Proceedings of the 5th International Conference of Fluid Flow, Heat and Mass Transfer (FFHMT'18)
Niagara Falls, Canada – June 7 – 9, 2018
Paper No. 172
DOI: 10.11159/ffhmt18.172

Characterization of a 3D Printed Mold for a Cell Culturing Microfluidic Device

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Abstract **–** The application of channels with multiple thicknesses is a major area of interest within fields of tissue engineering and microfluidics. 3D printing facilitates cost-effective fabrication of PDMS-based microfluidics using 3D printed molds. In this work, the limitations and the accuracy of using 3D printed templates for microfluidic applications with commercial SLA and FDM 3D printers, are presented. A 3D microfluidic cell culturing device that contains multiple thicknesses is proposed and the accuracy of printed parts in three dimensions is demonstrated. The reusable molds can be printed in less than two hours, with the average cost of 0.35 US$, which lead to fast and cheap fabrication compared to conventional fabrication methods which require clean room facilities. The surface roughness of these 3D printed molds are 0.25 and 1.12 for flexible and clear resins.

Keywords**:** Microfluidics, 3D Printing, Cell Culturing, 3D channel, Polydimethylsiloxane (PDMS).

1. Introduction

Microfluidics is a multidisciplinary area coupling several disciplines such as biology, chemistry and tissue engineering, which lead to manipulation and analysis of fluidics through micro channels. Polydimethylsiloxane (PDMS) is the most common elastomer utilized in microfluidic applications[1]. However, the most common way for the fabrication of microchannels relies on photolithography for master fabrication. This cleanroom-based microfabrication method is time-consuming and needs expensive facilities which leads to limited growth of this field in many research labs. On the other hand, this method is restricted to Planner channel in which the aspect ratio through the channel is uniform and causes improper microenvironment and cell-to-cell interactions and consequently induce ineffective drug delivery in microfluidic systems[2]. All of these factors, make other fabrication methods such as 3D printing interesting for microfluidics. 3D printing is fast becoming a key method in the fabrication of microfluidic devices. The fabrication cost and time could be reduced significantly by commercialized 3D printers. However, direct fabrication of 3D channels by a 3D printer is limited due to curing resin inside the channel. Hence, using a 3D printed mold for fabrication of microfluidic channels is a feasible and accessible method.

In recent years, there has been an increasing interest in 3D printing in the field of microfluidics. 3D printed microfluidic molds have been studied by many researchers using different 3D printers and a number of them have sought to determine the feasibility of this method. In 2013, Comina et al. published a paper in which they used 3D printed templates which were fabricated by a DLP 3D printer for glucose sensing[3]. PDMS casting and characterizing the roughness of 3D printing has been demonstrated in a report by Bonyar et al [4] and new method for fabricating helical channels by 3D printing the structure, casting PDMS around it and removing the structure from the molds was used by Hwang et al [5]. In this research, a microfluidic device for cell stimulation was achieved by 3D printed templates [6]. Chan et al. proposed three-step procedures for post-treating of 3D printed masters due to their issue for direct molding [7]. In addition, a novel capillary-driven microfluidic device was fabricated by Olanrewaju et al. [8]. Also, Kang reported a soluble 3D printed master for PDMS casting and created channels which were smaller than 200 μm [9]. This method can overcome the low resolution of 3D printers. Moreover, Didar lab has recently demonstrated a method that reduced the roughness of microfluidics channels which were fabricated by a 3D printed mold from 2 μm to 0.2 μm [10]. In view of all

that has been mentioned, 3D printing can improve and accelerate research in the field of microfluidics. In addition, low cast 3D printed molds can make it accessible to more research labs.

In this paper, the limitations and applications of 3D printed molds are studied. Two common methods of 3D printing which are fused deposition modelling (FDM) and stereolithography (SLA) are investigated and the benefits of each method is reported. Finally, by using this method a 3D microfluidic device for cell cultring is fabricated.

