Rapid prototyping of microfluidic devices with a wax printer[†]

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We demonstrate a rapid and inexpensive approach for the fabrication of high resolution poly(dimethylsiloxane) (PDMS)-based microfluidic devices. The complete process of fabrication could be performed in several hours (or less) without any specialized equipment other than a consumer-grade wax printer. The channels produced by this method are of high enough quality that we are able to demonstrate the sizing and separation of DNA fragments using capillary electrophoresis (CE) with no apparent loss of resolution over that found with glass chips fabricated by conventional photolithographic methods. We believe that this method will greatly improve the accessibility of rapid prototyping methods.

1 Introduction

Microfluidics, as an integral component in micro total analysis systems (µTAS), is gaining importance in a wide range of applications, particularly in medical diagnostics. Poly(dimethylsiloxane) (PDMS), a bio-compatible elastomeric material, is widely used to fabricate microfluidic devices using the soft-lithography¹ approach. This approach of building PDMS chips was a significant shift from the Si/glass fabrication process, and away from conventional micromolding/injection techniques.^{2,3} However, using this approach there is still the need to perform photolithography within the fabrication cycle. We believe that the elimination of expensive equipment and materials in fabrication (mask aligners, spinners, photoresist etc.) could aid in the fabrication of microfluidics within academic and possibly industrial settings. The current concept-to-device time period for PDMS-based devices is already impressive (~ 24 hours¹), with the bulk of the time spent in the mold preparation within a clean room (fabrication facility). We demonstrate here the complete fabrication of a microchip, within hours, with a method that completely eliminates the need of a clean-room facility. The present 'print, pour, peel, press and run' method could further application-driven research in microfluidics by significantly decreasing the turn around time inherent in fabricating a new design.

PDMS devices are typically fabricated using soft-lithography by patterning photoresist on glass¹ or silicon⁴ substrates or by etching into Si⁵ using conventional fabrication approaches. A tremendous advantage was obtained by generating the required photomask from high resolution printers (~ 3500 dpi).¹ Subsequently, inexpensive low resolution office printers (~ 600 dpi) coupled with photographic

reduction techniques⁶ and the use of a photoplotter⁷ were demonstrated for generating photomasks for building PDMS microchips. Each of these demonstrations eliminated the need for a metal mask from the soft lithography process. However, standard cleanroom-based photolithographic processing was still required.

There have been various efforts in eliminating or modifying the photolithographic step in the fabrication process. One such effort produced molds using a solid-object printer,⁸ however this is useful only for features >250 um, and requires expensive and specialized equipment. The fabrication of molds by precision CNC machining⁹ methods requires the use of specialized equipment in a double-cast approach wherein the CNC mold is used to create another mold that is then used to fabricate a PDMS-based device. This approach is not optimal for frequent design alterations, and requires substantial infrastructure and time. Printed circuit boards (PCBs) have also been used as molds in¹⁰ to build PDMS chips. Moreover, an elegant refinement of the PCB approach¹¹ demonstrated DNA separations (~ 50 bp resolution). However, either PCB process required the use of a photolithographic step and substantial processing.

Several 'print-and-cast' approaches were recently demonstrated wherein the master was made by a printer and then used to cast PDMS devices with feature heights of 7-11 µm.¹²⁻¹⁴ Although these approaches demonstrated significant improvements in rapidity and ease of use, the resulting surfaces had substantial roughness introduced by the choice of printing technology. Such roughness can preclude high-resolution capillary electrophoresis (CE). One such print-and-cast demonstration used a laser printer to selectively deposit a toner layer on a polyester film that was subsequently laminated against another printed polyester film, thereby creating channels.¹² Another demonstration used a photocopying machine¹³ to make a master on a transparency, resulting in feature depths of 8 to 14 µm for casting PDMS. In ref. 14 a laser printed transparency served as master with the toner ink acting as the mold to cast PDMS to build a device (for CE). In that work the roughness of the channels was a large fraction of the channel width (e.g. Fig. 1 or Fig. 2 of ref. 14) and although the resulting device was able to separate

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Fig. 1 Printed features on transparencies as imaged with an optical microscope. Images (a)-(f) are each at the same microscope settings and were printed by a Phaser 8500. Images (a), (c) (e) and (g) are from an untreated transparency and show raster marks and gaps in ink coverage, along with background speckles that are inherent to the mylar film (i.e. not caused by the printing). After the thermal treatment, in images (b), (d) (f) and (h), reflow of the ink (wax) is seen to have occurred leading to smoother channels/features with no apparent spots or raster marks. As seen in (f), the vendor-specified films are free of speckles. (a) Intersection of two 250 micrometre wide lines before and (b) after reflow. (c) Channel inlet (ca. 1.5 mm diameter) and channel before and (d) after reflow. (e) Channel inlet (ca. 1.5 mm diameter) and channel before and (f) after reflow. (g) The topmost portion of several thick wax-printed lines at higher magnification as viewed with an optical microscope in transmitted light (i.e. illuminated from below). Each line is 7 pixels wide and was printed on a Phasor 8400 at 600 dpi to form a 300 µm wide line with increased roughness effects. Evidence is seen of the interface between pixels with several voids that extend through the line. (h) After being placed, covered, on a hot-plate for 4 min at 100 C the voids seen in (g) have been filled and the lines are featureless in transmitted light, and almost featureless in reflected light (shown here).

