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Foil assisted replica molding for fabrication of microfluidic devices and their application *in vitro*

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We present a simple, rapid, bench-top, Foil Assisted Rapid Molding (FARM) method for fabrication of microfluidic devices. This novel technique involves the use of aluminium foil, pen and an X-Y plotter to create semi-circular or plano-concave, shallow microchannels. It is an easy do-it-yourself (DIY) technique for creating a microfluidic device in three simple steps: (1) creating channel design using CAD software, (2) plotting the patterns on aluminium foil and (3) using the reverse of engraved foil as a mold to create microfluidic devices. In this report, we present a detailed study of the proposed method by varying a range of parameters such as foil thickness, tip material, tip sizes and their effect on creating channels of differing geometry. Furthermore, we have demonstrated the cytocompatibility of these devices *in vitro*.

Introduction

The micromachining and patterning techniques used in microelectronics such as photolithography, direct etching and deposition, stereo-lithography and electron-beam lithography are widely used in the development of complex microfluidic devices using photopolymers, glass and other semiconductor surfaces[1,2]. These fabrication techniques demand significant investment and ongoing operational costs. In addition to these techniques, there are several other non-photolithography fabrication techniques used for fabrication of micro- as well as nano-structures and devices[2]. Several non-conventional[3] fabrication techniques are cost-effective and can be used for prototyping. The most popular technique in the field of microfluidics is soft lithography[4]. The term soft lithography is used to describe a set of techniques where elastomeric masks, masters and/or stamps are used to create micro- and nano-patterns or channels. For fabrication of microfluidic devices, replica molding is one of the most popular soft-lithography techniques[5,6]. However, apart from the need for a clean room facility, fabrication of an appropriate master prior to soft lithography is a major limitation. In recent years multiple non-traditional[3] methods have been reported such as cutting tapes[7], shrinky-dink microfluidics[8,9] and xurography methods[10]. The embedded template method[11] is another simple but promising method to create semi-circular and circular channels, but making complex device with long channels remains a challenge[12]. Thus, making shallow, semi-circular or plano-concave channels is still a complex and laborious task using either conventional or non-conventional techniques for applications such as creating valves[13]. In this work, we propose a novel, facile method, Foil-Assisted Replica Molding (FARM) that can be used to fabricate plano-concave channels with varying dimensions under standard laboratory conditions without the need of any sophisticated instrumentation. The FARM technique employs a plain, non-patterned piece of aluminium (Al) foil and a pen plotter to make masters/molds. Application of these FARM devices has been tested in cell culture experiments.

Experimental

The designs used in this present work were generated using CorelDraw® Suite X6, Coral Corporation, Canada (Figure 1 (A)). Thereafter designs were transferred to a plain, non-patterned piece of Al foil placed on the pen plotter (MP4400 series, Graphtech Corporation, Japan) to create master (Figure 1 (B)). Two types of Al foils were used in this study with thickness of 11 µm and 25 µm (Cat. No. Z185159) obtained from Hindalco Industries Ltd., India and Sigma-Aldrich, St. Louis, MO, respectively. Prior to patterning, the foil was carefully laid flat using a soft cloth material. Due to the flexible nature of the foil, engraved marks made on the dull-side are reflected as positive projections on the shiny-side (Figure 1 (C)). After printing the design, a piece of one side cemented transparent polyester sheet (SC40 and SC42, REGULUS GmbH, Germany) carefully adjoined with the foil provided stability and ruggedness to the foil master. Post-plotting the reverse of the engraved Al foil can be used as a master/mold to fabricate microfluidic devices using soft lithography (Figure 1 (D)). A microfluidic device with the letters "CCMB" on a Polydimethylsiloxane (PDMS) device fabricated by a mold made using FARM technique is shown in Figure 1 (E-F). These experiments clearly demonstrate that FARM is a facile, inexpensive method which allows for rapid prototyping. Pens with different tip sizes were used to create designs of varied

dimensions. Both fibre tip and metal tip pens with different tip sizes were used in this study. The pens used and optical images of the tips are shown in Fig. S1†.

