Soft lithography: masters on demand†

Mohamed Abdelgawad,[a] Michael W. L. Watson,[b] Edmond W. K. Young,[a,c] Jared M. Mudrik,[b] Mark D. Ungrin[c] and Aaron R. Wheeler*[b,c,d]

Received 10th March 2008, Accepted 21st May 2008
First published as an Advance Article on the web 24th June 2008
DOI: 10.1039/b804050h

We report an ultra-rapid prototyping technique for forming microchannel networks for lab-on-a-chip applications, called masters on-demand. Channels are produced by replica molding on masters formed by laser printing on flexible copper printed circuit board (PCB) substrates. Masters of various designs and dimensions can be individually or mass produced in less than 10 minutes. Using this technique, we have fabricated channels as narrow as 100 μm with heights ranging between 9 μm and 70 μm. Multi-depth channel fabrication is also reported, using a two-step printing process. The functionality of devices formed in this manner is verified by performing in-channel electrophoretic separations and culture and analysis of primary mammalian cells.

Introduction

The field of microfluidics dates back to 1992;[1,2] in those early days, lab-on-a-chip research was limited to a few groups with access to clean-rooms equipped with sophisticated microfabrication facilities. In the late 1990s, the Whitesides group at Harvard developed an alternative fabrication method, called soft lithography.[3–7] In soft lithography, an elastomeric material, typically poly(dimethylsiloxane), is cast against a positive-relief master, which is itself formed from patterned features in photoresist. This was an important development, as soft lithography made microfluidics much more accessible than it had been previously. Although soft lithography is less expensive and much faster than conventional microfabrication techniques, it suffers from several drawbacks, the most important being that photolithography is used to produce the master, which typically requires a clean-room facility.

Since the late 1990s, a variety of new fabrication methods have been developed, improving upon soft lithography. These newer methods can be divided into two categories—those that use photolithography (and related techniques), and those that do not. Examples of the first category of methods include: liquid phase photopolymerization of masters or channels,[8,9] the use of dry film photoresists to form masters,[10,11] and the formation of masters from printed circuit board (PCB) substrates pre-coated with photoresist.[12] Examples of the second category of methods not requiring photolithography have primarily relied on laser printers to print micron-sized features of laser toner or wax, which can be used directly[13–20] or indirectly[21,22] to form microchannel structures. While most of these methods are limited to modest channel depths that can be formed by thin layers of laser toner, the "Shrinky-Dink" process[23] can condense toner-features to achieve microchannels as deep as 80 μm. These examples represent the variety of innovative, creative, and low-cost techniques that have made microfluidics accessible to everyone. But while each of these methods is an improvement over soft lithography, they are all hindered by either (a) the requirement of photolithography facilities (first category above) or by (b) material incompatibilities and/or limited channel depths (second category above).

Here, we present a new ultra-rapid prototyping method for forming microfluidic devices that combines the advantages of each of the two categories of techniques described above. The new method does not require photolithography, and is used to form robust, metal masters with channel depths ranging from 9–70 μm. In developing this method, which we call masters on-demand, we built on the idea reported by Sudarsan *et al.*,[12] in which PDMS devices are cast against copper masters formed from PCB substrates. But rather than patterning etch masks on PCB substrates by photolithography, we used printed-toner features, essentially trading a UV exposure system for a desktop laser printer. This exchange reduces the time required to fabricate masters from a few days (including the process of obtaining a photomask from a commercial source) to few minutes, which makes the new technique ideal for on-demand applications where new designs are required on an on-going basis.

To demonstrate the functionality of devices formed using this technique, we fabricated microchannels with a range of dimensions and applied them to electrophoretic separations of amino acids and to the culture and analysis of primary

[a]*Department of Mechanical and Industrial Engineering, University of Toronto, 5 King's College Road, Toronto, ON, Canada M5S 3G8*
[b]*Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, ON, Canada M5S 3H6. E-mail: awheeler@chem.utoronto.ca; Fax: +1 (416) 946-3865; Tel: +1 (416) 946-3864*
[c]*Institute of Biomaterials and Biomedical Engineering, University of Toronto, 164 College Street, University of Toronto, Toronto, ON, Canada M5S 3G9*
[d]*Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, ON, Canada M5G 1L6*

† Electronic supplementary information (ESI) available: Methods and materials used to implement electrophoretic separations and cell culture and analysis. See DOI: 10.1039/b804050h

endothelial cells. This technique, in addition to being inexpensive and rapid (*i.e.*, masters can be formed in ~7 minutes), combines the accessibility of laser printing-based fabrication with the advantages of robust masters and the capacity to form deep channels.

