths of a Y-shaped channel and the lengths are defined as 7X and 6Y.](image)

2.2 Fabrication and evaluation of mold and microchannel

We fabricated a flow channel with a height of 0.3 mm by the following process (Fig. 2). First, a 0.3-mm thick master mold was prepared with a cutting plotter (Fig. 2a-d). (1) We tested two materials of cast-coated paper (Size A4, thickness 0.28 mm, <180>, Daigen Corporation) and silicone rubber (Sirius ultra-thin silicone rubber, transparent, thickness 0.3 mm, hardness 50, Fuso Rubber Co.) for a mold and fixed it on a cutting mat (CUT-MAT-12-J, Graphtec) (Fig. 2a). (2) Design patterns were created in a mold sheet with a cutting plotter according to the cutting condition described in Section 2.1 (Fig. 2b). (3) The engraved sheet was peeled off from the cutting mat and unnecessary cut-out parts were removed from the sheet (Fig. 2c). (4) The mold was pasted to one side of a double-sided tape (Nitoms, for general use, SJ 0 750,
white, 75 mm × 20 mm) to make a bottom substrate and to form a master mold. The other side was stuck to a polystyrene petri dish (Fig. 2d). (5) To improve the mold release from the master mold, the sample was treated with a vapor of trichloro (1H, 1H, 2H, 2H-perfluorooctyl) silane (PFOCTS). This process reduced the free energy of the surface and facilitated peeling of the cured PDMS from the master mold. 10 µL PFOCTS was vaporized on a hot plate heated at 80°C for 3 min.

Subsequently, the master mold was transferred to a PDMS negative mold (Fig. 2e, f), and finally we obtained a PDMS microchannel (Fig. 2g, h). (6) PDMS base polymer (Silpot 184, Dow Corning Toray) and curing agent were mixed together in a 10:1 by weight ratio. The mixture was poured onto the master mold and cured at 50°C for about two hours to transfer to a PDMS negative mold (Fig. 2e). (7) The negative mold was released from the master mold (Fig. 2f). (8) The negative mold was treated with an air plasma for 60 s with a plasma treater (YHS-R, Sakigake-semiconductor) to make this PDMS mold available for molding. The PDMS mold was transferred to the final PDMS microchannel under the same PDMS curing condition (Fig. 2g). (9) The chip was peeled off from the PDMS negative mold and treated with plasma for 30 s and bonded to a glass substrate to form a microchannel (Fig. 2h).

Various sizes of I-shaped PDMS flow channels were fabricated and observed with an optical microscope (VHX-2000, Keyence) equipped with a lens (VH-Z100UW, Keyence). The influence of each process was evaluated by comparing designed and measured values. Fig. 2(d) is a schematic image of measurement points that are set away from corners. The equivalent positions of each sample were observed for characterizing the processing accuracy.

Fig. 2 Schematic diagram of channel fabrication processes using a cutting plotter. (a)–(d) Master mold fabrication. Top view showing measurement points of the I-channel. (e), (f) Pattern transfer to PDMS. (g), (h) Fabrication of a PDMS channel.

2.3 Experiments using fabricated PDMS flow channels

A suspension of fluorescent particles L4530-1ML (average particle size 2.0 µm, Sigma Aldrich) and deionized (DI) water were introduced into a Y-shaped flow channel for the verification of a flow channel function (Fig. 3a). A medium solution containing spherical algae, *Volvox carteri* (about 200 µm in diameter, NIES-865), was introduced into an I-shaped flow channel (Fig. 3b). The purpose of using *Volvox* is to check that a created flow channel is suitable for multicellular organisms. *V. carteri* was cultured according to our protocol (Nagai, et al., 2015).

A tube was connected to a syringe for introduction, and a flow was introduced at a speed of 10 µL/min with a syringe pump (KDS 200, KD Scientific). The experiments were observed an inverted microscope (ECLISPE TE2000-U, Nikon) equipped with a 4× objective lens and a CCD camera (Digital Sight DS-2Mv, Nikon).

