Rapid fabrication of circular channel microfluidic flow-focusing devices for hydrogel droplet generation

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A rapid method to fabricate a flow-focusing microfluidic device with circular channels for droplet generation is presented. To create the desired 'polydimethylsiloxane (PDMS)' channel geometry, moulds using a rapid and cheap three dimensional (3D) printing process comparing with conventional microfabrication method have been fabricated. The 3D printer with a 16 μm layer resolution utilised to fabricate 100 μm half circular microfluidic moulds. The finished moulds were baked and silanized prior to casting to avoid the incomplete curing problem of the 'PDMS' castings. Casted 'PDMS' halves were aligned and bonded together to build complete microfluidic chips by an oxygen plasma treatment. Due to the resolution limitation of 3D printing, the channels were not perfectly circular rather than elliptical. A liquid 'PDMS' injection process was used and optimised to create fully circular channels, and also address challenges regarding the misalignment of the upper and lower halves of the microfluidic chip. Circular channels were successfully formed in the flow-focusing microfluidic device through the post 'PDMS' injection process without any blockage of the cross junction. The device functioned well to create ~200 μm droplets.

1. Introduction: Microfluidic devices have been widely used to create microdroplets for various applications such as tissue engineering [1], drug delivery [2] and microreactors [3]. These systems are high-throughput and cost-effective [4]. There are different approaches to generate droplets in microfluidic systems, namely; T-junction, co-axial, and flow-focusing methods [5–8]. In each of these methods, two immiscible fluid phases (disperse and continuous phase) are typically infused from two different inlets and flow into a junction. When these phases meet at the junction, the viscous force of the continuous fluid overcomes the surface tension of the disperse fluid. As a result, the disperse fluid breaks up to form droplets [6] (see Fig. 1).

A photolithography microfabrication method [9] has been used to fabricate a master mould to cast microfluidic channel devices for droplet generation. However, the cross-sectional shape of the channels fabricated by the photolithography technique is limited to a rectangular shape. The rectangular channel has several drawbacks to generate droplets [10]. In the rectangular channel, the disperse phase fluid wets the top and bottom walls at a low flow rate of continuous phase fluid. Wetting walls has two disadvantages: (i) Cells mixed in disperse phase tend to attach to the walls or are damaged because of shear force at surface [11]; and (ii) Capillary instability is changed and in result droplet break up is not controllable [12, 13]. By increasing the flow rate of a continuous phase, wetting surface of disperse phase might be eliminated; however, another problem occurs such as device leaking and cell damage because of high shear force. Another important factor in droplet generation is the uniformity of droplets. To generate uniform droplets, a uniform velocity profile on the cross-sectional direction is required. Since the circular microfluidic channel is truly symmetrical unlike the rectangular cross-sectional channel, it can generate the uniform velocity profile in the cross-sectional direction [14]. Circular channels that squeeze the disperse fluid in all direction prevents disperse fluid to wet the walls. This results in creating well controllable, monodisperse droplets. Moreover, rectangular channels are more likely to clog due to aggregation of synthesised droplets [15].

Due to these disadvantages, alternative fabrication methods have been developed to fabricate circular channels. For example, one method involves a trench imbedded in a silicon wafer. The trench is then filled with a thick layer of doped silicon oxide, followed by heating, which closes the trench, and creates a circular channel [16]. However, this technique is able to only fabricate small-sized silicon channels (a few micrometres in diameter).

Another method for making cylindrical channels utilised the capillary rise of liquid 'polydimethylsiloxane (PDMS)' inside an open channel in a 'PDMS' slab [17]. This method is not practical for fabricating circular channels smaller than 100 μm. Abdelgawad *et al.* [10] introduced a new method of fabricating circular channel by injecting liquid 'PDMS' through a rectangular channel, fabricated by the microfabrication soft lithography method, followed by passing air stream in channel filled with liquid 'PDMS'. 'PDMS' injection fills the corner of rectangular channel which can make simple circular channel. In addition to these methods, standard fabrication methods such as three dimensional (3D) printing [18], micro-cross construction [19] and laser machining [20] have also been used for fabricating circular channels. Moreover, the circular microfluidic channel was created using the combination of micromoulding and softlitoghraphy [21] and photolithographic reflowable photoresist [22]. The mould fabrication using these methods often requires many processing steps. The fabrication methods for circular microfluidic channels using a metal wire removal process [23] and monofilament threads [24] have been also reported. However, these techniques have limitation to fabricate the cross junction for the flow-focusing droplet generation. Among these methods, due to the emergence of high resolution 3D printers, 3D printing provides a simple and efficient tool for creating the moulds (with micro-sized features) that can be used for fabricating microfluidic channels with circular cross section.