Experimental

Fabrication materials

Flexible, copper-coated films (~$19 per sq. ft.) were obtained from DuPont Electronic Materials (Research Triangle Park, NC, USA). Four types of films were used: AP7164E (double sided 12 μm copper on 25 μm polyimide film), LF7002R (single sided 35 μm copper on 13 μm polyimide film), LF9110R (single sided 35 μm copper on 25 μm polyimide film), and AP9242E (double sided 70 μm copper on 50 μm polyimide film). Copper etchant CE-100 was purchased from Transene Company Inc. (Danvers, MA, USA). Dicing tape used for etch protection was from Semiconductor Equipment Corporation (MoorPark, CA, USA). Sylgard-184 poly(dimethylsiloxane) (PDMS) (Dow-Corning, Midland, MI, USA) was obtained from Paisley Products of Canada Inc. (Toronto, ON, Canada). Acetone, concentrated sulfuric acid, and hydrogen peroxide (30%) were from Fisher Scientific Canada (Ottawa, ON, Canada). Piranha solution was prepared as a 3 : 1 (v/v) mixture of sulfuric acid and hydrogen peroxide.

Master laser printing

A schematic depicting the laser printing fabrication procedure is shown in Fig. 1. Channel designs were generated using Adobe Illustrator (Adobe Systems Incorporated, San Jose, CA, USA) and copper-clad sheets were trimmed into A6 format (105 mm × 148 mm) and loaded into the manual feed tray of a Xerox Phaser 6360N color printer (Xerox Corporation, Wilsonville, OR, USA). For two-sided substrates (copper on the front and back), prior to printing, one side was stripped by immersing in CE-100 (60 °C, 2–8 min depending on copper thickness) while the other side was protected with dicing tape. After printing, substrates were cut to size (typically 25 × 75 mm) and immersed in CE-100 (60 °C, 2–8 min), until the exposed copper had been etched away. During etching, samples were continuously agitated to achieve uniform etch rates. After rinsing and drying, the remaining toner was removed using a wipe dampened with acetone. 2-D and 3-D profiles of the masters and surface roughness measurements were generated using a Wyko optical profilometer (Veeco Instruments Inc., Woodbury, NY, USA).

Channel casting and bonding

Prior to casting PDMS to form microfluidic devices, masters were affixed to glass slides using double-sided tape. This step can be performed before or after etching; however we found it was best to do it before etching for thin polyimide substrates (*i.e.*, 25 μm) to avoid wrinkling in later steps. Moreover, it is recommended to affix the tape only to the outline of the polyimide film (leaving the area under the copper pattern free) to avoid wrinkling in cases when the tape warps from heating during PDMS curing. After affixing, masters were placed into Petri dishes, and immersed in ~3–4 mm of freshly mixed, degassed PDMS (10 : 1 monomer : curing agent). Devices were cured in an oven (70 °C, 1 h), and the PDMS slab was peeled from the master, trimmed, and inlet holes were punched with a single hole paper puncher.

Two methods were used to irreversibly bond PDMS microfluidic devices to glass slides: oxygen plasma[4] or PDMS glue.[24] In

