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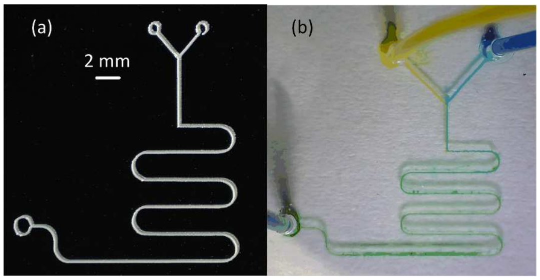

Preparation of PDMS microfliudic devices based on drop-on-demand generating of wax molds

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Abstract Polydimethylsiloxane (PDMS) microfliudic devices have been widely used in Micro total analysis system ($\mu - \mathrm{TAS}$) for various types of analytical, diagnostic and therapeutic applications. With the increasing use of these systems in various fields, the development of low-cost and easy manipulate method for fabricating PDMS micro devices is emerging as an important challenge in their widespread use. This article described a simple method for wax molds used in the preparation of PDMS microfluidic devices based on a PZT (Lead Zirconate Titanate) drop-on-demand droplet generator. Wax was jetted in the form of droplet, linked with each other and formed into wax molds on a glass sheet with the PZT actuator and a glass nozzle. The glass nozzle fabricated by a home-made micronozzle puller without complicated fabrication technology was low cost, simple and easy-made. Coefficient of variation of the jetted wax droplet diameter was under 4.0% which showed well reproductivity. The geometry and surface topography of wax mold were experimentally studied by changing the driving voltage, nozzle diameter, degree of overlapping and wax layer, and wax molds with aspect ratio ranged from 1 to 2.6 were prepared through multi layer of wax droplets. PDMS channels with depth of 200-700 $\mu\mathrm{m}$ and width of 200 and 250 $\mu\mathrm{m}$ were prepared by replication of the wax molds. A micro mixing of blue and yellow dye was realized with the prepared PDMS microfluidic devices. The wax droplet generating system supplied a low-cost, simple, easy-to-use and fast fabrication method for PDMS microfluidic device.

keywords PDMS microfliudic devices, drop-on-demand generating, wax mold, micro mixing

1. introduction

Micro total analysis system ($\mu-\mathrm{TAS}$) compared with systems of conventional size could reduce consumption of samples and reagents, decrease the analysis time and have the greater sensitivity and portability that allowed in situ and real-time analysis and considerable research was focused in the development of $\mu-\mathrm{TAS}$.[1-4] Polydimethylsiloxane (PDMS), a silicon-based polymer exhibiting tunable mechanical, chemical and optical properties has been widely used in $\mu-\mathrm{TAS}$ for the fabrication of micro fluidic devices as it has the advantages that PDMS is low cost compared to traditional MEMS substrates such as silicon or glass, it could be bonded to itself or other substrates easily, it is optically transparent, durable, non-fluorescent, biocompatible, chemically inert and nontoxic, and sealing of PDMS is much simpler than sealing glass or silicon. Therefore PDMS is often the preferred material for prototyping and testing of various microfluidic devices.[5-6]

The most commonly used technique for fabricating PDMS devices is soft lithography, which is a non-photolithographic approach to micro and nanofabrication and based on self-assembly and replica molding.[7-10] PDMS micro devices can be made by curing the PDMS resin over a master mold, which can be fabricated by conventional micromachining techniques or a photosensitive polymer such as SU-8[11-16]. As these conventional methods require complicated equipment and are time consuming, the development of simplified methods to fabricate master molds is very essential for simple, low-cost and rapid fabrication of PDMS microfluidic devices. Many simplified micro fabrication techniques have been developed for creating master molds. These include UV exposure on photosensitive polymer such as WA210 or solder resist by screen printing[17-18], laser localized rapid curing,[19-20] Shrinky-Dink or toner printing on flexible film,[21-22] etching of brass or copper sheet by toner printing mask,[23-24] ice water patterning by hot embossing of PMMA, [25]liquid molding on wax patterned paper,[26] wax printing on paper [27]or rapid prototyping of wax [28-29]or plastic[30], and so on. However, these methods have a number of limitations. The UV exposure requires photo mask and organic solvent and the equipments are complicated and require ultra clean room. The etching of copper or brass also needs masks and uses toxic etching solutions and additional etching and rinsing steps. Shrinkage occurs after the thermal treatment of the laser printing method and specific dimension of the structure is affected. Ice patterning requires another PMMA mold and needs evaporation and freezing steps. The liquid molding method is not well suited for fabricating structures with high aspect ratio due to the surface tension of the liquid. The smallest size of thermoplastic rapid prototyping is up to 250 μm, and the plastic mold is rigid and not suit to bend or sacrifice. Wax is a kind of cheap and easy available material and wax could be melted after the curing of PDMS which could enable some complicated PDMS micro structures. The wax printing process relies on specialized printers and the height of the channel replicated is less than 20 μm. These print and cast demonstrations are significant improvements to the fabrication of PMDS devices, but the development of low cost and easy method is still essential.