2. Experimental

2.1. 3D Printing Of The Mold

The CAD model of microchannels was designed using Solidworks (Dassault Systèmes) (Fig 1.a) and the designed models were exported as an STL file. The models were printed using Formlabs 2 and LulzBot TAZ 6 which are SLA and FDM 3D printers, respectively and printing of each part takes approximately 2 hours. Two different types of stereolithography resin were tested. The roughness of the flexible resin is significantly better than the clear resin. The resolution of the 3D printer according to its datasheet is 150 μm in the XY plane and 25 μm in the Z axis. In addition, clear filament (NGEN clear, colorFabb Inc.) was used for the FDM 3D printer and the default setting on the 3D printer was applied. A thickness of 2 mm was considered for the base to prevent the model from bending.

2.2. Post-Processing Of 3D Printed Molds

The 3D printed molds were washed with isopropanol for 2 minutes after printing and then nitrogen gas was used to dry them and then they were exposed to UV light (Stratalinker® UV Crosslinker 2400) for 6 min. The molds' roughness and accuracy were inspected using confocal microscopy. Since the PDMS cannot be polymerized into a cured part in contact with the surface of the molds, they are treated with oxygen plasma at high power (Harrick Plasma, Inc.) before molding for 2 min and then the parts are coated with fluorinated silane (Trichlorosilane, Sigma-Aldrich, Inc.) for 6 hours at 60 °C. Consequently, the cured parts will be removed easily from the master mold and the surface will not be not sticky (Fig 1.b)[7] .

2.3. Comfocal Microscopy

Surface profile was obtained by using a Confocal Laser Scanning Microscope (Olympus Inc.). In contrast to SEM, there is no need to prepare the part. 5x and 20x lenses were used for confocal microscopy. The cutoff value (λ_c), is set to 0.8 mm. Area of 2.4 mm by 1.2 mm was scanned with the ultrafine setting. The scanning of this area takes 60 min. The surface roughness, height profile and geometry were analysed and 2D and 3D images were exported by the microscope software for future analysis.

2.4. 3D Microfluidic Device for Cell Culturing

Several molds were fabricated and pretreated for cell culturing. These molds contain 31 rods on a channel with a cross-section of $1000 \times 60\ \mu m^2$. The diameter and height of the rods are 200 μm and 60 μm, respectively (Fig. 1.c-e). This design can be used to monitor cell culturing in the microfluidics channel. The effects of the environment such as light and temperature to cell culturing can be studied by using this design. It was observed that most of the algae cells stick to the rods due to their larger surface area. PDMS components were purchased from Dow Corning Crop (USA). Mixed polymer at 10:1 ratio (w/w) degassed in a vacuum chamber for 30 min prior to casting. Then, the polymer was poured into the master mold. The PDMS was cured in an oven at 70 °C for 4 hours. Afterwards, the PDMS part was peeled off from the mold and cleaned with IPA. The required holes for fluid inlet and outlet were made prior to plasma treating and thereafter, a glass slide and the PDMS part were treated with oxygen plasma for 50 sec and were bonded to each other. The fabricated devise is illustrated in Figure 1.f.

3. Results and Discussion

A stereo lithography (SLA) and fused deposition modeling (FDM) were used in this work, to investigate the feasibility of each method for microfluidic applications. SLA is an additive manufacturing method that converts photopolymer materials into solid parts by curing them using an ultraviolet (UV) laser. FDM is a thermoplastic filament technology which deposits a thermoplastic material through an extruder, layer by layer. In FDM printers, the resolution is a factor of the nozzle size and the positioner. In contrast, SLA printers' resolution is dictated by radial

beam scattering [11]. Here, we study the use of two affordable 3D printers to fabricate molds for microfluidic applications.