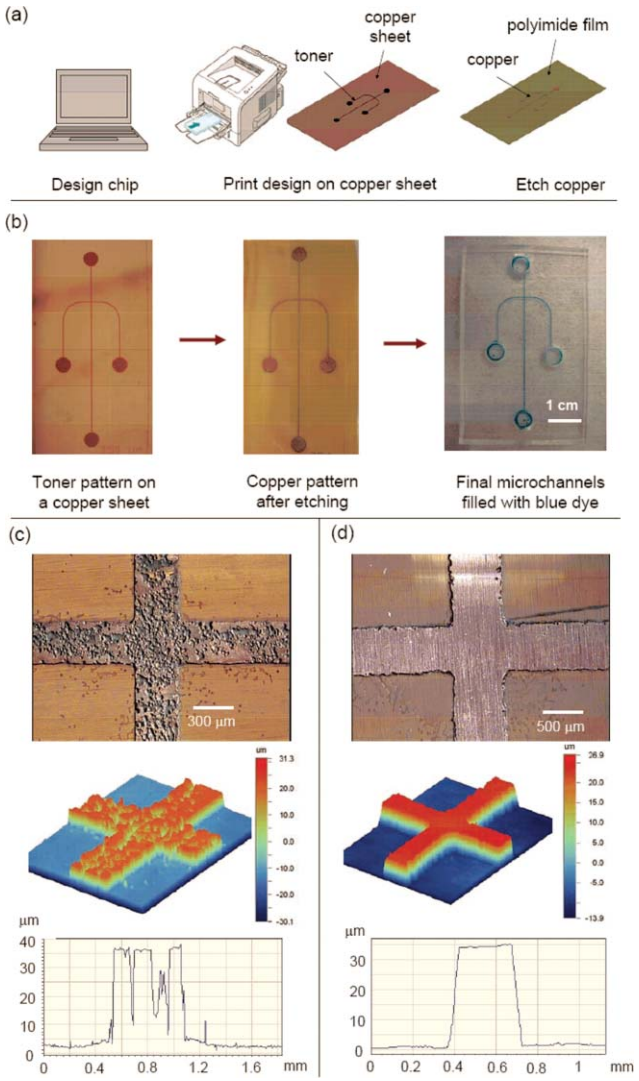

Fig. 1 Masters on-demand fabrication method for forming microfluidic devices. **a**. Schematic depicting the formation of copper-on-polyimide masters. First, a channel design is drawn using CAD software; then the design is printed on a flexible PCB substrate; finally, the exposed copper is etched away and the remaining toner removed. **b**. Pictures of the actual fabrication process showing the toner pattern on the copper sheet, the copper pattern after etching, and the resulting channel bonded to a glass slide and filled with a blue dye. **c**./**d**. Pictures and 2D and 3D profilometer scans of the resulting masters. **c**. A single layer of toner cannot fully protect a 35 μm-thick copper feature during etching, and surface pitting is observed. **d**. When a registration black toner mask is used, the toner is dense enough to resist dissolution in etchant. Masks formed from registration black can provide protection for sufficient time to etch up to 70 μm-thick copper with no pitting. Note the slightly trapezoidal nature of the sidewalls.

the first method, PDMS devices were cleaned in isopropanol and glass slides in piranha solution and then dried and exposed to an oxygen plasma (Harrick Plasma, Ithaca, NY, 90 s, 500 mTorr). Immediately afterwards, the PDMS and glass pieces were assembled while applying moderate pressure by hand for few seconds. In the second method, freshly mixed, degassed PDMS (10 : 1 monomer : curing agent) was spin coated on glass slides (6000 rpm, 1 min) and partially cured in an oven (70 °C, 5 min) to form a tacky layer of PDMS, against which the PDMS channel was then sealed by curing in an oven (70 °C, 1 h). After forming, channels were visualized using food color dye from McCormack Canada (London, ON, USA). Beads (30 μm dia.) used to pack multi-depth microchannels (see results section) were obtained from Rohm and Hass (Marlborough, MA, USA), and suspended in DI water with 20% ethanol.

Applications

The methods and materials used to implement electrophoretic separations and cell culture and analysis can be found online in the supplementary information.†

Results and discussion

Effect of toner density

The central feature of the new on-demand master fabrication technique is the use of a desktop printer to transfer laser-toner patterns onto flexible, copper-clad films. As described previously for other applications,[25] when immersed in ferric chloride etchants, laser toner (a mix of iron oxide and styrene-acrylate copolymer)[26] protects covered features, while exposed copper is etched away. When optimizing this method for fabricating microchannels, we noticed that a single layer of toner was not always dense enough to resist dissolution when exposed to etchant for long periods of time. While a single layer of toner works well for 9 μm-thick copper (etching time ~1–2 min), performance is poor when a single toner layer is used with thicker copper substrates (35 μm and 70 μm, with etch times of ~5 and ~8 min, respectively). In the latter case (Fig. 1c), we observed that etchant penetrated the toner, which had the effect of causing pinholes to form on the copper feature's surface. This problem was solved by using multiple layers of toner to form a thicker etch mask; this is readily achievable using color laser printers.

Color laser printers can print up to four different toner layers (namely cyan, yellow, magenta and black) on top of each other, with excellent alignment, to produce the specified color. Multilayer toner is not appreciably thicker than single-layer toner; rather, the salient feature is density—lines formed by printing multiple layers have fewer pinholes. For example, red features are formed by printing a layer of yellow on top of a layer of magenta—we observed that this type of two-toner-layer mask provides sufficient protection to etch 35 μm thick copper layers. To protect 70 μm copper, a four-toner layer mask was required, which was achieved by printing "rich black", or "registration black".[27] In this mode, cyan, yellow, magenta, and black toners are printed on top of each other to produce features that are darker and shinier than black toner alone. Masks formed using rich black were capable of protecting all of the thicknesses of copper evaluated here to full etch depth.

In comparison to the photoresist masters (*e.g.*, SU-8) typically used in soft-lithography, which are fragile and sometimes suffer from poor adhesion to the glass or silicon substrates, the copper PCB masters are robust; it is almost impossible to scratch or otherwise alter the features during casting. We have used every master for at least 10 castings with no degradation in quality, but speculate that they can be used indefinitely. Moreover, PCB masters formed in this manner might prove useful for hot embossing of microchannels in poly(methylmethacrylate) (PMMA),[28] or in other thermoplastic substrates.[12]

PDMS bonding

To form enclosed microchannels, micro-molded PDMS devices are typically sealed to cover-plates formed from glass or PDMS. The classic bonding technique uses oxygen plasma to form reactive silanol groups on two surfaces which condense into siloxy bonds when brought into contact.[4] We observed that this technique works well for masters formed from rolled-annealed substrates (LF9110R and LF7002R) which have a smooth polyimide film (average surface roughness of 40 nm, comparable to the ~1–3 nm for glass[29]) underneath the copper. In contrast, we observed that masters formed from electrodeposited copper substrates (AP7164E and AP9242E), which have a relatively rough polyimide film (average surface roughness of 420 nm) under the copper, did not result in PDMS devices capable of forming reliable, permanent seals by the oxygen plasma technique. This phenomenon was reported previously for devices formed from PCB-masters.[12] To overcome this problem, PDMS devices formed using electrodeposited copper masters were permanently bonded to glass slides coated with a partially cured PDMS layer[24] (see experimental section). If a spin-coater was not available, such substrates could be made compatible with oxygen plasma by wet-etching the copper layer to an intermediate depth because wet-etched copper is smoother (average roughness of 100 nm) than the polyimide backing. Regardless of the bonding method used, the devices exhibited strong, permanent seals, and could withstand electroosmotic and pressure-driven flows as described in the following sections.