In this paper, we developed a method for the fabrication of wax mold used in the replication of PDMS microfluidic devices based on drop-on-demand wax droplet generating. Firstly the steady jetting motion of wax droplet generating was experimentally studied, and then the influence of wax droplets on the geometry and surface topography of wax mold was researched. The micro channels on PDMS with various depth were replicated with the wax molds. Finally, the PDMS device was bonded, a two-channel PDMS micro mixer was prepared and the mixing of blue and yellow dye was realized on the devices.

2. Materials and method

2.1. Materials

Paraffin wax 56-58 was purchased from Shanghai Specimen and Model Factory. Sylgard 184, Dow

Corning was supplied by Dow Corning Corporation. Ethanol (AR, 95%), Erioglaucine disodium salt (BR, 85%, 3844-45-9) and tartrazine BS (85% 1934-21-0) were from Shanghai Jinsui Bio-tec Co., Ltd. PTFE (polytetrafluoroethylene) tube was from Shanghai Xinlong Plastic Co., Ltd. Quartz glass was supplied by Fuxin Optical Quartz Glass Factory.

2.2. Wax droplet generating system for preparation of wax molds

In the drop-on-demand wax droplet generating system, as shown in Fig. 1(a), the pulse influencing driven force was supplied by a PZT actuator P-844.10, produced by Polytec PI, Inc. and the photo of the PZT actuator with a connector and a glass micronozzle was shown in Fig. 1(c). The glass nozzles were fabricated through pulling process with a home-made glass micropipette puller [31] and forging process with Sutter MF-900. The drop-on-demand wax droplet generating was proposed on the friction coupling effect between the microfluidic boundary layer and solid wall of the micro channel. As shown in Fig. 1(b), the piezoelectric actuator connected to the solid wall of the glass micro nozzle exerts a periodicity disturbing on the solid wall and the boundary flow obtains a movement along with the solid wall. Then the viscous force F_1 within the fluids transfers the movement and the microfluidic body obtains a velocity v_1. When the applied pulse voltage decreases rapidly to zero in magnitude, the PZT actuator contracts and the liquid inside the micro-nozzle obtains a pulse inertia force F_2 relative to the micro-nozzle. When the inertia force F_2 is large enough in magnitude, the inertia force F_2 exceeds the viscous force F_1, a droplet of the liquids will be thrown out of the micro-nozzle drop by drop in the direction of the inertia force F_2.

Various kinds of wax molds could be formed on the polishing quartz glass sheet when the worktable moved along the patterns. Two Nickel-cadmium alloy (Cr20Ni80) resistance coils were fixed outside of the glass nozzle to melt the wax. The one with 1.5 cm diameter was used to heat the reservoir part of the nozzle and the Ø0.5 cm one was used to heat the nozzle tip. With the drop-on-demand wax droplet generating system, the melted wax was jetted on the glass sheet in the form of wax droplet, and the droplets linked with each other to form into wax molds.

2.3. Replication of wax mold on PDMS

Firstly, a Ø4 cm polishing quartz glass sheet was cleaned with AR ethanol in ultrasonic wave cleaner for 10 minutes, and then washed by de-ionized water and dried in drying oven. Then the glass sheet was laid on the worktable and the wax droplets were generated and linked with each other to form the wax pattern mold. 6 ml of degassed PDMS liquid composed of A and B with ratio of 10:1 was poured on the glass sheet with an aluminum ring as the dam. After that, the glass sheet was kept in 40 ℃ for 16h to cure the PDMS. Then, the PDMS sheet was separated from the glass sheet, drilled, ultrasonic cleaned in ethanol for 5 minutes and dried in nitrogen gas. Finally, the PDMS devices were boned with another quartz glass by pressing and keeping in 75 ℃ for 30 minutes. The whole process was schematically shown in the Fig. 2.

