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Rapid Prototyping of Poly(methyl methacrylate) Microfluidic Systems Using Solvent Imprinting and Bonding

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Abstract

We have developed a method for rapid prototyping of hard polymer microfluidic systems using solvent imprinting and bonding. We investigated the applicability of patterned SU-8 photoresist on glass as an easily fabricated template for solvent imprinting. Poly(methyl methacrylate) (PMMA) exposed to acetonitrile for 2 min then had an SU-8 template pressed into the surface for 10 min, which provided appropriately imprinted channels and a suitable surface for bonding. After a PMMA cover plate had also been exposed to acetonitrile for 2 min, the imprinted and top PMMA pieces could be bonded together at room temperature with appropriate pressure. The total fabrication time was less than 15 min. Under the optimized fabrication conditions, nearly 30 PMMA chips could be replicated using a single patterned SU-8 master with high chip-to-chip reproducibility. Relative standard deviations were 2.3% and 5.4% for the widths and depths of the replicated channels, respectively. Fluorescently labeled amino acid and peptide mixtures were baseline separated using these PMMA microchips in <15 s. Theoretical plate numbers in excess of 5000 were obtained for a ~3 cm separation distance, and the migration time relative standard deviation for an amino acid peak was 1.5% for intra-day and 2.2% for inter-day analysis. This new solvent imprinting and bonding approach significantly simplifies the process for fabricating microfluidic structures in hard polymers such as PMMA.

1. Introduction

Microchip electrophoresis has likely been the most successful analysis approach in lab-on-a-chip systems, providing minimal sample and reagent consumption, rapid and efficient separations, and straightforward integration and automation [1,2]. The increasing demand for low-cost microdevices has led to the development of reproducible protocols for their manufacture using inexpensive substrates such as polymers [3]. Indeed, a wide variety of polymeric materials, such as poly(dimethylsiloxane) (PDMS) [4], poly(methyl methacrylate) (PMMA) [5], poly(cyclic olefin) [6], polycarbonate [7], poly(tetrafluoroethylene) [8], poly(ethylene terephthalate) [9], polyethylene [10], polystyrene [11], etc., have been evaluated for fabricating microchips. These polymers can be used to create microstructures with various optical, mechanical and surface properties [12].

In contrast to the cleanroom-intensive fabrication techniques needed to make glass microchips, polymer microstructures can be produced in high volumes with much simpler technologies such as hot embossing [13] or injection molding [5]. The most widely used,

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and perhaps the easiest fabrication approach, is that of PDMS casting in rapid prototyping [14]. Following the creation of a microchannel substrate, a cover must be sealed to the patterned piece to enclose the microcapillaries. Several methods for bonding polymers in this step have been reported, including elevated temperature annealing [15], lamination [10], plasma oxidation [16], and the use of adhesives [17] or solvents [18]. These sealing steps are far easier to accomplish with polymeric devices than glass substrates that must be annealed at ~600 °C for several hours.

Although rapid PDMS prototyping is easy and inexpensive, the electrophoresis separation performance of the resulting devices can be less than optimal, due to strong, nonspecific interactions with the hydrophobic PDMS surface [19]. On the other hand, rigid polymers such as PMMA offer superior separation performance to PDMS, but device fabrication is not as simple. To date, hot embossing has been the most popular method for PMMA microstructure fabrication [15]. In this approach, features are transferred from a template to heat-softened PMMA, and then thermal sealing encloses the channels. This approach is much faster (1–2 h fabrication time) than making glass microdevices, but is still time consuming. In addition, the etching of a silicon template for hot embossing polymer microchips is a rather slow process. Thus, alternative templates, such as SU-8 patterned substrates typically used for rapid PDMS prototyping, need to be explored for patterning hard polymers.