Channel characterization

The height of channels formed by the new method is defined by the thickness of the copper layer, while the widths and shapes of the channels are determined by the geometry of the printed design and the isotropic nature of the etching process. The smallest line width that could be produced using our printer was 200 μm, which generated channels ranging from ~100 to ~180 μm for the substrates used here (*i.e.*, 100 μm-wide features for 70 μm-thick copper and 180 μm-wide features for 12 μm-thick copper). There is no upper limit on the widths of channels formed by the new method, except for the universal constraint of PDMS sagging at low channel aspect ratio (height/width).[30,31] As shown in Fig. 1d, channels formed by the new method have trapezoidal side walls, which has been observed previously.[12]

The primary limitation of the new technique is the low resolution of the desktop printer. Two consequences of the low resolution are limited channel widths and heterogeneous channel walls. As described above, channels with widths as low as 100 μm can be formed reproducibly. Widths as small as 65 μm

can be formed by increasing the etch times; however, channels formed in this manner have increased surface heterogeneity caused by the extended etching process. Additionally, the limited resolution of the printed lines results in channel walls with some waviness. Nonetheless, the efficiency of fabrication (as fast as ~7 min for the complete process from design to master) makes this an attractive method for on-demand device prototyping, and devices formed in this manner work well for a range of applications, as described below.

Applications

PDMS devices formed as described above were evaluated for compatibility with chemical separations, a mainstay application in microfluidics. In such applications, it is often advantageous to use shallow channels to limit the current and Joule heating. In this work, we used 35 μm-deep channels to separate fluorescent dyes as well as labelled amino acids by micellar electrokinetic chromatography (MEKC). Typical results are shown in Fig. 2. In each case, all analytes were baseline (or near-baseline) resolved in less than two minutes.

To further demonstrate the utility of devices formed using the reported technique, microchannels were used to culture primary endothelial cells for cell–protein adhesion studies. In such applications, it is often advantageous to use deep channels to prevent clogging during cell loading. In this work, aortic endothelial cells isolated from pig hearts were seeded into 70 μm-deep microchannels *via* syringe, and were incubated under typical culture conditions to allow cell adhesion and spreading onto pre-adsorbed protein. Immediately after seeding, cells appeared rounded (as in suspension), and were uniformly dispersed throughout the channel (Fig. 2c). After 3 hours of incubation, cells were observed to spread and form networked cell islands, which is a positive indicator of endothelial cell health[32] (Fig. 2d).

Devices formed using the new technique had similar functionality to (conventional) PDMS–glass devices generated by soft lithography. As with conventional devices, the microchannels formed by the new method were robust and versatile, able to withstand multiple injections of solutions while permitting live-cell fluorescence imaging. When exposed to flow rates of ~1 mL min^{-1}, the channels withstood pressures of ~50 kPa with no leaking. One disadvantage of this method is that the roughness of the PDMS channel walls degrades the quality of phase-contrast images (not shown). But, as demonstrated in Fig. 2, this channel characteristic has no effect on fluorescence images or brightfield images (not shown). Because of the rapidity with which devices can be formed, this new technique would be well suited for screening the effects of microchannel dimensions (width, length, or height) to investigate diffusional factors on cellular behavior in different microenvironments.[33] Using conventional soft lithography, the process of iterating through masters with many different depths (in which each experiment suggests the next device parameter) would require several days of work.

Multi-depth channels

The applications described above are well-suited for devices with uniform channel depth; however, there are many applications in which multiple channel depths are required.[34–37] When using conventional photolithography-based techniques, fabrication of multi-depth channels is challenging and time-consuming, requiring at least two photomasks and optimization of exposure time.[38–40] In contrast, when using the new laser printing technique, fabricating multi-depth masters is straightforward, as depicted in Fig. 3. First, a toner mask is printed to cover areas in which the feature height is to be kept at the original copper thickness (*i.e.*, 12, 35, or 70 μm according to the substrate used). Then, the exposed copper is etched to the intermediate height, which can be any value between zero and the full thickness, as a function of etch rate and time.[12,41] After removing the toner, channel patterns were printed on the (now) multi-thickness substrate which was then etched again to remove