3. Results and discussion

3.1. Generating of wax droplets

To avoid that liquid wax would exude when the glass micro nozzle was overloaded and that generating would not happen when the liquid in the glass micro nozzle was not enough, there was a range for the height of wax liquid in the glass nozzle in steady jetting motion, and the liquid height varied with the sizes of the nozzle tip. Three kinds of glass nozzle were prepared with nozzle diameters of 120 μm, 140 μm and 180 μm. The amplitude of driving signal was 40 V which was the minimum amplitude to jet out wax droplets. The maximum length of wax liquid in the micro nozzle was shown in Fig. 3(a), and the maximum length ranged from 2.4 cm to 3.8 cm varying with inner diameters of the nozzle tip.

The sizes of the wax droplets were measured after being jetted on glass slide with each kind of micro nozzle in different voltage *V*, and results showed that the wax could not be jetted with *V* less than 40 V.

Diameters of the wax droplets jetted by 140 μm nozzle formed on the slide glass increased from 200 μm to 320 μm, and the 30° scanning electron microscope photo of wax droplets jetted with *V* of 50 V, 60 V, 70 V, and 80 V were shown in the Fig. 3(b). The distance between each droplet was set as 400 μm, and the jetting frequency was 3 Hz. The amount of wax increased with the amplitude of electrical driving signal. The shapes of wax droplets were treated as a circle equaled in the area to make the measurement easy as the wax mold by the linking of wax droplets was slightly influenced by the shape of a single droplet. The mean diameters jetted by 140 μm nozzle with *V* of 50 V, 60 V, 70 V, and 80 V were respectively 202.1 μm, 250.0 μm, 290.2 μm, and 323.5 μm, and the stand deviation were respectively 5.6, 7.0, 5.2, and 6.0. The coefficient of variation (*CV*) is defined as the ratio of the standard deviation to the mean diameter, and it shows the extent of variability in relation to mean of the population. The table used for the complete set of measurements was shown in the table S1, and *CV* of wax droplets were all under 4.0% which showed well uniformity of the wax droplets.

The sizes of the wax droplets jetted with other types of nozzles on driving voltage from 40 V to 80 V were measured with the same method, and the results were shown in Fig. 3(c). The smallest droplet prepared was 150 μm and the droplet sizes ranged from 150 μm to 400 μm.

3.2. Influence of wax droplet generating on wax mold

3.2.1. Wax mold formed by the linking of wax droplets

As shown in Fig. 4(a), two assumptions were taken, of which one was that the wax droplet was a piece of cylinder, and the other was that the overlapping area of two neighbor droplets was determined only by the overlap distance. k referred to the ratio of overlapping area between two neighbor droplets and the area of a whole wax droplet. Thus *k* could be written as equation (1), where *L* was the length of the overlap area (μm); D_b was the diameter of a wax droplet (μm); *a* was the distance of each jetting step of the work stage (μm).

$$k = \frac{L}{D_b} = 1 - \frac{a}{D_b} \quad (1)$$

The wax mold for straight PDMS micro channel was formed by the linking of wax droplets, and the surface topography of the wax mold was influenced by the degree of overlapping (*k*). When the overlapping was less than 0 which meant that none of the wax droplet linked with each other, the PDMS channel would be blocked. 0-30% of *k* resulted a fluctuating surface on the side of the wax mold as shown in the Fig. 4(b). When *k* increased to 50%, a straight wax mold with smooth top surface could be produced, as shown in Fig. 4(c). The bottom of wax line extended a bit by 50-80% of *k*, and it took more time to prepare the same length of wax mold. When the degree of overlapping was more than 80%, wax droplets grown from the slide into an inclined pillar.