To overcome several disadvantages of existing methods for micromachining hard polymers, while taking advantage of their improved properties relative to PDMS, we have developed a fast, solvent-based polymer imprinting and bonding method. This approach enables rapid prototyping of microfluidics in hard polymeric materials. Instead of heating a material above the glass transition temperature, we apply a solvent on the surface to soften it for pattern transfer. Moreover, solvent imprinting provides a PMMA surface wet with acetonitrile for straightforward bonding to a cover plate. The whole microdevice patterning and bonding process can be completed within 15 min, significantly faster than thermal imprinting and bonding of polymers. Microchips made using these procedures have been employed in the separation of fluorescently labeled amino acids and peptides, offering rapid analysis with good separation performance.

2. Experimental

2.1 SU-8 template fabrication

75 mm×50 mm×1 mm glass microscope slides (Fisher Scientific) were dehydrated at 200 °C for 30 min. Patterned SU-8 photoresist features were formed on glass substrates as follows. SU-8 25 (Microchem, Newton, MA, USA) was spun on a glass slide at 1400 rpm for 2.5 min, which yielded a thickness of 16 µm, followed by baking at 50 °C, 65 °C and 95 °C for 5 min each. After exposing the photoresist to UV light through a patterning mask for 30 s, the glass slide was baked at 65 °C and 95 °C for 5 min each. Then, the SU-8 pattern was developed in SU-8 developer (Microchem) for 2 min, and lastly the glass substrate was diced into smaller pieces for imprinting templates.

2.2 Channel imprinting and PMMA bonding

A schematic overview of the imprinting and bonding process is shown in Figure 1A–G. Laser cut 4.8 mm×2 mm×0.4 mm PMMA pieces (Acrylite FF, CYRO Industries, Rockaway, NJ, USA) were cleaned with soapy water, rinsed and dried under nitrogen. Next, a uniform layer of acetonitrile (~300 µL) was applied onto two PMMA substrates (Figure 1A–B). After 30–120 s, the SU-8 patterned glass slide was pressed into one solvent-wetted surface until the stamp cavities were filled such that no bubbles were visible in the interface, and a C-clamp was used to hold the assembly together for a period of 6–60 min (Figure 1C).

The other PMMA piece (after 30 – 120 s acetonitrile exposure) had a glass microscope slide placed on top (to limit solvent evaporation) with ~500 kPa applied for 8 min (Figure 1D). The imprinted and top piece were each separated from their respective glass substrates (Figure 1E–F), and gentle pressure was applied manually to bond the solvent-wetted surfaces together (Figure 1G).

2.3 Separation and detection procedures

The amino acids were from ICN Biomedicals (Aurora, OH, USA), and the peptides were from Sigma-Aldrich (St. Louis, MO, USA). The amino acids and peptides were labeled fluorescently using fluorescein-5-isothiocyanate (FITC, Molecular Probes, Eugene, OR, USA) as in our prior studies [18]. The microchip layout is shown in Figure 1H; the channel linewidths (defined from the SU-8 pattern) are 55 μm , and the injection intersection offset is 100 μm . Electrophoresis procedures were similar to previous work.[18] 10 mM Tris (pH 8.5) with 0.5% (w/v) hydroxypropyl cellulose (HPC, Sigma-Aldrich) was used as the running buffer. Sample was placed into reservoir 1, while the other reservoirs were filled with running buffer. To load sample into the injection intersection, reservoirs 1, 2 and 4 were grounded, while 500 V were applied to reservoir 3. During separation, reservoir 2 was grounded, reservoirs 1 and 3 were maintained at 500 V, and 2000 V were applied to reservoir 4.

The laser-induced fluorescence detection system has been described previously [20]. Briefly, excitation of the FITC-labeled compounds was achieved with the 488-nm line from an air-cooled Ar ion laser, which was focused ~2 mm from the end of the separation channel using a 20 $\times$ 0.45NA objective. Fluorescence was collected using the same objective, and stray light was excluded by confocal spatial filtering with a 200- μm -diameter pinhole. A photomultiplier tube detected photons passing through the aperture, and the detector output was recorded on a computer at a 10 Hz sampling rate.