For fabrication of a Polydimethylsiloxane (PDMS) (Sylgard 184, DOW Corning, Minland, MI.) device, the foil master was transferred to a petri dish and fixed using double-sided sticky tape. Thereafter, a thoroughly mixed and degassed mix of PDMS prepolymer and curing agent in the ratio of 10:1(wt/wt) was poured onto the Petri dish containing the foil mold and cured at 60 °C. Once cured, patterned PDMS was peeled off from the master and access holes were created using a biopsy punch. The patterned PDMS slab and a precleaned glass slide were subjected to corona treatment for 30 seconds using a handheld laboratory corona surface generator (BD-20AC; Electro-Technic Products, Chicago, IL) after which the surfaces were bonded

For testing the suitability of these devices for cell culture experiments, we used IMR-32 (human neuroblastoma cell line) and green fluorescence protein (GFP) tagged MDA-MB (adenocarcinoma cell line from human mammary glands) cells obtained from CCMB's central cell and tissue culture facility. The cells were cultured in cDMEM (complete Dulbecco's Modified Eagle's Medium) with low glucose medium supplemented with L-glutamine (2 mM), sodium bicarbonate (1.5 g/L), 10% FBS (Fetal Bovine Serum) and 1% antibiotic (penicillin/streptomycin/gentamycin) solution. Cultures were then maintained in a humidified atmosphere of 5% CO_2 at 37 °C. Cells were harvested at 80-90% confluency using 0.1% trypsin EDTA (Sigma Aldrich, St. Lucia, MI). These harvested cells were loaded into the FARM microchannels. All the devices and tubing materials used in cell culture studies were autoclaved prior to use. Later, the devices were further sterilized with ethanol to eliminate microbial contamination as well as to remove the blockage within the channels. The devices and tubing were sterilized by exposing them to UV radiation for 15 minutes. All the devices used in this study were coated with poly-L-lysine (50 µg/mL). This solution was passed through the device using a peristaltic pump maintained at a flow rate of 43 µL/min. Later, the poly-L-lysine-coated devices were incubated for 2 hrs at 37 °C. After incubation, the devices were flushed with Milli-Q water followed by phosphate buffer saline (Sigma Aldrich, St. Lucia, MI).

Prior to loading the cells into the FARM microfluidic channels, plated cells were trypsinized with 1 mL trypsin-EDTA (0.1%) and centrifuged at 800 rpm at 4 ºC. The cell pellet thus obtained was resuspended in 2 mL of complete medium in a sterile tube. The cell suspension ($1x10^6$ cells/mL) was later pumped into the microfluidic device using a peristaltic pump (43 µL/min) until the cell suspension was seen at the other end of the channel. The presence of IMR-32 cells and GFP-MDA-MB cells in the microfluidic channels was confirmed using optical light microscopy and fluorescence microscopy, respectively. These FARM devices loaded with cells were incubated at 37 °C with 5% CO_2. During each experiment, cell culture medium inside the channel was changed at regular intervals (every 1 hr) for a period of 34 hrs. The polyether ether ketone (PEEK) tubing was directly inserted into the inlet and fresh medium passed through the channels.

In another device with a T-junction design, the cells were inoculated from the bottom of the T-junction through an inlet well ($1x10^6$ cells/mL) and incubated at 37 °C with 5% CO_2. As in the previous case, the medium was changed every hour and this experiment was carried out for 34 h.

Results & Discussion

The process of fabricating microfluidic devices using the FARM method is a simple and rapid approach to make working prototypes. Taking advantage of the large working area of a flatbed pen-plotter, large numbers of masters/molds can be made in a single sheet of aluminium foil with varying channel sizes ranging from 150-500 µm (Fig. S2†). Several parameters were investigated to optimize the process to fabricate microfluidic devices with varying geometry of the channels.