3.2.2.Multi height of wax mold

The wax mold formed by single row of wax droplets was usually used for the fabrication of PDMS channel, and the depth of the replicated PDMS channel relied on the height of the wax mold. The aspect ratio of wax mold cross section increased with the repeated generating times as that one layer of wax droplets would be generated on the wax mold when repeated jetting one time on the same pattern. However, the width of the wax mold did not change with the layers, as the top of the wax mold was round and the solidification of wax droplets was very fast. The wax molds with 1-5 layers using the micro nozzle of 140 μm outlet diameter, driving voltage of 50V, frequency of 6 Hz, overlapping of *k*=50%, were prepared, and the wax molds with 2, 3, 4, and 5 layers of wax droplets were shown in the Fig. 5(a-f). The wax could be precisely jetted on the same position with the work table, and the number of layers could be read from the surface topography of the wax mold as that there were lamellar structures on the wall side of each layer. Wax molds with more than five layers of wax droplets were tried, and too many layers of wax droplets made the wax mold become a thin wall with high aspect ratio standing on the glass slide. The relation between the height of the wax mold and repeated jetting times was shown in the Fig. 5(g), and the aspect ratio of the wax mold ranged from 1 to 2.6.

3.3. PDMS micro channel by replication of wax mold

Firstly the Ø4 cm quartz glass sheet with thickness of 1.9 mm was washed by ultrasonic cleaner in de-ionized water for 15 minutes and then laid in drying oven to remove the water. Then, the designed wax pattern was jetted on the glass sheet to prepare the wax mold. The sylgard 184 silicon elastomer A and B were mixed by the quality ration of 10:1 by a stirrer with speed of 800 rotations per minute for 10 minutes. After the PDMS solution was degassed by a vacuum drying oven, an annular dam was bonded on the glass sheet with glue and the PDMS was poured on the glass sheet with wax mold. Then the glass sheet with wax mold and uncured PDMS was put in drying oven of 40 ℃ for 36 hours. The cured PDMS was heated for 1 minutes in 100 ℃ to separate the wax from the PDMS, and then the PDMS sheet was taken off from the glass sheet, and washed by boiling de-ionized water to remove the wax from the micro channel. Three holes were drilled in the PDMS sheet with a hole punch (supplied by Suzhou Wenhao Devices Technology Co., Ltd.) and then the PDMS devices and glass slide were washed by ultrasonic cleaner in de-ionized water and ethanol respectively for 15 minutes. After being dried with nitrogen gas, the devices were bonded. As the strength of the wax is weak, especially when the wax mold is composed of multi layers of wax droplets, and the surface of the wax mold is a little rough, the wax mold prepared could be used only once for the replication of PDMS micro channels. To use the mold repeatedly, the micro channels on the PDMS could be replicated by other thermal plastic material to prepare reusable molds.

The PDMS micro channel replicated by the wax mold with *k* of 10% and 50% were shown in the Fig 6 (a) and (b). The boundary of micro channel with *k*=10% was rough with zig-zag pattern, and the wax mold by *k*=50% could replicate PDMS micro channel with smooth surface.

The micro channels with depth of 200 μm, 250 μm and 370 μm were shown in the Fig. 6 (b), (c) and (d) respectively. The widths of all the channels were 200 μm and their boundary surfaces were almost the same, whereas the color of the channel with depth of 370 μm was deeper. We cut the channels with different depth into sheets with thickness of 3 mm perpendicular the flow direction and the cross sections were shown in the Fig.6 (Photos were taken by scanning electron microscope). The depth of channel increased with the height of wax mold which was determined by the size of the wax droplets and generating layers. As the wax mold was almost entirely replicated, the relation between the depth of the micro channel and the repeated generating layers could be read in the Fig. 5(g), and the aspect ratio ranged from 1 to 2.6. Compared with direct laser writing which was a convenient and time-saving method for the PDMS micro channels, the width of the micro channel did not have to change with the channel depth as the width of the wax mold did not change with the wax layers.[32-34] This kind of method was inspired by the 3 dimensional rapid prototyping method, and the difference was that the mold was formed by the wax droplets. The resolution was improved to 200 μm, and it was able to sacrifice the mold to make more complicated structures. This method still had some disadvantages such as that the surface of the channel was a little rough and the size of the microchannel was up to hundreds of micro meters. In further work, the rough surface may be improved by additives in the liquid wax and smaller wax droplets were expected to be prepared by glass micro nozzles with smaller tip diameters.