Fig. 3 Schematic of experiments using fabricated PDMS flow channels. (a) Fluorescent particles and DI water were introduced into a Y-shaped channel. (b) *Volvox* was introduced into an I-shaped channel.
3. Results and Discussion

3.1 Production of 0.3-mm-high flow channel

Cut and molded samples were obtained using a cutting plotter and double casting (Fig. 4). 0.3-mm-high PDMS flow channels with widths larger than 350 µm were successfully formed (Table 1). Fig. 4(a) shows the whole image of a cast-coated paper master mold cut out with a cutting plotter. An engraved sheet was easier for handling than a small piece and our study is superior to the previous study (Pinto, et al., 2015). Fig. 4(b) is the image of a PDMS rectangular flow channel obtained as a result of the twice pattern transfer. PDMS Y-shaped channels were created as Fig. 4 (c) and (d) show a PDMS mold and PDMS channel, respectively.

Fig. 4 Images of a paper master mold and PDMS flow channel. (a) Overview of the paper master mold. (b) A photo of I-shape PDMS channels in the boxed area. (c), (d) Microscope images of Y-shaped channels made with cast coated paper. (c) PDMS mold (X = 928 µm). (d) PDMS channel (X = 1002µm).

Table 1 Widths of failure patterns after the fabrication processes

|                         | Master Mold | Negative PDMS Mold | PDMS Channel |
|-------------------------|-------------|--------------------|--------------|
| Cast-coated Paper       | 50–200 µm   | 50–200 µm          | 50–350 µm    |
| (Thickness 0.28 mm)     |             |                    |              |
| Silicone Rubber         | 50–200 µm   | 50–350 µm          | 50–350 µm    |
| (Thickness 0.3 mm)      |             |                    |              |

Fig. 5 shows transitional microscopic images of a master mold with a designed channel width of 450 µm and a corresponding negative mold and flow channel. The master molds were made of a sheet of cast-coated paper or silicone rubber. The straight section on the long side had better shape formation than the corners, where clear shapes were lost. In the master mold made of the paper (Fig. 5a–c), the state of the all surfaces were rough and residues affected the shape of flow channels. In the negative mold (Fig. 5b) and the flow channel (Fig. 5c), the angles of the wall surfaces were about 15° from the vertical direction. The shape of the cutting edge of paper can be explained by the cutting-edge angle of a cutter blade (Fig. 6). The blade in the stage of Fig. 6(b) shows 30° and it faced the cutting direction, which was also along the long side of a rectangular. The sectional shape of the long side agreed with the cutting-edge angle. In the master mold made of the silicone rubber (Fig. 5b), the vertical wall surface was created, and the edge was clear.

The heights of PDMS microchannels were 280 µm (Fig. 5c) and 330 µm (Fig. 5f), respectively. According to the specifications of this plotter, it is capable of cutting various materials from thin paper up to 0.5 mm thick. This information suggests the possibility of making a 0.5-mm-high microchannel with a 0.5-mm-thick sheet.
3.2 Characterization of cutting accuracy and pattern transition

The process transition of rectangular widths is displayed in Fig. 7, where these samples were made with a 0.3-mm-thick sheet of cast-coated paper or silicone rubber. The horizontal axis represents setting widths and the vertical axis represents measured widths. Four samples were measured for each size pattern. Linear regression lines clearly show that widths increased as increasing designed values. $R^2$ values of the six relationships were over 0.9, which shows a good fit of the lines to the data. The pattern transfer from a paper mold remained much the same. In case of a silicone rubber mold, widths decreased after each step. We consider this decrease was caused by poor PDMS peeling from the vertical side wall and thermal expansion of silicone rubber during PDMS curing. As Table 1 presents the sizes of failure patterns after the fabrication processes, rectangles with less than 250 µm width were not cut out with this cutting plotter. After the molding process, sizes less than 400 µm were not formed. These results indicated that the minimum feature width was 0.4 mm for a 0.3-mm-thick sheet of paper and silicone rubber.
Fig. 7 Width transition during the processes from a master mold to a microchannel starting from a sheet of (a)–(c) cast-coated paper and (d)–(f) silicone rubber. Four samples were measured in each size. Linear regression lines and equations are displayed. (a), (d) Master mold. (b), (e) Negative PDMS mold. (c), (f) PDMS channel.