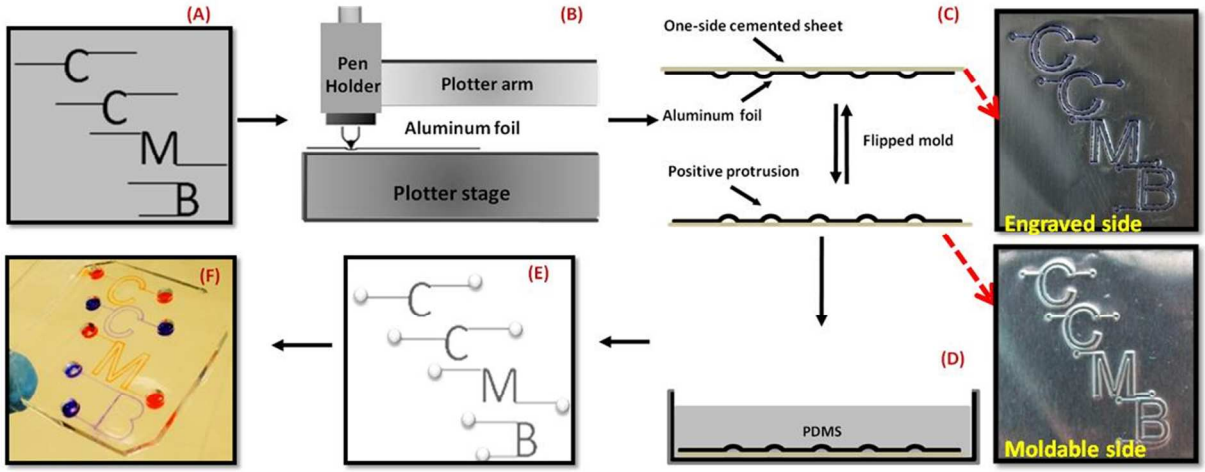

Fig. 1 Schematic illustration of the process for fabrication of microfluidic device (A) CAD design, (B) pattern/s transferred onto the aluminium foil using the X-Y pen plotter, (C) polyester film supported patterned foil as master, (D) molding of PDMS against patterned aluminium foil mold (E) molded PDMS after punching the access holes (F) Final PDMS devices sealed on glass surface.

Aluminium foils that are commercially available consist of a non-shiny rough surface (RMS roughness 423 nm; Fig. S3 (A)†) and a shiny or polished smooth surface. Throughout, AFM images of the shiny side revealed line-like nano-sized patterns (RMS roughness 90 nm) on the surface (Fig. S3 (B)†). However, the PDMS replica made against the shiny side was transparent, while the replica created from the non-shiny side turned translucent due to greater roughness. Therefore, in the current work, patterns were engraved or written on the non-shiny side of the foil, so that positive protrusions were formed on the shiny side of the foil. We have used foils of different thickness and found that foil thickness affects the channel size. There was negligible difference in the roughness between the two brands of foils used in this study. However, it proved much easier and convenient to handle the thicker foils.

Table 1 The list of pens that were used in the experiment. M - Metal tip and F- fibre tip.

Pen No.	Pen	Tip Size (mm)	Average Channel Size (µm); n=5
1	Rotring(M)	0.3	150 ± 5.5
2	Pilot V5(M)	0.5	200 ± 6.7
3	Parker ultra fine navigator(M)	0.5	220 ± 4.7
4	Cello Techno tip(M)	0.6	250 ± 5.2
5	Pilot V7(M)	0.7	320 ± 5.0
6	Staedtler Lumocolor (F)	0.4	160 ± 10.2
7	Graphtech 0il based (F)	0.4	200 ± 12.8
8	Staedtler Lumocolor (F)	0.6	250 ± 11.0
9	Graphtech Water based(F)	0.7	420 ± 16.8

The key factor in the FARM method is the tip size and tip material of the pens used for transferring the patterns to the foil. To avoid any variation while writing, all the pen tips were mounted on pen holder and aligner (PHP32, GRAPHTEC, Japan (Fig S4†). In order to investigate the reproducibility and morphology of the structures, we have used multiple pens with fibre and metal tips. The sizes of channels were dependent upon the tip size. As expected, a smaller tip with 0.3 mm diameter created smaller channels (150 ± 5.5 µm) while pen tip of 0.7 mm produced larger channels (315-435 µm). The variation in channel size with tip size of 9 different pen tips used in this study is shown in Table 1. The data clearly indicates the reproducibility of the FARM method for fabrication of microfluidic channels. It is important to note that variations in the channel dimensions also depend on the tip material. It is clear from the data presented in the Table. 1 that a metal tip creates less variation (between 4.7 and 6.7 Relative Standard Deviation (RSD)) in sizes compared to fibre tip pens (between 10 and 16.8 RSD). The Scanning Electron Microscopy (SEM) images (Fig. 2) show that metal tips are more likely to produce uniform and smooth channels (Fig.2 (A)) compared to those made using fibre tips (Fig.2 (B)). Therefore, for the remainder of the work, metal tips were used to create foil molds. The cross-section (CS) view of the 150 µm and 400 µm channels shown in Fig. 2 (C) and (D) clearly indicated the plano-concave shape of the fabricated channels. Depending on the pen tip used, the depth of the channel achieved varies from 20 to 25 µm for metal tip, while in case of fibre tip pen this variation is about 19 to 29 µm. We have also observed that the depth of the channel also depends on pen speed of the plotter (result not shown). The SEM image shown in Figure 2 (C) shows channel depth of ~21 µm using a metal tip, while channel depth of ~28 µm was observed using a fibre tip (Figure 2 (D)) . The masters/molds fabricated by the traditional micromachining methods are in general square in shape. This square shape of the channels has several disadvantages: (1) corners of rectangular channels create problems of undesired sample trapping which could lead to severe contamination[14], (2) reduced flow speed near the breakpoint of droplet formation in the channel can lead to longer droplets than desired[15], (3) inefficient micro-check valve as the corner of square channel cannot be "sealed" properly[13] (4) also a square cross-section may lead to jamming of channels with micro-particles during the generation of polymer micro-particles.