3.4. Micro mixing experiments

A kind of wax mold used for two-channel micro mixing with width of 200 μm was prepared with 140 μm nozzle, driving voltage of 40V, 6 Hz, and *k*=50%. And then with this kind of wax mold, the PDMS micro mixer was prepared following the procedure described in 2.3. PTFE tubes were inserted as inlet and outlet channels, and the inlet channels were separately connected with a syringe of 1 mL filled with 800 μM erioglaucine (blue dye) and 1870 μM tartrazine (yellow dye) respectively. The flow rate of per inlet channel was 10 μL/min. The blue dye and yellow dye solution flowed into the PDMS micro channel through the PTFE tube. Firstly the channels emerged blue and yellow color respectively, and then when the two kinds of solutions mixed in the junction position, a green color emerged because of the additive color mixing effect. As the two kinds of liquid kept flowing through the channel, a light green color appeared in the end of the channel which showed that the two solutions were fully mixed. This method supplied a fast and easy fabrication method for the preparation of wax mold used in the replication of PDMS micro devices, and other kinds of PDMS micro devices could also be made with this kind of method.

4. Conclusion

In this work, we demonstrated a fast and low cost method for the fabrication of wax mold used in the replication of PDMS micro fluid devices based on drop-on-demand wax droplet generating. A PZT stack actuator was used as driving source to provide enough pulse inertia force for wax droplet generating. The glass nozzle fabricated with borosilicate glass tube had advantages of easy-made, cheap, good chemical resistance, low friction and simple. The wax used was cheap and easy available and the wax mold was formed directly without any other multiple steps or templates. The wax droplet size could be easily changed by changing the micro-nozzle outlet diameter and the amplitude of the applied pulse voltage. The width of the wax mold determined by the size of the wax droplets ranged from 150 μm to 400 μm and multi height of wax molds ranged from 200 μm to 650 μm could be prepared by multi-layer generating of wax droplets. PDMS micro channels with depth ranged from 200 μm to 650 μm were replicated. A two-channel PDMS micro mixer was prepared with the wax mold and a color change reaction from blue dye and yellow dye into green was realized with the mixer. In further work, other kinds of planar or three-dimensional (3-D) PDMS device could be prepared for the $\mu-\mathrm{TAS}$ and two or more nozzles could be fixed on one PZT actuator and more than one wax patterns could be produced at the same time for large scale production.

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Figure legend

Fig. 1 (a) Schematic of wax droplet generating system for preparation of wax molds (b)Principle of micro fluidic driving (c) Photo of the PZT actuator and glass micro nozzle