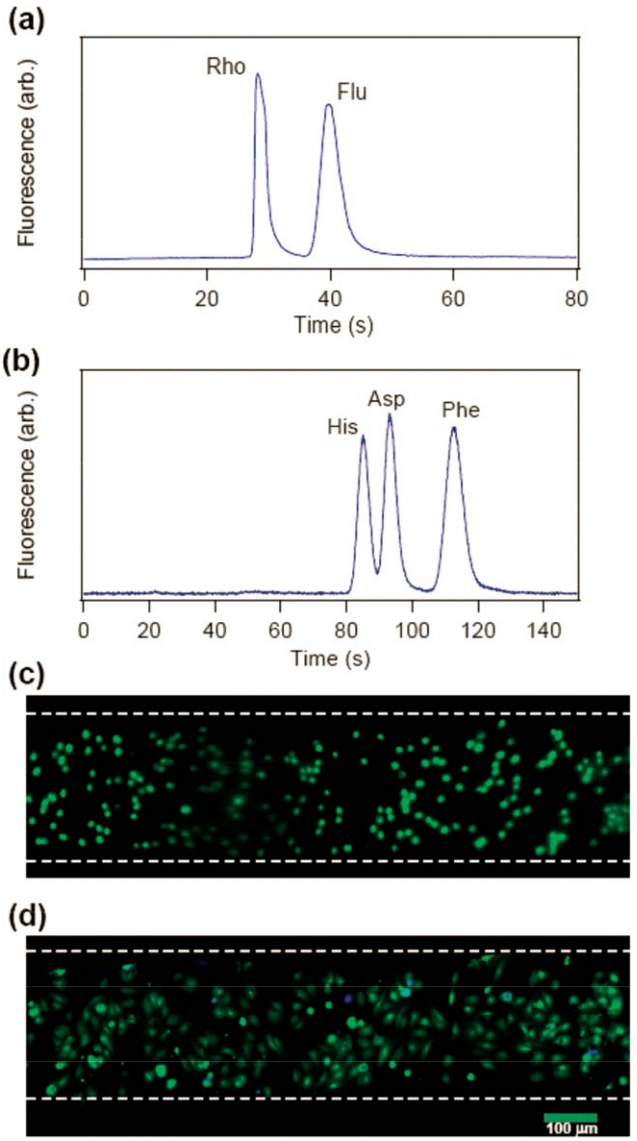

Fig. 2 Applications implemented in devices formed by the new method. **a**. Electropherogram of rhodamine 123 (Rho) and fluorescein (Flu). **b**. Electropherogram of NDA-labeled histidine (His), aspartic acid (Asp) and phenylalanine (Phe). **c**. Porcine aortic valve endothelial cells (PAVECS) distributed evenly throughout channel immediately after seeding. **d**. After 3 hours of incubation, cells spread and formed networks within the microchannel. Scale bar = 100 μm.