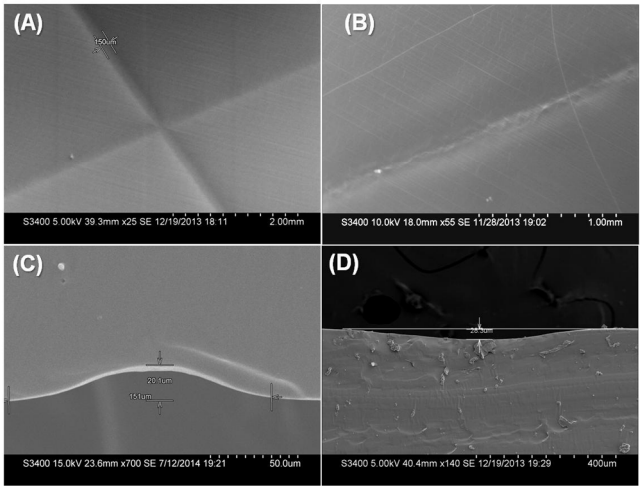

Fig. 2 SEM images of (A) PDMS replica of channel structure created by metal tip (B)) PDMS replica of channel structure created by fibre tip and CS of shape of channel prepared using (C) metal tip and (D) fibre tip.

Creating channels of semi-circular or plano-concave shaped channels is important for various applications such as plumbing or creating valves for lab-on-a-chip devices. Usually, such shapes of channels are only possible using complex methodologies[13].

To study cytocompatibility of the FARM devices, we have successfully demonstrated the growth of two different cell lines (GFP-MDA-MB, IMR-32) in microfluidic devices fabricated using the novel FARM method. The cells that were entrapped in the microchannels showed proliferation and differentiation with little/no signs of mortality, even after 34 hrs of incubation with continued flow of perfusion medium flow suggesting the cytocompatibility of the device. The GFP-tagged MDA-MB cells that were entrapped in the microchannels appeared viable which was reflected by the strong green fluorescence of gene expression (Fig 3 (A,B,C)). In parallel, the IMR-32 cells were seen to adhere in the microchannels and appeared bright (Fig 3

(D,E)). The overall percentage of cell survival in both the cell lines was recorded above 75% (data not shown). We suggest that our novel devices fabricated using the FARM technique could be exploited to study cell behaviour and characteristics in a constrained environment. Moreover, the FARM devices are easy to fabricate and can be used for a range of biological applications. Though they are cost-effective and simple, they function at par with the devices fabricated using sophisticated instrumentation. Our engineered devices should address challenges associated with cell dynamics in a constrained environment.

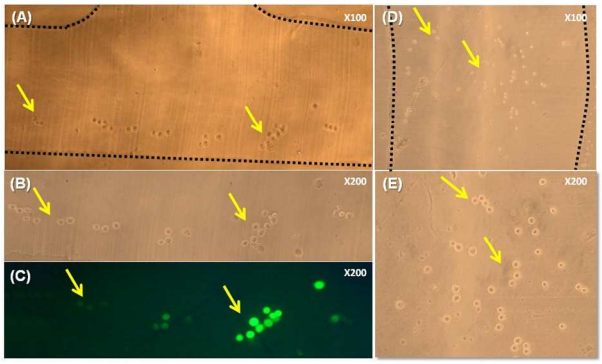

Fig.3 The gfp MDA-MB (A, B, C) and IMR-32 (D, E) cells within the FARM microchannels. Arrow represents the entrapped cells in the microchannels. The dotted line indicates the boundary of the micro-channels.