Fig. 2 Process for preparation of PDMS microfluidic devices

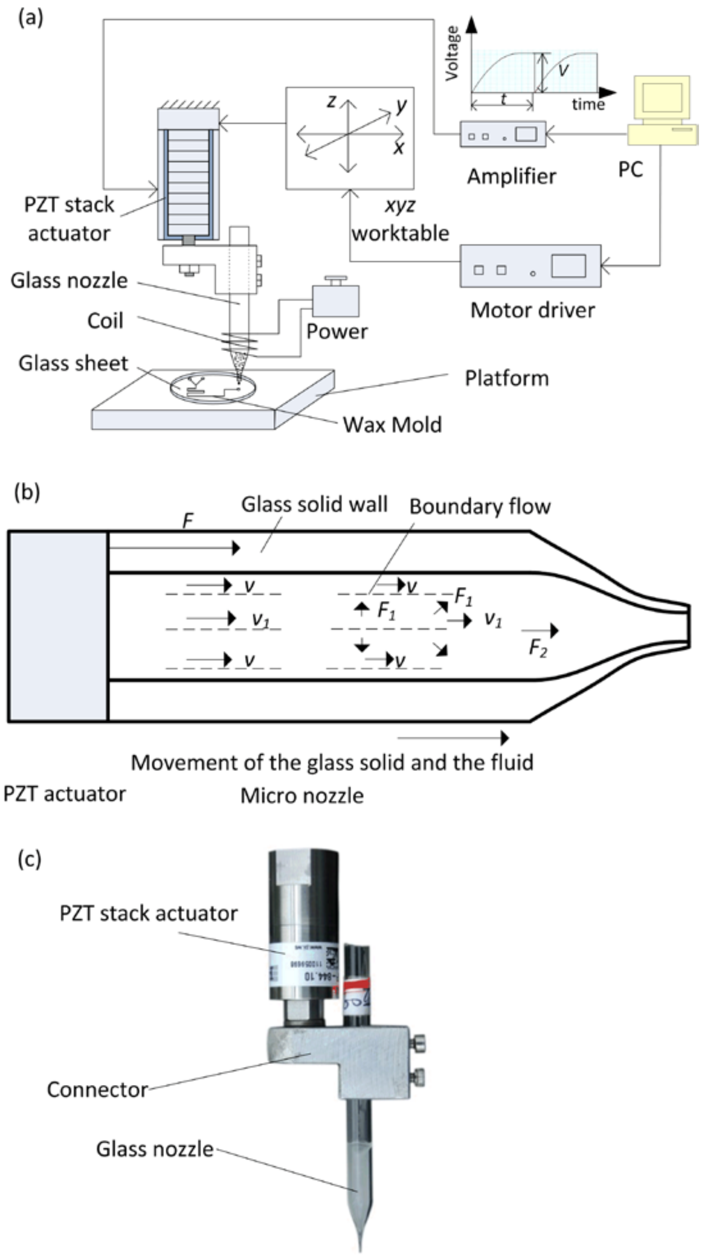
Fig. 3 (a) Maximum length of wax liquid in micronozzle on steady jetting motion. (b) Photo of 4×4 wax droplet arrays jetted by micronozzle of 140 μm outlet diameter. (c) Relationship between droplet sizes and driving voltage for nozzles with outlet diameters from 120 μm to 180 μm.

Fig. 4 (a) Schematic diagram of the wax droplets, (b) Photo of wax mold with k=10%, (c) Photo of wax mold with k=50%,

Fig. 5 (a, b) SEM Photo of wax mold with 2 layers of wax droplets, (c) SEM Photo of wax mold with 3 layers of wax droplets, (d) SEM Photo of wax mold with 4 layers of wax droplets, (e, f) Photo of wax mold with 4 and 5 layers of wax droplets (Taken by Nikon D2x in a controlled light source), (g) Relation between the height of wax mold and layers of wax droplets

Fig. 6 (a) Photo of PDMS micro channel replicated with k=10% (b) Photo of PDMS micro channel with k=50% and depth of 200 µm (c-d) Photo of PDMS micro channels replicated by wax molds with height of 250 µm and 370 µm (k=50%)

Fig. 7 (a) Photo of wax mold for the replication of PDMS micro mixer, (b) Photo of micro mixing of blue dye and yellow dye with the bonded PDMS micro mixer



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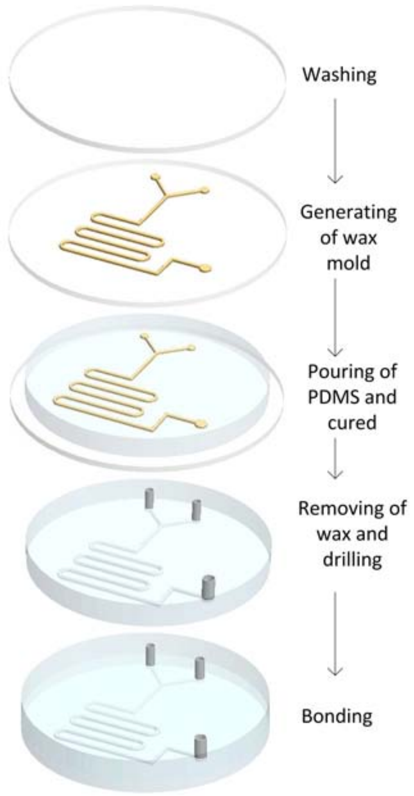