In one of the first applications of 3D printing to microfabrication, McDonald *et al.* [25] utilised a low resolution (above 250 μm) 3D printer for the fabrication of microfluidic devices. Thomas *et al.* later investigated the print and peel microfabrication method. To fabricate a 3D printed master mould, they simply printed a mould for casting the polymer, and then assembled a 3D element on the mould [26]. Boynar *et al.* [27, 28] used a high resolution (16 μm) 3D printer to fabricate microfluidic master mould with different shapes of channels. Recently, 3D printing has been used to fabricate circular microfluidic channels for flow focusing applications [29]. However, the fabricated circular channels were not perfectly

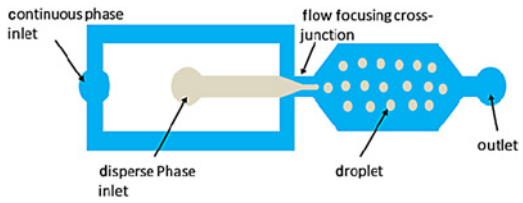


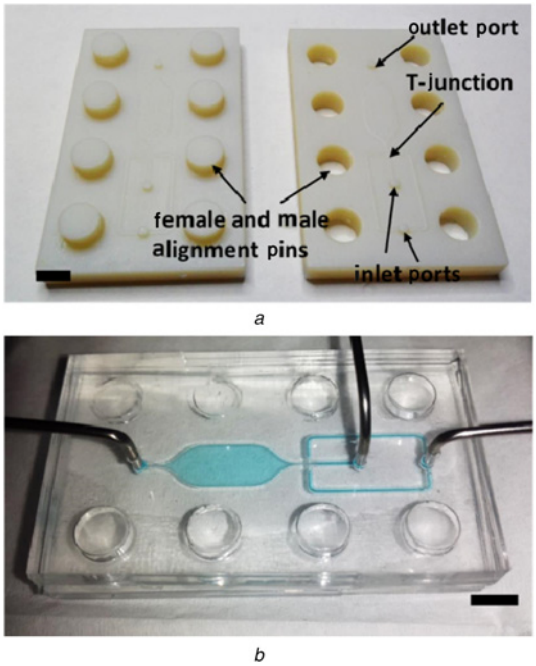
Fig. 1 *Schematic of microfluidic flow-focusing device*

circular. Therefore, although the resolution of 3D printing has increased, creating a perfect circular microfluidic channel for micro droplet generation remains a challenge.

In this Letter, a fast, low cost technique is presented for fabricating circular channel flow-focusing microfluidic devices using a 3D printing method. Challenges regarding the use of 3D printed moulds in the peel-off process, removing cloudy surfaces finishes in the castings, getting fully circular geometries, and fixing misalignment have been addressed. The flow-focusing channels are used to generate hydrogel droplets ~200 µm in diameter, with the diameter of droplet being controlled by the flow rates of the fluid.

2. Methods and materials

2.1. Mould fabrication: The 'PDMS' mould is designed using SolidWorks® (Dassault Systems, Vélizy-Villacoublay, France) software. Two identical moulds are designed with similar geometry except for the alignment pins. The male and female alignment features are made so that the two halves of the microfluidic chip would align as desired [29]. An STL format of the mould is then printed (see Fig. 2*a*) with a poly jet 3D printer (Object500 Connex, Stratasys Ltd., Eden Prairie, USA) using VeroWhite-FullCure®830 (Stratasys Ltd., Eden Prairie, USA) materials at 16 µm layer resolution in Z direction and 45 µm build resolution in X-Y direction. The moulds were fabricated with a glossy surface finish to allow smooth surface with the roughness of 3.8 µm previously reported in [30]. After printing, the mould is baked at 80°C overnight. The part is then silanized with Trichloro (1H, 1H, 2H, 2H-perfluro-octyl) silane (Sigma-Aldrich, St. Louis, USA). Salinization is performed by adding 50 µl of the silane solution to a petri dish and placing the petri dish and the moulds into a desiccator for 30 min. These processes are necessary to easily peel the cured 'PDMS' channel from the mould made by the VeroWhite material [31].