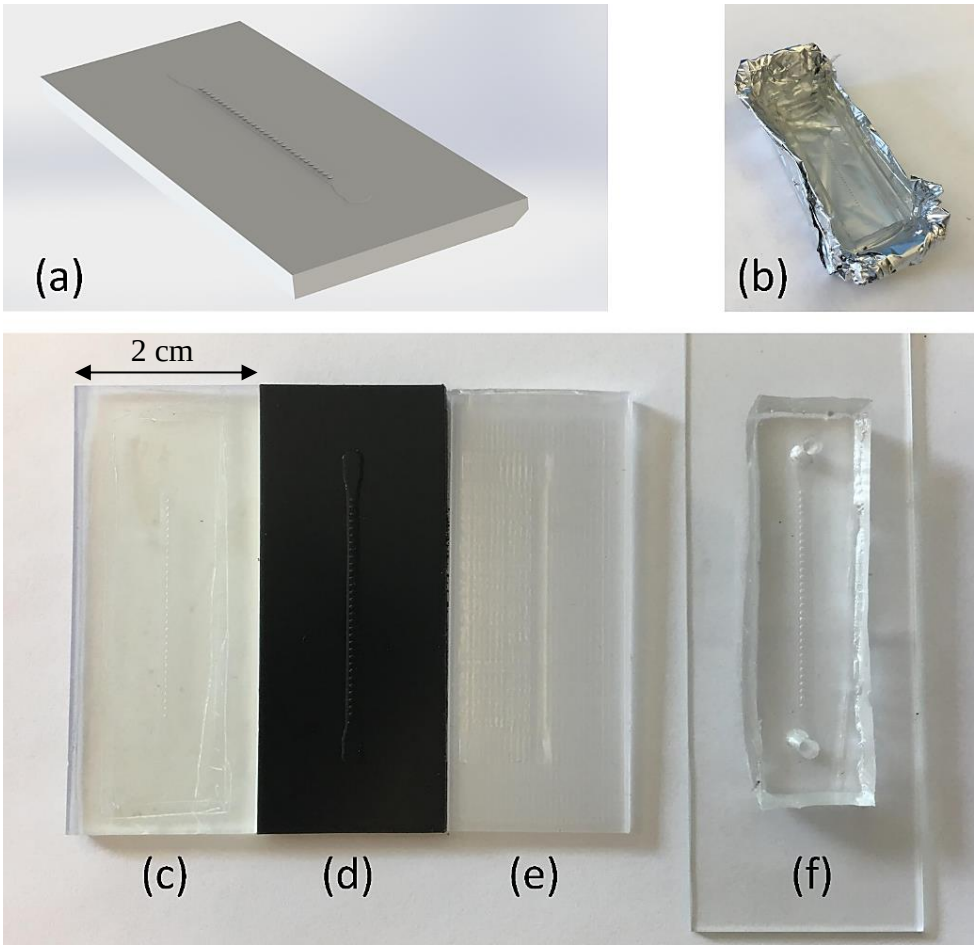

Fig. 1: Rapid prototyping PDMS-based microfluidic device by 3D printed mold. (a) Design of a microfluidic channel with 1 mm width and 60 μm height which consists of 31 rods with 200 μm radius and 100 μm height. (b) The reusable mold for fabrication of PDMS-based microfluidic device fabricated by clear resin. 3D printed molds by clear resin (c), flexible resin (d) and FDM filament (e). (f) Picture of fabricated microfluidic device is depicted.

3D printing enables the fabrication of microfluidic chips with multiple thicknesses. In contrast to photolithography, there is no need for multiple masks and alignments. CAD models can be printed simply by 3D printers which make them ideal for fast prototyping. A comparison of printing cost of a 2 cm × 5 cm × 2mm template for two different methods is presented in table 1.

Table 1: Comparison of printing cost and time for different resins.

Resin type	Cost	Printing time
Clear (Formlabs)	0.30 US	114 min
Flexible (Formlabs)	0.40 US	113 min
NGEN Clear Filament (colorFabb)	0.05 US	21 min