3.3 Evaluation of a flow channel

Two solutions were introduced to a Y-shaped flow channel made with cast-coated paper or silicone rubber (Fig. 8). Fig. 8(a)–(d) and (e)–(h) show the state of flows made with cast-coated paper and silicone rubber, respectively. The movement of aggregated particles were encircled. Two syringes containing a suspension solution of fluorescent particles and DI water were connected and pumped at a flow rate, \( Q = 10 \mu\text{L/min} \). We measured the average flow speeds after the junction by tracking particles and the speeds were 1.4 ± 0.5 mm/s (n=3; cast-coated paper) and 3.3 ± 1.0 mm/s (n=3; silicone rubber). The average flow speed, \( V \) through a channel after the junction with a cross-section area, \( A \) is calculated by \( V = 2Q/A \). For the channel made with cast-coated paper, substitution of \( 2Q = 20 \mu\text{L/min} \) and \( A = 0.3 \text{mm}^2 \) leads to \( V = 1.1 \text{mm/s} \). The measured flow speeds agreed with the calculated results. Laminar flow occurred as the particles moved along straight lines parallel to the channel wall from the inlets to the outlet. As an exception, flow was unsteady near a protrusion on the side wall, where the width of the channel was reduced (Fig. 8a). The boundary of two flows was observed around the central part. The bonding between the PDMS flow channel and the glass substrate was sufficient, and no leakage was observed. Although the surface roughness near the edges was observed, flow was steady in most parts of the channel.

Fig. 9 shows the results of solution introduction containing spherical *Volvox* (diameter of about 200 µm) in an I-shaped channel. They flowed at an average speed of 4.2 ± 0.1 mm/s (n=3) without clogging as yellow circles represent the movement of *Volvox* colonies every 67 ms. The channel had enough height for applications in biological samples, especially multicellular organisms. This process allowed for the fabrication of practical flow channels, because the leakage and stagnation of flow did not occur in the flow channel and a laminar flow was generated. Although the bonding between glass and PDMS was achieved by vacuum plasma treatment, this bonding equipment can be replaced with a low-cost corona treater (Haubert, et al., 2006, Nagai, et al., 2013b).
Fig. 8 Fluorescent particles showing flows in a Y-shaped flow channel. Single aggregated particles are encircled. Flow channels made with (a)–(d) cast-coated paper (channel width: 960 µm) and (e)–(h) silicone rubber (channel width: 620 µm). A protrusion near the junction is denoted by a red triangle.

Fig. 9 Time lapse microscopy images of the transport of Volvox through an I-shaped flow channel (channel height: 280 µm, width: 470 µm). This channel was made with silicone rubber.

4. Conclusions

We fabricated the 0.3-mm thick master mold made of cast-coated paper and silicone rubber using a low-cost cutting plotter and double casting. A PDMS micro flow channel was created by twice molding of PDMS. The dimensional transition between two mold materials was investigated. The process characteristics of cast-coated paper and silicone rubber are summarized here. The paper-mold-based process had the following characteristics: (1) almost same patterns were transferred from a master mold to a PDMS chip. (2) residues on a cut surface affected the edge of the patterns. (3) 15° slope appeared on the sidewall of paper. The silicone-rubber-based process had the following features: (1) the patterns gradually decreased after the transfer. (2) The edge of the master mold was clearer than the paper mold. (3) The side wall was vertical, and the height of the fabricated channel was about 0.3 mm, which was determined by the starting sheet. The prepared flow channel had sufficient height for introduction of multicellular organisms.

Our process was low-cost compared to photolithography in a clean-room environment using a photomask and exposure equipment. The fabricated PDMS chip was suitable for observation inside the channel. It was easier to handle an engraved sheet than small pieces. This study showed that our process solved the previous researches using xurography. We believe our development will provide access to rapid and low-cost microfabrication methods for biological scientists studying animal and plant cells (∼0.1 mm) and improve the development cycles of testing new chip designs and research ideas in microfluidics.

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