a

b

Fig. 2 *Device fabrication using 3D printed moulds*
a Pictures of 3D printed moulds for the microfluidic chip castings with features labelled
b Assembled microfluidic chip with light blue dye shows channels with no leakage observable. Scale bar = 5 mm

2.2. 'PDMS' casting: The casting process creates the two halves of the microfluidic channel device with their internal geometries. It is important that the reusable moulds are first properly prepared by cleaning the surface with isopropanol to ensure that the surface is particle and residue free. The two halves of the microfluidic chip are made by pouring the liquid 'PDMS' (SYLGARD 184, Dow Corning Co., Midland, USA) over the moulds in a petri dish. The 'PDMS' is made of an elastomer and a curing agent mixture with a 10:1 ratio. Once the liquid mixture is poured, it is placed in a desiccator for approximately 90 min or until all the bubbles have dissipated. The mould is then placed in an oven at 70°C for 3 hours to solidify the 'PDMS'. Once the 'PDMS' has cured, it can be peeled from the petri dish and mould.

2.3. Assembling the microfluidic chip: The procedure for assembling the two halves of the microfluidic parts into a chip are as follows. First, the holes for the inlet and outlets are cut using a hole punch. To get a strong bond between the two halves of the microfluidic chip, the surfaces of both chips must be clean. To clean the 'PDMS' parts, they are placed in an ultrasonic bath with reverse osmosis (RO) water for approximately 5 min. When the parts are dry, they are exposed to a hand-held corona device (BD-20, Electro-Technic Products, USA) for 5 min (at 30 s/cm²) which is necessary to achieve a strong bond [32]. Finally, the two halves are put together and clamped for 72 h to allow the bond to cure. The bonding of device is checked with the injection of coloured liquid shown in Fig. 2*b*.

2.4. Liquid 'PDMS' injection: To obtain circular channels, a 'PDMS' injection coating method [10] is adopted. A 2:1 liquid heptane/PDMS mixture is injected into the microfluidic chip, and then allowed to cure while an air stream flowed through the channels. The 'PDMS' is the standard 10:1 base to curing agent ratio. After the heptane/PDMS is fully injected into the internal geometries of the chip, the chip is placed on a hotplate for 60 s at 100°C. After 60 s, air is pumped into the chip at a constant flow rate of 24 ml/min. Air is pumped into all inlets and allowed to escape through all outlets. It is important that the air is forced through all the channels to prevent blockages in the device. The chip should remain on the hotplate, with constant air flow, for 10 min. This process can be repeated to obtain smaller diameter channels with slight variations to curing times, baking temperatures and air flow volumes. Circular channels ranging from 5 to 200 µm were successfully fabricated by Abdelgawad *et al.* [10].

2.5. Hydrogel synthesis and droplet generation: Photocrosslinkable 'gelatin methacrylate (GelMA)' hydrogel was used to test the droplet generation and synthesised by the method described in [8]. Briefly, 5 g of gelatin was dissolved into 45 ml of dimethyl sulfoxide at 50°C. Afterward, 0.5 g of 4-(dimethylamino)-pyridine was dissolved in the solution. Then, 0.5 g of glycidyl methacrylate was slowly added to the solution while it was stirring at 50°C. The reaction was kept at 50°C for 48 h. After that, the solution was dialysed against RO water by using a dialysis membrane tube (molecular weight cut off: 12000–14000 Da; Fisher Scientific) for 3 days, while the deionised water was being changed twice a day. Finally, the dried hydrogel was made through the lyophilisation process. 'GelMA' prepolymer solutions were prepared by dissolving 4 wt% of 'GelMA' with 2 wt%

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of 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropio-phenone in phosphate buffered saline. The microfluidic flow focusing device is used to generate spherical 'GelMA' microgels. The 'GelMA' prepolymer solution was used as disperse phase. The continuous phase consisted of mineral oil supplemented with Span 80 as a surfactant (20%), which decreases the surface tension between oil and prepolymer solution. Unless stated otherwise, all materials were purchased from Sigma-Aldrich, St. Louis, USA.

