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TECHNICAL INNOVATION

Ultra-rapid prototyping of flexible, multi-layered microfluidic devices via razor writing

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The fabrication of microfluidic devices is often still a time-consuming and costly process. Here we introduce a very simple and cheap microfabrication process based on "razor writing", also termed xurography, for the ultra-rapid prototyping of microfluidic devices. Thin poly(dimethylsiloxane) (PDMS) membranes are spin-coated on a flexible plastic foil and cut into user-defined shapes with a bench-top cutter plotter. The PDMS membranes can then be assembled into desirable microdevices via plasma-bonding. The plastic foil allows manipulating exceptionally thin (30 - 300 μm) PDMS layers and can be readily peeled off after fabrication. This versatile technique can be used to produce a wide variety of microfluidic device prototypes within just a few hours.

Introduction

The ever-increasing application of microfluidic technology in chemistry, biology and medicine that we are witnessing, calls for simpler and cheaper fabrication methods that do not rely on expensive clean room-based processes. Razor writing, or xurography has emerged as an attractive alternative for the fabrication of micro-structured templates for replica molding¹, or stencils for glass etching². Despite a substantial reduction in the resolution compared to photolithography (ca. 10 μm versus 30 nm), this method drastically reduces the time and cost for microfluidic chip fabrication. More recently, xurography has been used to generate microfluidic systems for sensor applications by cutting poly(dimethylsiloxane) (PDMS) layers that were coated on double-sided tape³. However, the use of such tapes to bond microfluidic chips suffers from several drawbacks such as cell toxicity and a poor resistance to high pressure. Moreover, this technique does not allow the fabrication of flexible devices that are for example relevant for cell culture applications that involve mechanical stimulation.

To address these issues, we here present a simple and cost-effective xurography-based method for the ultra-rapid prototyping of flexible microfluidic devices. Micron-sized PDMS layers are first spin-coated on a plastic foil and then patterned with user-defined micro-scale structures via xurography. In a final step, the microstructured PDMS layers are plasma-bonded onto various substrates such as glass or PDMS in order to generate a desired microfluidic device. We show by way of several examples how this method can be used to generate novel microfluidic chips, including flexible devices and three-dimensional (3D) multi-layered microfluidic chips.

Materials and Methods

Spin coating of PDMS layers

PDMS (Sylgar 184 silicone elastomer, Dow Corning) chips were prepared at two ratios of elastomer to curing agent (10:1 and 5:1) and degassed for 20-30 minutes. The PDMS mix was poured onto a plastic foil that was placed within a Petri dish, and spin coated for one minute at variable rotational speeds. The PDMS layers bound to plastic foils (common plastic transparency, Sigel, Switzerland) were baked for one hour at 80°C and then stored in a dust-free environment before use. The thicknesses of the resulting PDMS layers on plastic foil were measured using a material thickness gauge.

Microfluidic device fabrication

A robotic cutter plotter (Robo Pro CE5000-40-CPR) was used to cut user-defined features into the PDMS layers. Any desired design can be created with the dedicated ROBO Master-Pro software. The PDMS layer was placed on a supportive double-sided adhesive (100 μm thick) and fed to the cutter plotter. The cutter plotter was set at the lowest possible cutting speed, with an off-set of zero and a force from 6-10 N, depending on the substrate thickness. Excess cut PDMS was removed manually with tweezers and then stored in a dust-free environment before use. The cut PDMS layers were first plasma bonded on glass or PDMS substrates, and then baked for one hour at 80°C. The plastic foil was manually removed. A PDMS slab (or thin film on plastic foil depending on the application) was plasma-bonded on the construct before backing it at 80°C for one hour. Inlets and outlets were then punched with a blunt needle. The microfluidic devices were stored in a dust-free environment before use.

PDMS membrane perforation

For the microfluidic 3D water-in-oil droplet generator, holes were fabricated by laser ablation⁴ using a laser micro-dissection microscope (PALM, Zeiss). PDMS layers (<100 μm thick), plasma bonded to the partially assembled microfluidic chip, were perforated as follows: (1) the device was placed under the laser micro-dissection microscope, membrane facing down, (2) the software was utilized to define the cutting area (circle $\varnothing = 50 \mu\text{m}$) and set to cut the designed area three times at 90-95% intensity with no off-set, (3) the laser ablation was initiated and monitored live, and (4) the perforated chip was recovered and PDMS particles blown away with an air gun.