dopamine and catechol, the peak widths were a significant fraction of the overall run time ($\sim 10\%$). These print and cast demonstrations are significant improvements to the rapid prototyping methodology, but their effectiveness is challenged by the employed technology—in particular the generation of rough channels due to the master being formed of discrete particles of toner. This is problematic for microfluidic applications such as CE and there have been no reports to date of DNA-based CE using devices fabricated using a printand-cast approach. In this paper, wax is used to form the features of the master, and this forms smooth features that are eminently suitable for microfluidic applications. We demonstrate CE using these chips and achieve comparable resolution as for photolithographically-defined glass-based CE chips.

2. Materials and methods

The design is printed using a wax printer (Xerox Phasor, 8500 DN) at the highest resolution setting (2400 FinePoint[®]) resolution) and printed on either a Mylar film (Fuji Photo Film Canada Inc., ON, Canada) or the transparency film (Phaser[®] solid ink professional transparency film) recommended by the vendor. The design was drawn in L-Edit v3.0 (MEMS Pro 8, MEMS CAP, CA, USA). Both the films have excellent adhesion to wax, resulting in features with sharp edges as compared to the laser printer technology. The precision in the feature formation is highly repeatable with no noticeable dependence on temperature and humidity. Although smooth, the features of the master can be improved by an additional thermal treatment (described below).

PDMS (Sylgard 184, Dow Corning, NC, USA) is mixed in a 10: 1 ratio of monomer to the curing agent, and poured onto the transparency to form a 1.2 mm thick layer. The PDMS is subsequently thermally cured for 3 h at 60 °C. A room temperature cure of the PDMS can also be performed, thereby eliminating a heat source, but requiring longer cure times. The glass was cleaned using ethanol and dried with N2. The PDMS layer is then peeled off the transparency, cleaned with ethanol and thoroughly dried prior to sealing with unpatterned borofloat glass (Paragon Optical Company, PA, USA), thereby creating microchannels within a hybrid PDMS/glass device. For this work, it was convenient to also degas (in a vacuum system) the PDMS prior to pouring and to irreversibly bond the PDMS by using a UV lamp. Neither of these two steps was required. Without degassing, the device is more likely to contain bubbles that may interfere with operation. In order to irreversibly bond the structure, a 6 min UV exposure in a custom-built UV ozone (185 nm) system is made and the PDMS is brought in contact with the glass and left untouched for ~ 2 h to irreversibly bond PDMS with glass or with PDMS. We have found that reversible sealing (i.e. same procedure but without the UV exposure) has no effect on CE except that greater care must be taken in handling in order not to delaminate the structure. To form the reservoirs, punched holes were made in the PDMS layer, these were 1.5 mm in diameter and have a volume of $\sim 3.5 \,\mu l$.

Fragment analysis (using CE) is performed with the microfluidic tool kit, μ TK (Micralyne, Edmonton, Canada) and all operations were automated using a compiled LabVIEW interface running on a PC *via* a serial connection. The μ TK provides the optical detection and high voltages needed to perform CE with confocal laser-induced fluorescence (LIF) detection. The LIF system uses excitation at 532 nm and detection at 578 nm. A 60 s injection at 0.4 kV was applied followed by a separation voltage of 6 kV. LIF detection was performed at 50 mm from the channel intersection. CE is performed using a denaturing sieving

matrix (POP6, Applied Biosystems, Foster City, CA) along with a DNA size standard (GS500, Applied Biosystems, Foster City, CA) for sizing. Further details on the system and its use in CE can be found elsewhere.¹⁵

3. Results and discussion

By optical inspection it was found that the as-printed features are smoother than have previously been demonstrated by methods that utilize toner particles, with smooth variations (Fig. 1) in the width of straight channels that we estimate to be about 5 percent, or about 10 micrometres. By comparison, the similarly sized lines shown in Fig. 1 or Fig. 2 of ref. 14 show rapid variations of channel width that are somewhat larger, about 40 micrometres or about 20%. Not only are these variations several times larger than in the present work, the fact that the channel is not smoothly varying in width is likely to degrade electrophoretic performance further.