Conclusion

In this study we have demonstrated for the first time a facile, foil assisted replica molding method which can be used without sophisticated infrastructure, to fabricate microfluidic devices of varying sizes. FARM is a low cost relatively simple laboratory technique for rapid prototyping of microfluidic devices with complex structures which can be made within 2-3 hrs. A silicon mold made by conventional methods costs ~ US$ 125 to 1500, depending on cost of wafers, photoresists, photomask/s and other chemicals used in the process. On the other hand, a 6 inch diameter foil mold will cost < US$ 1, keeping in mind that a 12 inch X 100 foot roll of Sigma aluminum foil costs ~ US$ 190. We suggest that with further advances in pen-plotters much improved resolutions can be achieved and far better devices can be fabricated. Also, using small metal tip pens with 0.1 mm tip size, employed in the field of architecture, can lead to smaller channels. We are currently exploring the possibility of altering the depth of channels by exploiting the Z–axis of the plotter. In doing this, one can create multilayer devices by programming the pen plotter. The micro-channels created from the FARM method could mimic the microenvironment *in vivo*, and our new miniature devices could be used as a potential alternative for controlled and better understanding of cell dynamics more precisely *in vitro*. We are currently exploring more biological applications with this simple fabrication method. We strongly believe that this is a promising technique which does not require any state-of-the-art facilities for fabrication of micro-devices. Further investigations are needed to develop affordable microfluidic devices for biological, chemical and clinical applications.

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Notes and references

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† Material, chemicals and various aluminium foil molds, PDMS devices, pen and tips used and AFM image with roughness date are shown in ESI.

Electronic Supplementary Information (ESI) available: [Images of various aluminium foil molds and corresponding PDMS device, AFM image and roughness data of aluminium foil, image of pen holder used for aligning the tips are included in ESI]. See DOI: 10.1039/b000000x/

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Foil assisted replica molding for fabrication of microfluidic devices and their application *in vitro*

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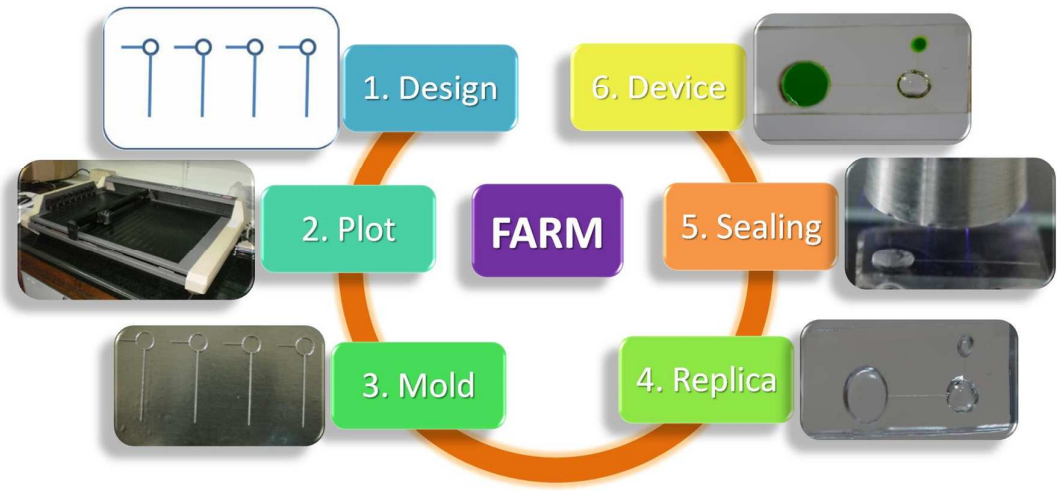

In this report, we present a non-traditional, facile, rapid, benchtop and cost-effective way to fabricate microfluidic devices in laboratory atmosphere with minimal infrastruture. We have named this method as "foil assisted replica molding" (FARM) for fabrication of microfluidic devices. This novel technique involves use of aluminium foil, pen and an X-Y plotter to create plano-concave and shallow microchannels of varing sizes. It is an easy do-it-yourself (DIY) technique for creating microfluidic devices. Furthermore, we have demonstrated the cytocompatabiliy of these devices *in vitro*.