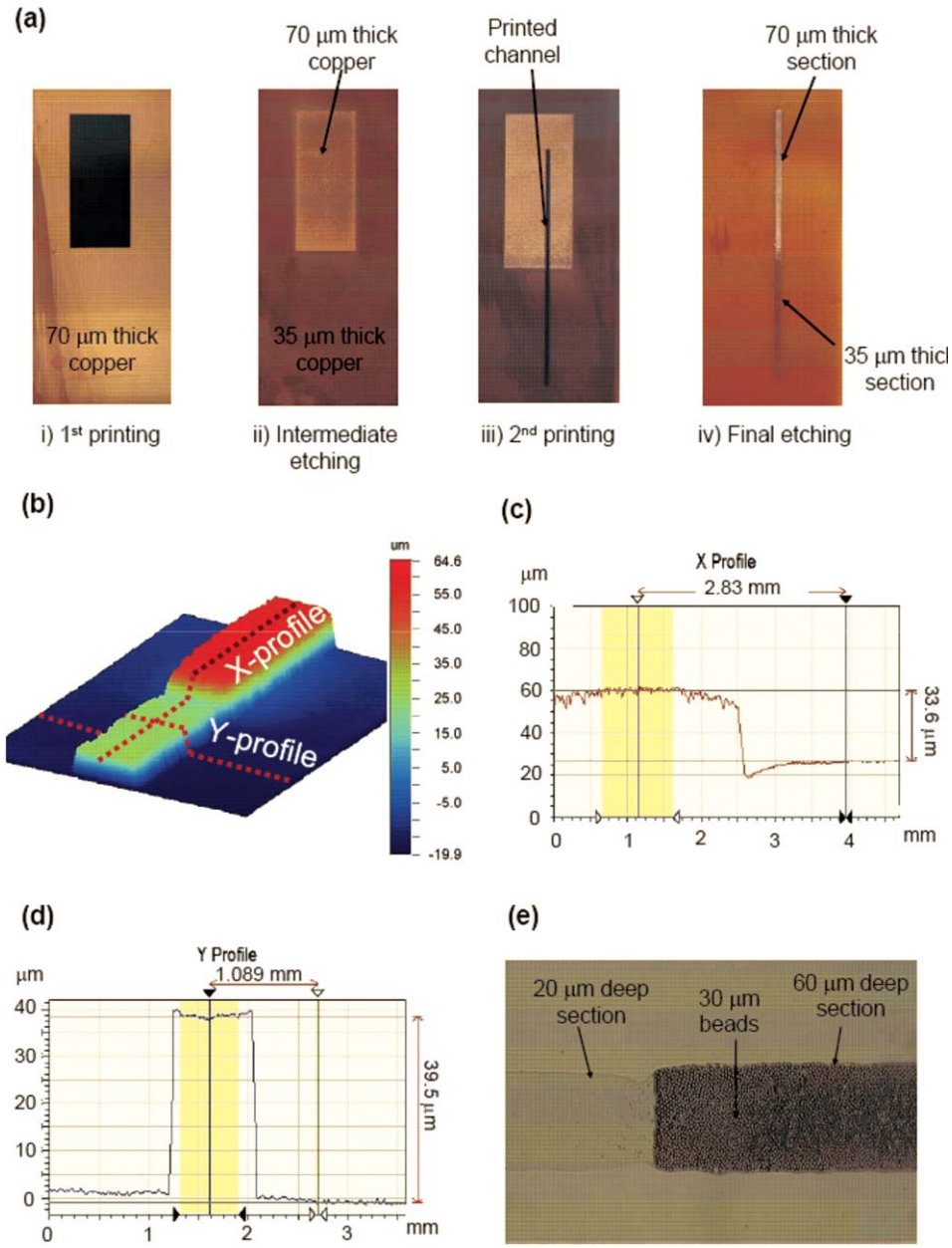

Fig. 3 Multi-depth channels. **a**. Pictures of the four steps in the fabrication of multi-depth masters: (i) portions of the channel which are to have maximum depth (in this case, 70 µm) are covered with toner; (ii) exposed copper is etched to the desired thickness (in this case, 35 µm, requiring 3 min of etching) and the toner is removed; (iii) the channel pattern is printed on the master such that portions of the channel overlap the different thicknesses of copper; (iv) the remaining exposed copper is etched away and toner is removed. **b**. 3D profilometer scan of a master with two heights. **c**. 2D profilometer scan of the master shown in b along the *X*-profile. The masked portion of the channel-master is 33 µm higher than the etched portion. **d**. The *Y*-profile plot shows the height of the etched portion to be 39 µm. e. Picture of 30 µm dia. beads packed inside a multi-depth microchannel formed using the new method. The beads can pass though the deep section of the channel, but are blocked from passing through the shallow section. In this case, the original master was 70 µm and 35 µm deep as in **b**–**d**, but was etched for an additional 1 min after removal of the second toner mask to form a 60 µm/20 µm master.

all of the exposed copper. As shown in Fig. 3a, we typically used large features (several mm wide) for the first etch, which made alignment of the channel pattern for the second etch straightforward. As shown in Fig. 3c, an unintended "dip" is formed on the master at the interface between the two channel depths; we believe this is caused by reduced toner coverage on the heterogenous interface (resulting in increased etching). To demonstrate the utility of devices formed in this manner, we packed 30 µm dia. beads into a 60 µm/20 µm multi-depth microchannel (Fig. 3e); similar structures have been used to perform on-chip solid phase extraction[36,37] and separations.

Conclusion

Rapid microchannel prototyping by on-demand master fabrication is a straightforward and low-cost method to fabricate

microchannel networks for lab-on-a-chip applications. Channel masters can be produced in less than 10 minutes without requirement of sophisticated facilities. Channels as narrow as 100 μm can be produced by this technique with channel depths ranging between 9 and 70 μm. Microchannels produced using this method are compatible with both electroosmotic and pressure-driven flows and are compatible with electrophoretic separations and cell culture and analysis. Multi-depth channels can also be formed by this method using a two-step printing process, which allows for the rapid formation of packed columns of beads. Thus, masters on-demand represents a useful new rapid-prototyping method for a wide range of lab-on-a-chip applications.

Acknowledgements

We thank DuPont for contributing free samples of their flexible PCB substrates, and Prof. Craig Simmons (U. of Toronto) for access to PAVECs and associated reagents. We thank the Natural Sciences and Engineering Research Council (NSERC) and the Canada Foundation for Innovation (CFI) for financial support. M. Abdelgawad thanks the OGS program for an Ontario Graduate Scholarship. ARW thanks the CRC for a Canada Research Chair.