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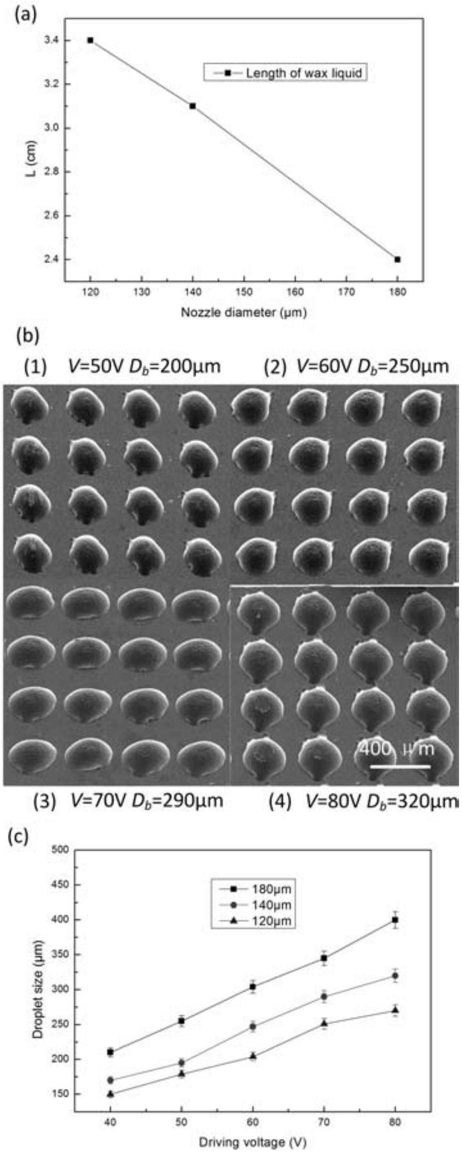

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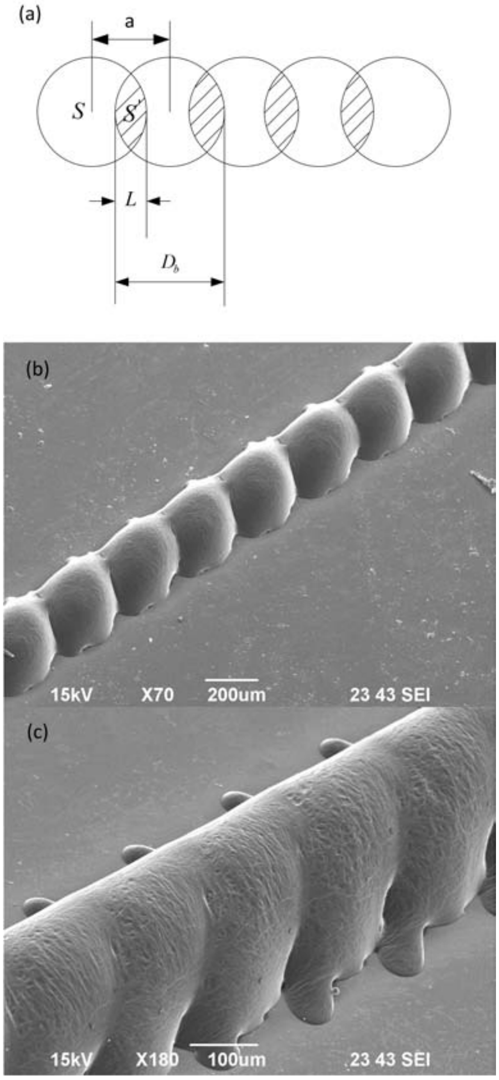