Microscopy

Microscopy (Bright-field and fluorescence) was performed using a Zeiss Axio Observer (Metamorph).

Cell culture

C2C12 myoblasts were cultured in expansion medium DMEM (Gibco) supplemented with 15% fetal bovine serum (Gibco), 1% penicillin/streptavidin, 1 mM sodium pyruvate (Gibco) and 10 mM HEPES (Gibco), or differentiation medium DMEM, supplemented with 2% horse serum (Gibco), 1% penicillin/streptavidin, 1 mM sodium pyruvate (Gibco) and 10 mM HEPES (Gibco). Live/dead staining (Invitrogen) was performed according to manufacturer's instructions.

Results and discussion

Resolution of PDMS xurography

Our bench-top method to fabricate microfluidic devices is very simple and versatile as shown in **Figure 1a** and **Supplementary Video 1**. First, a PDMS layer is spin-coated onto a plastic foil and baked for one hour at 80°C. Two ratios of elastomer to curing agent were used (5:1 and 10:1). PDMS layer thicknesses between 34 ± 2 and $200 \pm 10 \mu\text{m}$ (at 5:1 ratio) and 138 ± 2 and $307 \pm 25 \mu\text{m}$ (at 10:1 ratio), respectively, can be obtained, depending also on the rotational speed (**Fig. 1b**). The deposition of PDMS coatings on the flexible plastic foil facilitates handling, especially for PDMS layers with a thickness below 100 μm . Importantly, the plastic foil can be easily peeled off once the PDMS is bonded to a substrate of choice.

To assess the resolution that can be obtained with our specific robotic cutter plotter (Robo Pro CE5000-40-CPR) and the different PDMS formulations, a series of structures of defined size and spacing were generated (**Fig. 1c-e**). Overall, more reliable structures down to 100 μm were obtained with a 5:1 curing ratio, whereas the same structures tended to get damaged with 10:1 formulations (**Fig. 1c**). In both cases, rectangles of 100 μm width of interspacing often ripped off (**Fig. 1d**, black arrow). However, it is possible to overcome this limitation in resolution by modifying the drawing of the microfluidic layout using the cutter plotter software. In this

case, small microchannel intersections down to $\sim 20 \mu\text{m}$ can be obtained (**Fig 1e**). Image analysis of the generated features (i.e. an array of 500 μm wide rectangles cut in 5:1 PDMS layers of 150 μm thickness) was used to assess the resolution of our method. This analysis showed that cut features correlate with $98.7 \pm 4.4 \%$ accuracy to the dimensions designed by the software. However, this accuracy dropped significantly with smaller feature sizes and channels below 100 μm could not be cut (data not shown). It should also be noted that the relatively low resolution compared to standard photolithography is sufficient for many applications, and that better a resolution can be obtained with more expensive cutter plotter models. Indeed, a cutter plotter with an addressable resolution down to 10 μm was reported elsewhere¹.