3. Results and discussion: The fabrication process explained in the previous section was used to fabricate a circular channel microfluidic flow-focusing droplet generator. The half circular channels were fabricated in 'PDMS', and bonded to each other. If the 'PDMS' is not fully cured (where it is in contact with the mould), it could indicate that the VeroWhite material was interfering with the curing process. As mentioned in the previous section, this problem can be mitigated by baking and silanizing the mould. Another potential problem with casting is a cloudy or rough surface finish. This can be mitigated by ensuring the mould is free of any residue. In addition, the curing temperature during the casting process has the potential to warp to mould. Therefore, to decrease the chance of warping the mould, it is important to keep any temperature during casting below 70°C.

As shown in Fig. 2*b*, the two parts were successfully attached to each other and no leakage was observed. Acceptable bonding strength between the two halves of this microfluidic chip can be difficult to obtain consistently. Thus, it is important that the surfaces between the two halves of the microfluidic chip are in full contact with one another. Warped moulds, or casting the 'PDMS' on uneven surfaces, can lead to problems with achieving full surface contact. Using a clamp is a good way to ensure full contact. The bond was found to be strong enough to carry out experiments without any leakage from the channels shown in Fig. 2*b*.

Due to the limitations of the 3D printer, the ∼200 µm channels fabricated using the 3D printed moulds were elliptic shaped as opposed to the desired circular. Fig. 3*a* shows a cross section of the 'PDMS' channel after the assembly. As can be seen, the width of the individual channel was extended and the depth of the channel was too shallow. The channels were elliptic shaped with pointed edges where the two halves of the 'PDMS' join together. This problem was caused by the limitations of printing at the micro scale, as the printer material cannot hold the desired half circle shape and collapses into the 'mound' shape. Even 3D printers with high resolution were not able to fabricate sharp edge of half circular channel. In result, a perfect circular channel was not created after bonding two 'PDMS' halves together. Therefore, post 'PDMS' injection was required to cover misalignment and create fully circular channel. To achieve circular channels, we injected the liquid 'PDMS' solution and cured it by pumping air flow through channels described in the method section. A common problem associated with this process was the air not reaching all of the outlets. Air can create a path to one outlet, and not

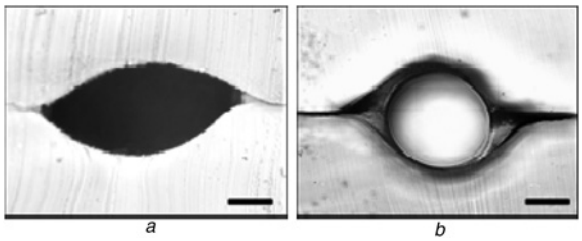

Fig. 3 *Optical microscope images of the cross-section of channels*
a Cross-section of an elliptical channel prior to PDMS injection
b a circular channel with 230 µm in diameter post PDMS injection and curing procedure. Scale bar = 100 µm

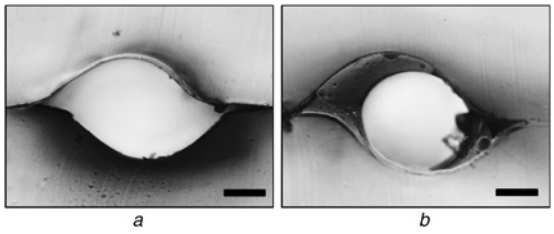

Fig. 4 *Optical microscope images of the cross-section of misaligned channels*
a Cross-section of misaligned channel prior to PDMS injection
b A circular channel with 250 µm in diameter post PDMS injection and curing procedure. Misaligned channel resulted in correction by the PDMS injection, Scale bar = 100 µm

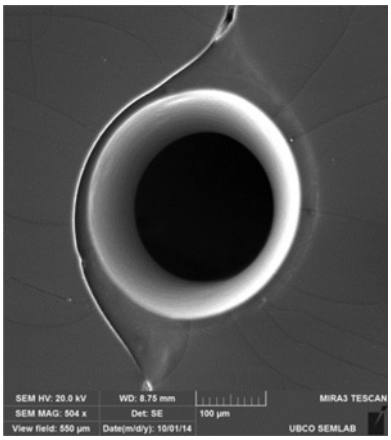

Fig. 5 *SEM of the cross section of a circular channel*

make a path to another. A simple fix was to plug the outlet that has the path, and to force the air to the other outlet. Once a path has been formed with the air to all outlets, it is important that air is allowed to flow freely through all of them. If air is not allowed to flow through a channel, the 'PDMS' will cure, and block the entire channel. Post 'PDMS' injection coating yields results were shown in Fig. 3*b*. The channels were perfectly circular as desired. The 'PDMS' injection coating also allowed us to correct misaligned channels (Fig. 4*a*). This misalignment can be present for various reasons; however, the 'PDMS' injection coating mitigated the problem significantly (Fig. 4*b*).