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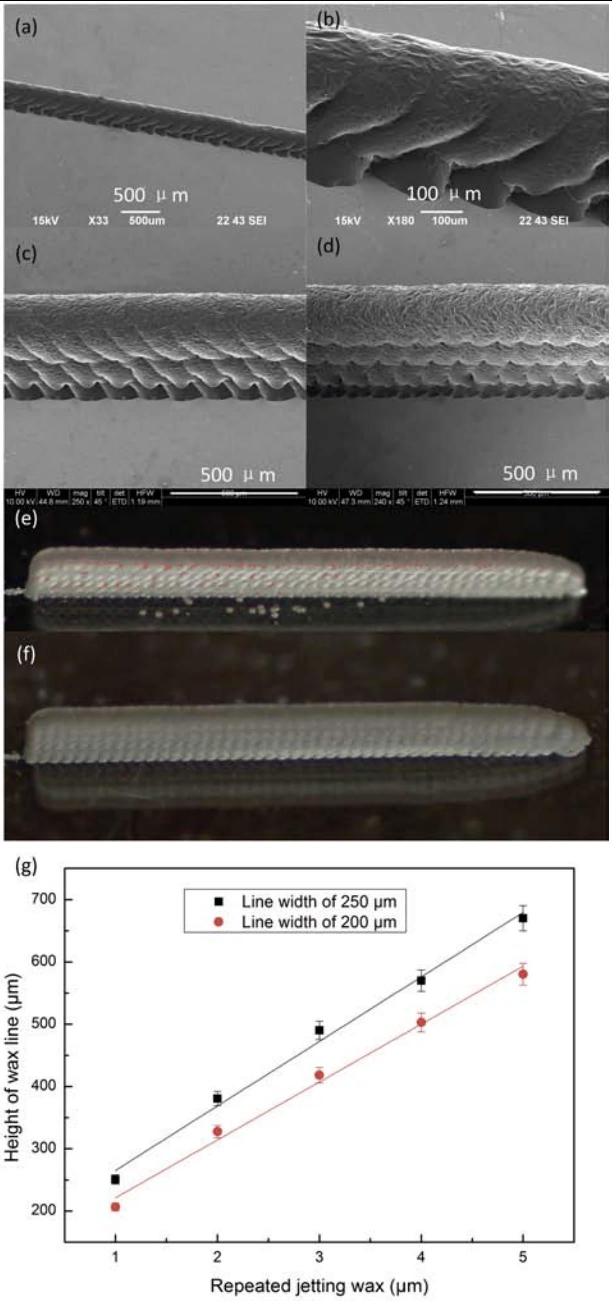

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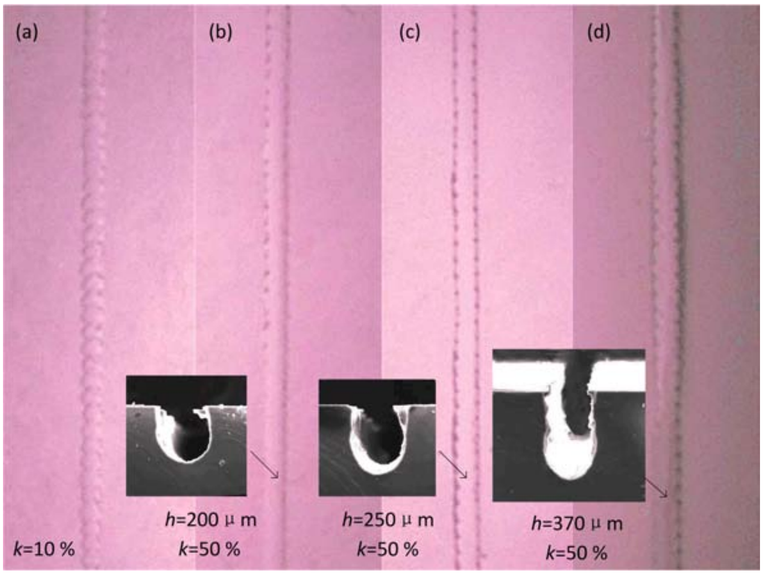
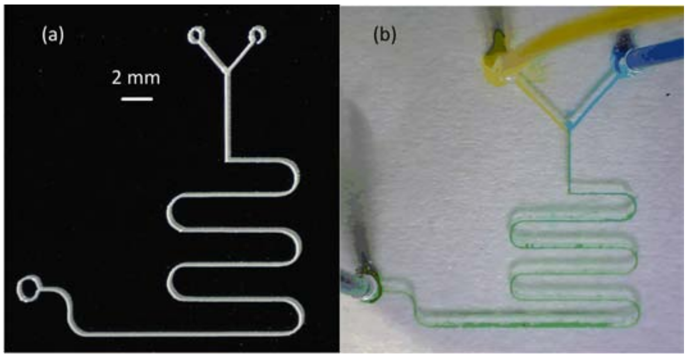


Fig. 6 (a) Photo of PDMS micro channel replicated with k=10% (b) Photo of PDMS micro channel with k=50% and depth of 200 μm (c-d) Photo of PDMS micro channels replicated by wax molds with height of 250 μm and 370 μm (k=50%)



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