References

1 D. J. Harrison, A. Manz, Z. Fan, H. Ludi and H. M. Widmer, Capillary electrophoresis and sample injection systems integrated on a planar glass chip, *Anal. Chem.*, 1992, **64**, 1926–1932.
2 A. Manz, D. J. Harrison, E. M. J. Verpoorte, J. C. Fettinger, A. Paulus, H. Ludi and H. M. Widmer, Planar chips technology for miniaturization and integration of separation techniques into monitoring systems. Capillary electrophoresis on a chip, *J. Chromatogr.*, 1992, **593**, 253–258.
3 D. Qin, Y. Xia and G. M. Whitesides, Rapid prototyping of complex structures with feature sizes larger than 20 mm, *Adv. Mater.*, 1996, **8**, 917–919.
4 D. C. Duffy, J. C. McDonald, O. J. A. Schueller and G. M. Whitesides, Rapid prototyping of microfluidic systems in Poly(dimethylsiloxane), *Anal. Chem.*, 1998, **70**, 4974–4984.
5 Y. Xia and G. M. Whitesides, Soft lithography, *Annu. Rev. Mater. Sci.*, 1998, **28**, 153–184.
6 J. R. Anderson, D. T. Chiu, R. J. Jackman, O. Chemiavskaya, J. C. McDonald, H. Wu, S. H. Whitesides and G. M. Whitesides, Fabrication of topologically complex three-dimensional microfluidic systems in PDMS by rapid prototyping, *Anal. Chem.*, 2000, **72**, 3158–3164.
7 J. C. McDonald, D. C. Duffy, J. R. Anderson, D. T. Chiu, H. Wu, O. J. A. Schueller and G. M. Whitesides, Fabrication of microfluidic systems in poly(dimethylsiloxane), *Electrophoresis*, 2000, **21**, 27–40.
8 C. Khoury, G. A. Mensing and D. J. Beebe, Ultra rapid prototyping of microfluidic systems using liquid phase photopolymerization, *Lab Chip*, 2002, **2**, 50–55.
9 J. B. Hutchison, K. T. Haraldsson, B. T. Good, R. P. Sebra, N. Luo, K. S. Anseth and C. N. Bowman, Robust polymer microfluidic device fabrication *via* contact liquid photolithographic polymerization (CLiPP), *Lab Chip*, 2004, **4**, 658–662.
10 P. Vulto, N. Glade, L. Altomare, J. Bablet, L. D. Tin, G. Medoro, I. Chartier, N. Manaresi, M. Tartagni and R. Guerrieri, Microfluidic channel fabrication in dry film resist for production and prototyping of hybrid chips, *Lab Chip*, 2005, **5**, 158–162.
11 K. Stephan, P. Pittet, L. Renaud, P. Kleimann, P. Morin, N. Ouaini and R. Ferrigno, Fast prototyping using a dry film photoresist: microfabrication of soft-lithography masters for microfluidic structures, *J. Micromech. Microeng.*, 2007, **17**, N69–N74.
12 A. P. Sudarsan and V. M. Ugaz, Printed circuit technology for fabrication of plastic-based microfluidic devices, *Anal. Chem.*, 2004, **76**, 3229–3235.
13 A. Tan, K. Rodgers, J. P. Murrihy, C. O'Mathuna and J. D. Glennon, Rapid fabrication of microfluidic devices in poly(dimethylsiloxane) by photocopying, *Lab Chip*, 2001, **1**, 7–9.
14 M. L. Branham, R. Tran-Son-Tay, C. Schoonover, P. S. Davis, S. D. Allen and W. Shyy, Rapid prototyping of micropatterned substrates using conventional laser printers, *J. Mater. Res.*, 2002, **17**, 1559–1562.
15 C. L. Do Lago, H. D. T. Da Silva, C. A. Neves, J. G. A. Brito-Neto and J. A. F. Da Silva, A Dry Process for Production of Microfluidic Devices Based on the Lamination of Laser-Printed Polyester Films, *Anal. Chem.*, 2003, **75**, 3853–3858.
16 W. K. T. Coltro, J. A. Fracassi da Silva, H. D. Torres da Silva, E. M. Richter, R. Furlan, L. Angnes, C. L. do Lago, L. H. Mazo and E. Carrilho, Electrophoresis microchip fabricated by a direct-printing process with end-channel amperometric detection, *Electrophoresis*, 2004, **25**, 3832–3839.
17 N. Bao, Q. Zhang, J.-J. Xu and H.-Y. Chen, Fabrication of poly(dimethylsiloxane) microfluidic system based on masters directly printed with an office laser printer, *J. Chromatogr., A*, 2005, **1089**, 270–275.
18 A. L. Liu, F. Y. He, K. Wang, T. Zhou, Y. Lu and X. H. Xia, Rapid method for design and fabrication of passive micromixers in microfluidic devices using a direct-printing process, *Lab Chip*, 2005, **5**, 974–978.
19 V. I. Vullev, J. Wan, V. Heinrich, P. Landsman, P. E. Bower, B. Xia, B. Millare and G. Jones Ii, Nonlithographic fabrication of microfluidic devices, *J. Am. Chem. Soc.*, 2006, **128**, 16062–16072.
20 G. V. Kaigala, S. Ho, R. Penterman and C. J. Backhouse, Rapid prototyping of microfluidic devices with a wax printer, *Lab Chip*, 2007, **7**, 384–387.
21 C. L. do Lago, C. A. Neves, D. Pereira de Jesus, H. D. da Silva, J. G. Brito-Neto and J. A. Fracassi da Silva, Microfluidic devices obtained by thermal toner transferring on glass substrate, *Electrophoresis*, 2004, **25**, 3825–3831.
22 W. K. T. Coltro, E. Piccin, J. A. Fracassi Da Silva, C. Lucio Do Lago and E. Carrilho, A toner-mediated lithographic technology for rapid prototyping of glass microchannels, *Lab Chip*, 2007, **7**, 931–934.
23 A. Grimes, D. N. Breslauer, M. Long, J. Pegan, L. P. Lee and M. Khine, Shrinky-Dink microfluidics: rapid generation of deep and rounded patterns, *Lab Chip*, 2008, **8**, 170–172.
24 A. R. Wheeler, G. Trapp, O. Trapp and R. N. Zare, Electroosmotic flow in a poly(dimethylsiloxane) channel does not depend on percent curing agent, *Electrophoresis*, 2004, **25**, 1120–1124.
25 M. Abdelgawad and A. R. Wheeler, Rapid prototyping in copper substrates for digital microfluidics, *Adv. Mater.*, 2007, **19**, 133–137.
26 X. Nie, J. D. Miller and Y. D. Yeboah, The effect of ink types and printing processes on flotation deinking efficiency of wastepaper recycling, *Environ. Eng. Policy*, 1998, **1**, 47–58.
27 E. R. S. Hodges, *The Guild Handbook of Scientific Illustration*, John Wiley and Sons, 2nd edn, 2003.
28 R. T. Kelly, T. Pan and A. T. Woolley, Phase-changing sacrificial materials for solvent bonding of high-performance polymeric capillary electrophoresis microchips, *Anal. Chem.*, 2005, **77**, 3536–3541.
29 Z. X. Jiang, Y. D. Huang, L. Liu and J. Long, Effects of roughness on interfacial performances of silica glass and non-polar polyarylacetylene resin composites, *Appl. Surf. Sci.*, 2007, **253**, 9357–9364.
30 E. Delamarche, H. Schmid, B. Michel and H. Biebuvck, Stability of molded polydimethylsiloxane microstructures, *Adv. Mater.*, 1997, **9**, 741–746.
31 T. Gervais, J. El-Ali, A. Gunther and K. F. Jensen, Flow-induced deformation of shallow microfluidic channels, *Lab Chip*, 2006, **6**, 500–507.
32 E. W. K. Young, A. R. Wheeler and C. A. Simmons, Matrix-dependent adhesion of vascular and valvular endothelial cells in microfluidic channels, *Lab Chip*, 2007, **7**, 1759–1766.
33 H. Yu, I. Meyvantsson, I. A. Shkel and D. J. Beebe, Diffusion dependent cell behavior in microenvironments, *Lab Chip*, 2005, **5**, 1089–1095.
34 L. Zhu, Q. Zhang, H. Feng, S. Ang, F. S. Chau and W. T. Liu, Filter-based microfluidic device as a platform for immunofluorescent assay of microbial cells, *Lab Chip*, 2004, **4**, 337–341.
35 K. Hyoung Kang, X. Xuan, Y. Kang and D. Li, Effects of dc-dielectrophoretic force on particle trajectories in microchannels, *J. Appl. Phys.*, 2006, **99**.