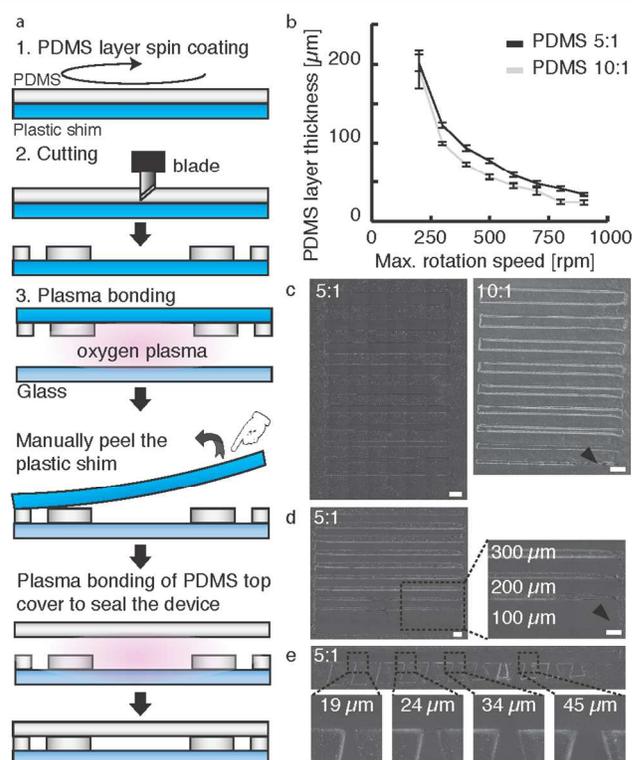


Figure 1: Scheme and resolution assessment of microfluidic device fabrication by direct PDMS layer razor writing. **a.** General method used to fabricate our devices. 1. PDMS is spin-coated on a plastic foil to obtain a thin layer. 2. A robotic cutter plotter is used to cut out the desired microfluidics designs. 3. The layer is bonded on the two sides with PDMS and glass substrates to obtain a ready-to-use microfluidic chip. **b.** Graphical representation of the dependence of PDMS layer thickness on the spin speed (1 minute). **c.** Representative stitched micrograph of features fabricated in PDMS layer by razor writing. Cut rectangles of 1 cm in length and a height from 500 μm to 100 μm in 5:1 PDMS. In 10:1 PDMS, rectangles with a height of 200 μm and above were obtained. **d.** Representative stitched micrograph of features fabricated in PDMS layer by razor writing. Cut rectangles (500 μm width and 1 cm length) with spacing from 500 to 100 μm . The cutter plotter enabled interspacing of 200 μm and above. No PDMS was left between cut rectangles of 100 μm spacing (black arrow). **e.** Representative stitched micrograph of features fabricated in PDMS layer by razor writing. Cut intersection of a rectangle and triangles, each triangle was cut 100 μm closer to the centre of the rectangular channel. Resulting intersection can be fabricated to sizes down to $\pm 20 \mu\text{m}$. PDMS thickness was set to $\sim 75 \mu\text{m}$ (600rpm) for assessing the resolution of the cutting procedure (Scale bar = 500 μm)

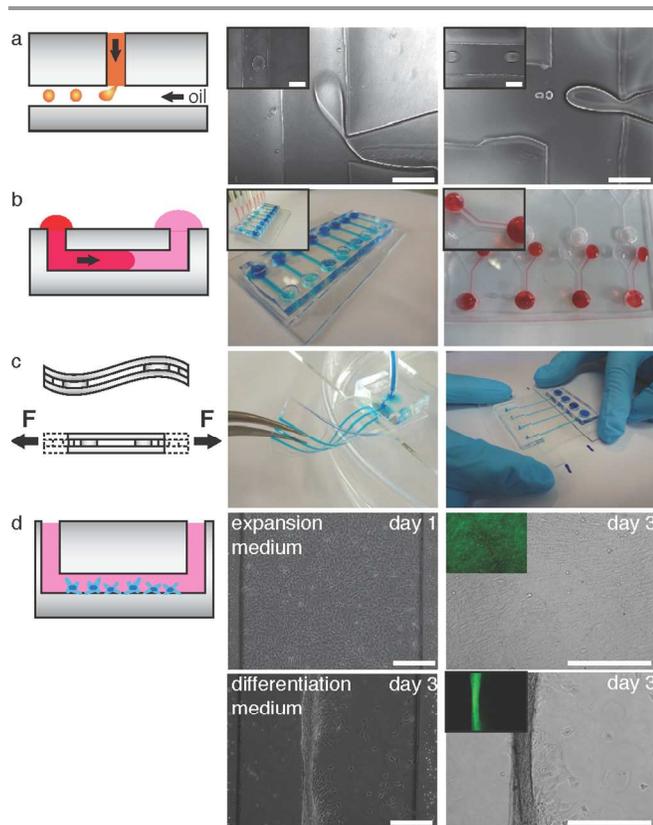


Figure 2: Examples of relatively simple microfluidic devices fabricated by direct PDMS layer razor writing. **a.** Microfluidic water-in-oil droplet generator. (center) Micrograph of water-in-oil droplet formation with a T-junction chip. (left) Micrograph of water-in-oil droplet formation with a flow-focusing chip. Microchannel dimensions: height: 75 μm , width: 200 μm . Nozzle width: 50 μm (left) and 20 μm (right). **b.** Arrays of parallel microfluidic networks. (center) Picture of an array of microchannels filled with a multichannel pipette. (left) Picture of an array of microfluidic Y-junctions operated pump-less, that is, via flow driven by surface tension. Microchannel dimensions, left panel: height: 75 μm , width: 500 μm , length: 2 cm; right panel height: 75 μm , width: 200 μm , length: 2 cm. **c.** Flexible and stretchable microfluidic devices. (center) Picture of a flexible microfluidic device. (left) Picture of a stretchable microfluidic device. Microchannel dimensions: height: 75 μm , width: 200 μm , length: 5 cm. Nozzle width: 50 μm (left) and 20 μm (right). Plain PDMS layers were 75 μm thick. **d.** Microfluidic chips for cell culture. C2C12 were cultured in our microfluidic chips for a period of 3 days. Inserts show fluorescent micrographs of a live (green) and dead (red) staining to assess cell survival on chip. (Scale bar = 100 μm)