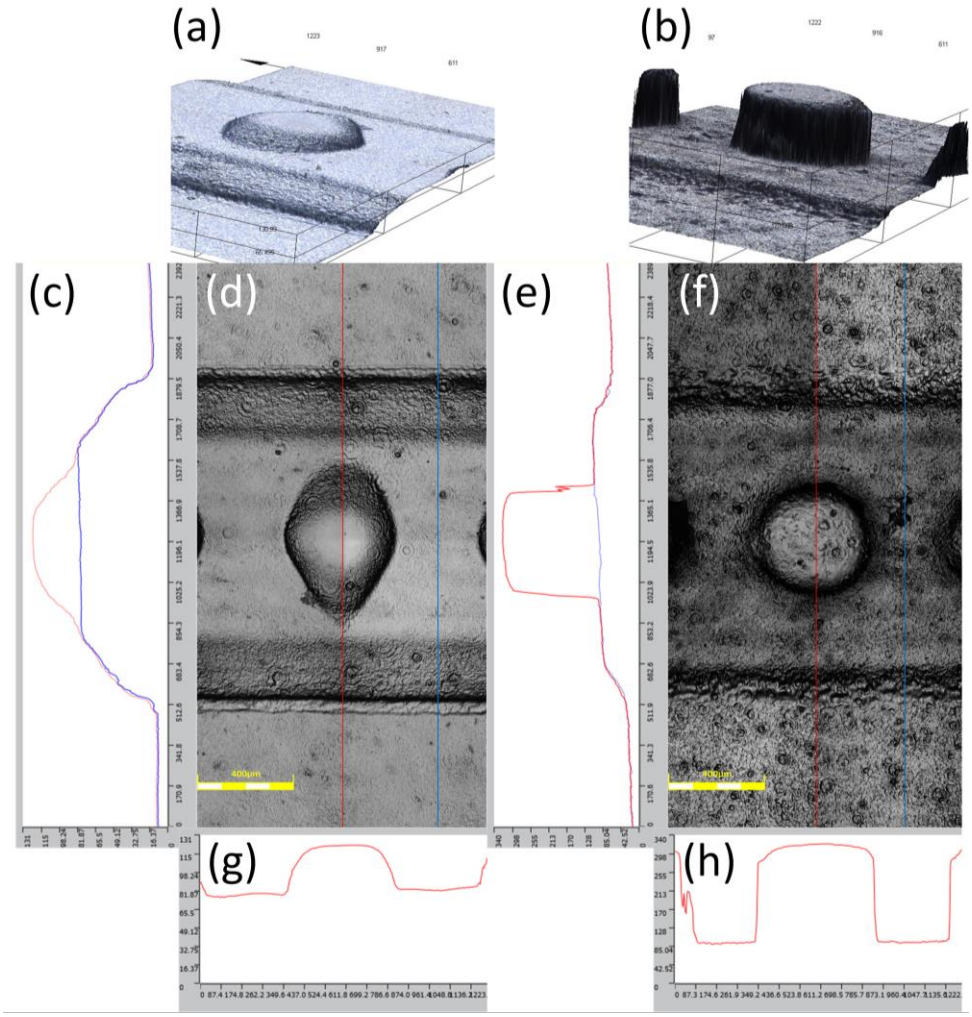

Fig. 2: Laser scanning microscopic images of 3D printed molds in flexible resin (*left*) and clear resin (*right*) using the Formlabs 2 3D printer. 3D scanning of the 3D printed mold by flexible resin (a) and clear resin (b). Measured surface roughnesses of molds, Sa, are 1.122 and 0.25 for the clear mold and the flexible mold, respectively. (c&e) The height profile of the channel by line scanning microscopy. The blue line shows the height of the channel in the 2D scanned image and the red line shows the height of the channel in presentence of a rod in the red line in the 2D scanned image. (d) and (f) present the surface of the mold. (g) and (h) shows the height and the profile of the rod.

The design contains rods with 400 μm diameter and 100 μm height on a channel with 60 μm height and 1 mm width. The demanded printing time is 113 min and 21 min for SLA 3D printer and FDM 3D printer, respectively. Printing cost and time for the FDM 3D printer is considerably lower. However, these factors are insufficient to make it a reasonable method for microfluidic devices due to this printer's low resolution. Printing time for clear and flexible resin is almost the same. However, the flexible resin's printing cost is slightly higher than clear resin's. Overall, 3D printing enables cost-effective fabrication of individual microfluidic devices.

Surface roughness was measured by a 20x lens of the confocal microscope and the cutoff value was set to 0.8 mm. The average roughness was 1.122 μm, 0.43 μm, and 7.61 μm for clear resin, flexible resin and FDM filament, respectively. It shows that SLA 3D printers are more suitable for microfluidic applications. The FDM 3D printer could not construct rods on the channel. In addition, the surface roughness and channel profile are inadequate for microfluidic devices. The result obtained from the microscope for parts printed with SLA 3D printer is illustrated in Figure 3.The surface roughness of flexible resin is 4 times better than clear resin. Therefore, fabricated channels with this resin are smoother and comparable with lithography. However, the resolution of clear resin is better than flexible resin in x and y-directions (Figure 2). As it is presented in Figure 2 shows both types of resin have a considerable error in the depth of the channel