36 Z. Long, Z. Shen, D. Wu, J. Qin and B. Lin, Integrated multilayer microfluidic device with a nanoporous membrane interconnect for online coupling of solid-phase extraction to microchip electrophoresis, *Lab Chip*, 2007, **7**, 1819–1824.
37 R. Zhong, D. Liu, L. Yu, N. Ye, Z. Dai, J. Qin and B. Lin, Fabrication of two-weir structure-based packed columns for on-chip solid-phase extraction of DNA, *Electrophoresis*, 2007, **28**, 2920–2926.
38 J. Carlier, S. Arscott, V. Thomy, J. C. Fourrier, F. Caron, J. C. Camart, C. Druon and P. Tabourier, Integrated microfluidics based on multi-layered SU-8 for mass spectrometry analysis, *J. Micromech. Microeng.*, 2004, **14**, 619–624.
39 B. Bohl, R. Steger, R. Zengerle and P. Koltay, Multi-layer SU-8 lift-off technology for microfluidic devices, *J. Micromech. Microeng.*, 2005, **15**, 1125–1130.
40 K.-S. Yun and E. Yoon, Fabrication of complex multilevel microchannels in PDMS by using three-dimensional photoresist masters, *Lab Chip*, 2008, **8**, 245–250.
41 O. Cakir, H. Temel and M. Kiyak, Chemical etching of Cu-ETP copper, *J. Mater. Process Technol.*, 2005, **162–163**, 275–279.