Fabrication of relatively simple microfluidic devices

To illustrate the versatility of our ultra-rapid prototyping method, we fabricated a range of microfluidic devices (Fig. 2) such as two variants of water-in-oil droplet generators, a T-junction and a flow focusing design (Fig. 2a). The T-junction yielded stable and controlled droplet generation (left insert Fig. 2a) while with the flow focusing design it was challenging to perfectly align the nozzle with the centre of the collecting channel. Nevertheless, stable droplet generation could be achieved with this design (right insert Fig. 2a). Parallelized microfluidic devices that are compatible with conventional multi-well plate formats were also fabricated with our method (Fig. 2b). To this end, arrays of straight channels or Y-junctions were fabricated. Inlets were designed to be compatible with standard multichannel pipettes which enables

operating these systems by flow that is driven by surface tension⁵.

Flexible or stretchable microfluidic devices can be of great use for advanced cell culture applications such as those that employ the mechanical stimulation of cells⁶. To demonstrate that such devices can be produced by our method, we replaced glass slides or thick PDMS slabs with thin spin-coated PDMS films. Indeed, flexible and stretchable microfluidic devices that can be perfused with liquid were readily obtained (Fig. 2c). Our devices were typically perfused with a flow rate up to 100 $\mu\text{L}/\text{min}$. No noticeable leak was observed (data not shown).

To show that our fabrication method is fully compatible with cell culture, we fabricated simple micro-culture devices for mammalian cell culture (Fig. 2d). For example, C2C12 myoblasts were cultured in microchannels in either expansion or differentiation culture conditions for three days. The chips can indeed be successfully used for myoblast culture under both culture conditions, as is evident from the micrographs shown in Figure 2d. A live/dead assay showed that after three days of culture no significant cell death occurred in these micro-scale in vitro experiments (inserts of Fig. 2d).

Fabrication of multi-layered microfluidic chips

We next sought to fabricate more complex, multi-layered microfluidic chips (Fig. 3). For example, arrays of overpass microchannels were fabricated by stacking three layers (Fig. 3a). The first and third layers comprised the fluidic layers, whereas the second layer was used to connect the bottom and

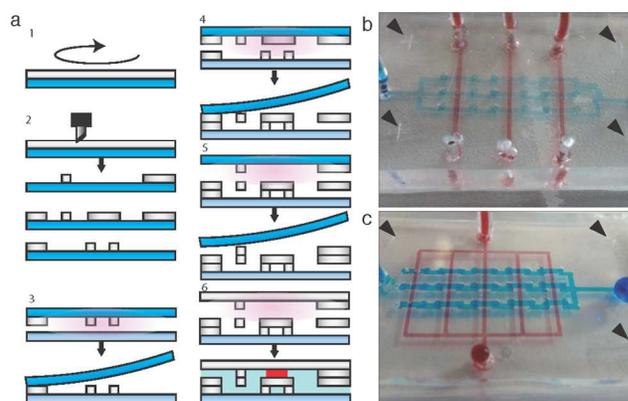


Figure 3: Images of multi-layer microfluidic devices fabricated by layer stacking. **a.** Schematic representation of multi-layer device fabrication. 1. PDMS layer spin coating on plastic foil. 2. Cut out the PDMS layer on plastic foil with desired features. 3. Plasma bonding of the first layer on a glass slide. 4. Alignment and plasma bonding of the second layer and removal of the plastic foil. 5. Alignment and plasma bonding of the third layer and removal of the plastic foil. 6. Plasma bonding of a PDMS slab onto the last PDMS layer and punching of the inlets and outlets yielding a functional microfluidic device. **b.** Simple multi-layer microfluidic chip with multiple overpass microchannels filled with coloured water for better visualization. Black arrow indicate alignment marks. **c.** More complex multi-layer microfluidic chip with multiple overpass microchannels filled with dyed water for visualization. Black arrow indicate alignment marks. Microchannel dimensions of each layer: Height 75 μm and width 200 μm . Fluids were perfused with a syringe pump at a flow rate of 100 $\mu\text{L}/\text{min}$.