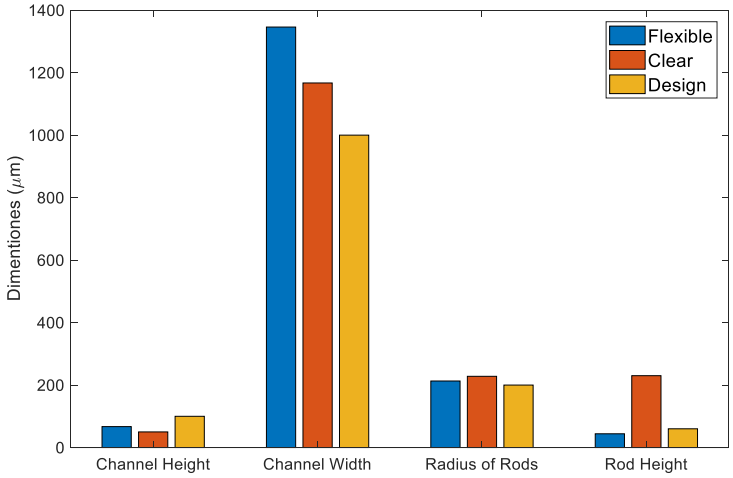

Fig. 3: Comparison of the designed model and the printed mold with different resins. The yellow bar represents the design dimensions.

compared to the CAD model. It is apparent from this Figure that rods have a circular shape compared to the flexible resin which has an elliptical shape (Fig 2.g and Fig 2.h). Furthermore, the height profile increases gradually in the flexible mold compared to the clear mold in which the height profile is comparable to the designed model. Heating Clear mold tends to bend them which result in a curved PDMS chip. In the molds that printed with flexible resin this would not be a concern due to their flexibility.

Figure 3 compares dimensions measured with the confocal microscopy and the designed mold. Comparing the two results, it can be seen that the rod's radius has an acceptable error with less than 15% for both resins. However, the shape of a rod with the clear resin has a smaller deviation from the circular shape of the designed mold. As Figure 3 shows, there is a significant difference between the designed and the printed parts in the Z direction. A considerable error can be seen in the results obtained for the rod's height which was printed with the clear resin. Considering the XY resolution, the width of the channel is wider for both resins. We can conclude that the printer is more accurate in XY plane compared to the Z direction. However, it is needed to be considered that the accuracy of printed parts also can be related to other factors such as alignment of the 3D printer and layer thickness.

Due to the smallest feature limitation, application of 3D printed molds is limited. However, it is a reasonable method for many applications. For instance, it can be used for particle sorting methods such as acoustophoresis and dielectrophoresis in which the channel size is usually larger than 100 μm [12]–[14]. In addition, it is needed to be taken into account that printed molds can be used several times.

4. Conclusion

The fabrication of PDMS-based microfluidic devices has been presented using commercially available SLA and FDM 3D printers. This study set out to determine the feasibility and simplicity of this method which does not require clean room facilities. In addition, it simplifies the fabrication of microfluidic devices with multiple thicknesses.

Reusable molds can be printed in 2 hours, at an average cost of 0.35 US$, which lead to fast prototyping and cost effective production. FDM printing is a cost effective method with a variety of thermoplastic filaments. Unfortunately, FDM printers are unable to print channels with features smaller than 150 μm with rough surface. These factors make SLA 3D printers appealing for microfluidics. However, PDMS cannot be polymerised in contact with 3D printed molds with photoreactive resins. Therefore, post processing is essential prior to PDMS replication.

The investigation of the printer accuracy has shown that it can construct multilevel microfluidic devices. A major limitation of this method is the low resolution and the difference between the CAD model and the printed part. Printed part by clear resin shows a more accurate result compared to other type of resin in XY direction. In addition, the Z profile of the printed part is closer to the CAD model. However, a significant error has been seen in the printed parts with clear resin, particularly in the Z direction.

In addition, we presented a 3D cell culturing platform fabricated by two different resins to monitor cell culturing as a function of environment. The chip fabrication with conventional methods needs multiple masks and aligning which is a time consuming method.

3D printing will almost certainly become more a common method for microfabrication in the future. There is a strong possibility that the resolution of 3D printers will improve in the long term. A considerable progress has been made in fabrication of channels with smaller features [15]. However, conventional lithography is still the most reliable and precise method, especially for mass productions.

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