In the present work, apart from variations in channel width, the features on both types of transparencies have some defects that can be removed by a simple procedure (described below). The first type of defect are the irregularities seen in Fig. 1(a,c,e,g) that are due to occasional gaps in ink-coverage. A second type of defect is due to the rastering of the printing process. Since part of the irregularities consist of raster lines that have a specific orientation, the distortions in the printed features were minimized by aligning them parallel or perpendicular to the direction of the raster lines. Hence, the edges of the straight lines (Fig. 1(a)) in raster-aligned features are sharper when compared to edges of the curved geometries (Fig. 1(e))-i.e. the circular features are rougher than the straight lines. However, for CE applications the resolution of the circle is not important, since it serves only to mark the regions to be hole-punched to form reservoirs. Also by optical inspection we found that the mylar film has random dark spots ('speckles') that are visible in the non-feature printed regions (Fig. 1(a-d)) and these are also present in the as-received mylar transparencies. However, these spots produce isolated voids that do not affect the functionality of the resulting devices. The films recommended by the vendor have negligible numbers of speckles (Fig. 1(e-f)).

It is apparent that the defects seen in Fig. 1(a,c,e,g) would be greatly suppressed if the wax were made (1) to reflow, in order to merge the neighbouring raster lines and (2) to wet the surface better, in order to fill the voids. We found that by warming the printed transparency for 10 min at 100 °C we could do this, removing all the gaps in wax coverage and all evidence of rastering, thus providing very smooth profiles, as shown in Fig. 1(b,d,f,h). The proposed mechanism of this thermal treatment is that both the viscocity and the surface tension (of the wax) decrease near the melting point, thus facilitating the spread of wax on the film. Although we do not know the composition of the proprietary wax used, it is thought to be paraffin based. Paraffin wax is known to demonstrate a sharp, large drop in surface energy (approx. 50%) near its phase transition temperature.¹⁶ Manzello and Yang¹⁷ note that as the melting temperature is approached, the paraffin wax surface becomes 'glassy', i.e. highly reflective as it becomes far smoother. As expected from Young's equation,¹⁸

the sudden decrease in surface energy of the wax, without significant change in other surface energies, will lead to a decreased contact angle, *i.e.* better wetting of the transparency by the wax in a reflow process that leads to a more homogenous film. At 100 °C, above the phase transition of the wax, this reflow may further be assisted by the volumetric expansion during heating. The optimum time and temperature for this heat treatment was experimentally established. After the thermal treatment we obtained a smooth wax pattern suitable for casting a microfluidic channels. (We also found that we could warm the printed film just after having poured the PDMS atop it-this allows a rapid curing of the PDMS while reflowing the wax. Although this gave a slightly less smooth result, the entire process could then be completed within an hour.) Measurements by a stylus profilometer (as shown in the ESI[†]), also demonstrated that the master was smoother following the thermal treatment and had a maximum height of about 10 µm, a depth useful for microfluidic applications. Further characterization of the PDMS features using a scanning electron microscope (SEM) corroborated the profilometer findings (data not shown).

As discussed above, channel roughness can degrade electrophoretic performance and there have been no reports to date of DNA-based CE using devices fabricated using a print-andcast approach. Such separations require smooth, well-defined microfluidic channels coupled with optimization of the electrophoretic conditions. To demonstrate the effectiveness of the present fabrication approach, we demonstrated microchip CE for DNA separations. Resolving a known DNA sequence (or fragment size) for the genetic basis of disease diagnosis has important clinical implications (e.g. as in ref. 19 where a strand of DNA is detected and sized using CE, thereby indicating the presence or absence of a virus in a patient sample). Electrophoretic fragment analysis was performed in a 95 mm long simple-cross microchip (of the same design as the glass ones in ref. 15, and as shown in the ESI⁺) using a modification of the procedure employed for glass-based chips,¹⁵ with the results shown in the electropherogram of Fig. 2. The resolution of the devices is approximately 8 base pairs, a value typical for separations in photolithographically fabricated glass chips of this same design. We attribute this remarkably good performance to the reflow of the wax features, thereby forming straighter lines with smoother features. This resolution is sufficient to run a DNA size standard typically used in medical diagnostic applications (Fig. 2(a)) or to size a genetically amplified DNA fragment by electrophoresing it along with the size standard (Fig. 2(b)). These hybrid CE chips have been found to be indefinitely reusable after water rinsing and polymer reloading.

4 Concluding remarks

We have demonstrated a simple procedure for the fabrication of PDMS-based devices using a wax printer in a 'print, pour, peel, press and run' procedure. The potential of such devices is demonstrated using CE for DNA separation with no apparent loss of resolution over that of conventional photolithographic microfabrication methods. We believe the simplicity, costeffectiveness, and rapid fabrication of polymeric devices is an



Fig. 2 Electropherogram representing time (*x*-axis) and relative fluorescence intensity (*y*-axis) of a PCR product separated using the hybrid PDMS/glass CE microchip fabricated using the rapid proto-typing approach. In (a) only the DNA ladder (GS500) is separated within the CE chip, and in (b) the PCR product that represents the presence of a DNA virus which is separated/detected, and additionally sized using the GS500 DNA ladder.

extremely useful research tool. The technique allows the fabrication of a new microchip design within a matter of hours (and as little as an hour) without the need of microfabrication facilities.

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