Scanning electron micrograph (SEM) of the cross section of a circular channel is shown in Fig. 5; this figure shows that a perfect circular channel was created after 'PDMS' injection and all misalignments were corrected.

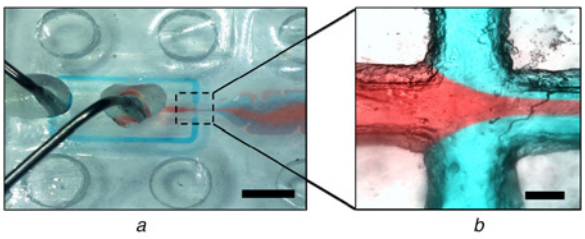

Fig. 6 *Junction of microfluidic chip after PDMS injection*
a Different coloured channels show clearly that there is no blockage of the junction during PDMS injection and circular channel formation. Scale bar = 5 mm
b An optical microscopic image shows the detail of the junction. Scale bar = 100 µm

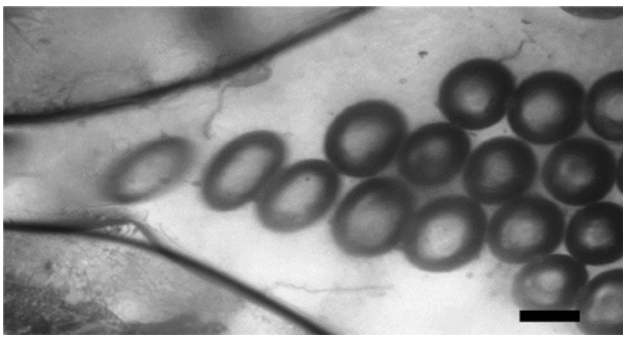

Fig. 7 *Droplet generation from the microfluidic chip. Scale bar = 200 μm*

In this microfluidic chip, two different phase of liquids are pumped into the centre channel (water phase) and the outer channels (oil phase). The fluids meet at the cross-junction where the viscous force of the continuous oil phase flow overcomes the surface tension of water phase flow, which causes water droplets to be created. The cross-junction is critical portion of this flow-focusing microfluidic chip. Figs. 6*a* and *b* demonstrated that the cross-junction was not affected by liquid 'PDMS' coating to form circular channels. Since PDMS channel intrinsically lacks chemical inertness to organic solvent, glass channel is more appropriate for transporting organic reagents [33]. However, PDMS can be further coated with glass using a sol-gel method to prevent the organic solvent absorption [34]. As shown in Fig. 7, the droplet generation was successful, and fluid flowed smoothly with no trace of blockage or leakage. Hydrogel droplets of different size were achieved through this process.

The 3D printed mould is a relatively simple manufacturing process to fabricate microfluidic channel devices. 3D printing technique has advantages to rapidly fabricate complex structures with high aspect ratio comparing with the conventional photolithography technique which requires photomasks and a series of microfabrication processes. Although the current resolution of 3D printers has a limitation to fabricate structures with hundreds of micrometres, in combination with the PDMS injection technique, 3D printed moulds will be a promising solution to provide the flexibility to fabricate various sizes of circular channels using the replica-moulding soft lithography technique.

Therefore, the developed fabrication method using 3D printed moulds can overcome the size limitation of microfluidic channel and will facilitate to use the 3D printing technique for fabricating a variety of microfluidic channel devices. For future work, this rapid fabricated device will be used to encapsulate cells to generate injectable microscale tissues and has many potential applications in biomedical engineering area.

4. Conclusion: In this Letter, a simple and low cost fabrication method is proposed for microfluidic droplet generators with circular channels. The 'PDMS' channels were successfully cured and peeled off the 3D printed moulds, and challenges regarding the interference of the 3D printed material as well as the cloudy (and rough 3D printed) surfaces finishes were addressed. Moreover, to create fully circular channels, and address challenges regarding the misalignment of the upper and lower halves, a 'PDMS' injection coating method was utilised and optimised. The channels were successfully fabricated, and a flow-focusing microfluidic device for creating microgel droplets was assembled. No leakage was observed, and due to the use of circular channels, the device successfully created droplets of different size without blockage. The developed methodology provides a simple and effective way of fabricating circular microfluidic channels for numerous biological and chemical applications.

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6 References

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