top layers with the use of via to link the upper and lower fluidic layers. Alignment was achieved by using simple alignment

marks (Fig 3b, black arrows) that were cut on the four edges of each layer. Afterwards, the stacked layers were bound onto a glass slide, a PDMS block was put on top, and access holes were punched to create inlets and outlets. The resulting 3D microfluidic networks were fully functional as demonstrated by dye perfusion experiments (Fig. 3b,c). However, it should be noted that our method to generate 3D microfluidic networks is limited in its scope in that the fabrication of microchannels with varying depths or the fabrication of micro-post arrays within microchannel is not possible.

Fabrication of a 3D water-in-oil droplet generator

To further illustrate the versatility of our method, we applied it in combination with laser ablation to yield a 3D water-in-oil droplet-generating nozzle (Fig. 4). To this end, a two-layer microfluidic chip was fabricated and laser ablation, used here because of the resolution limitations of the cutter plotter, that we had available was used to generate a small (*i.e.* 50 μ m in diameter) hole within the thin (<100 μ m) PDMS membrane that separated the two fluidic layers (Fig. 4a). This process is highly reproducible and reliable. The **supplementary video 2** shows the laser ablation process in detail.

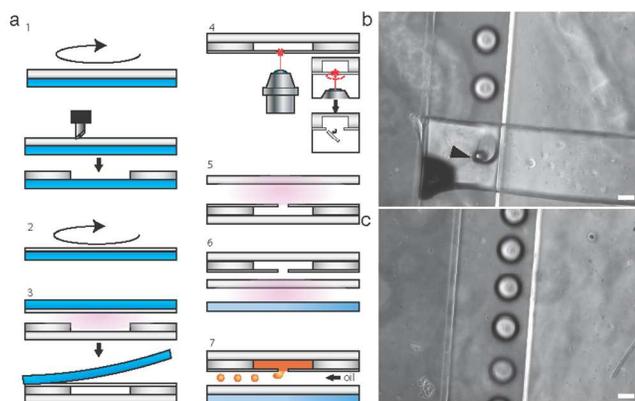


Figure 4: 3D microfluidic water-in-oil droplet generator. **a.** Schematic representation of 3D water-in-oil droplet generator fabrication. 1. PDMS layer are spin coated on plastic foil and then cut to generate desired features. 2. PDMS layer spin coating on plastic foil. 3. Plasma bonding of the thin PDMS layer onto the cut PDMS layer. 4. Laser ablation of a small hole through the thin PDMS layer. 5. Alignment and plasma bonding of the cut second layer on the perforated thin PDMS membrane. 6. Plasma bonding of the device on a glass slide and generation of inlets and outlets. 7. Perfusion of the device with oil and a water solution in their respective fluidic layer to generate water-in-oil droplets. **b.** Micrograph of water-in-oil droplet generation using the 3D T-junction chip. (black arrow) Laser ablated hole through the thin PDMS membrane. **c.** Micrograph of water-in-oil droplet generated using the 3D droplet generator. Oil was perfused at 50 μ L/min and the aqueous phase at 10 μ L/min. (Scale bar = 100 μ m)

To generate water-in-oil droplets, an aqueous solution was perfused in the upper fluidic level and the oil phase in the bottom layer. With the chosen microchannel configuration, the small hole enabled the fabrication of a 3D T-junction nozzle for stable water-in-oil droplet generation (Fig. 4b,c and **supplementary video 3** and 4). Image analysis performed to characterize droplet generation displays a narrow droplet distribution (Fig. S1). We believe that this system could be

easily parallelized⁷ to yield high throughput droplet generators for screening applications. This example shows that even more complex prototypes can be rapidly fabricated using our method.

Conclusions

We report a simple and versatile bench-top technique for the prototyping of microfluidic devices. Despite its limitation in resolution, our methods should be attractive for the fabrication of a wide variety of microfluidic devices. Our approach is ultra-rapid, taking only a few hours from the conception to the fabrication of fully functional microfluidic devices. The fabrication of functional microfluidic devices that are compatible with cell culture and resistant to high pressure was demonstrated, solving problems of state-of-the-art xurography techniques.

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Notes and references

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