3. Results and Discussion

3.1 Solvent imprinting and bonding

To precisely control the microstructure dimensions imprinted in the PMMA surface, we studied imprinting and solvent exposure times, both of which directly influenced the accuracy with which the features were replicated. Fig. 2 shows a comparison of channel shapes after different imprinting times, and measurements of channel dimensions are provided in Table 1. For shorter imprinting times, the cross section of the channel had a trapezoidal shape, with ridges at the channel edges ranging in height (h') from 3.4 μm at 6 min to 0 μm at 60 min. These ridges were either caused by PMMA displacement during imprinting; or template removal from PMMA that was still soft. For all imprinting times, the channel width at the bottom (w) was close to the 55 μm SU-8 linewidth. In contrast, the channel width at the top (w') was ~20 μm wider than the SU-8 linewidth for the shortest imprinting time (6 min), but drew closer to the pattern linewidth as imprinting time increased. A 60 min imprinting time provided the most faithful replication of the original features of the SU-8 template; however, by then, too much solvent had evaporated to allow bonding to the cover plate. As a compromise between replication fidelity and ease of bonding, a 10 min imprinting duration was chosen. Fig. 3 shows an imprinted injection cross region; very little channel roughness is visible at this scale. Moreover, the cross-sectional shape of the fabricated channel reflects the approximate dimensions of the SU-8 template.

The solvent exposure time prior to imprinting and bonding also affected the enclosed channel dimensions. When the exposure time was 30 s (Fig. 4A), the enclosed channel was shallower (13 μm) compared with a 2 min solvent exposure time (Fig. 4B), with a 16 μm

depth. Channel width for both solvent exposure times was essentially the same as that of the SU-8 linewidth (55 μm). We attribute the difference between 30 s and 2 min solvent exposure to the effective depth in PMMA that acetonitrile could penetrate. With a shorter exposure time of 30 s, the acetonitrile softened a PMMA layer that was thinner than the 16- μm -tall SU-8 features. Thus, the SU-8 template could not be inserted fully into the PMMA, which resulted in shallower channels. For longer times, this problem was diminished, so we chose a 2 min solvent exposure time prior to imprinting. When the solvent exposure time was increased beyond 3 min, the imprinted channels had a tendency to crack as the devices aged. Importantly, after bonding, the enclosed channel width and height corresponded very well to the template features (e.g., Fig. 4B). These results indicate that during the bonding process, the ridges (h') disappear and the trapezoidal cross section becomes rectangular again, such that the enclosed channel shape matches that of the SU-8 template. Using these optimum fabrication conditions, we could make up to 30 PMMA microchips from a single SU-8 master. Feature dimension reproducibility was also high, as the relative standard deviations of channel width and height were 2.3% and 5.4%, respectively. Although we utilized acetonitrile for imprinting and bonding, other solvents for PMMA, such as acetone and tetrahydrofuran, may also work.

3.2 Microchip separation

The analytical performance of our rapidly prototyped PMMA microchips fabricated by solvent imprinting and bonding was demonstrated through capillary electrophoresis separation of amino acids and peptides. To minimize electroosmotic flow and prevent analyte adsorption [21], 0.5% (w/v) HPC was incorporated in the buffer. Fig. 5A shows the separation of FITC-labeled glycine, phenylalanine, and arginine; the three peaks were well resolved in 10 s. The best separations were achieved at electric fields of 200–350 V/cm, although the PMMA microchips prepared by our method could endure electric fields as high as 700 V/cm. The theoretical plate numbers for the three amino acids in Fig. 5A ranged from 4500 to 5500. The migration time reproducibility was also evaluated by running 10 consecutive injections and separations at 2-min intervals. The relative standard deviation of the FITC-Arg migration time was 1.5% for intra-day and 2.2% for inter-day analysis, indicating excellent reproducibility. We also tested the microchips for usability in peptide separations. Electrophoresis of four peptides, Phe-Ala (PA), Phe-Leu-Glu-Glu-Ile (FLEEI), Ala-Leu-Ala-Leu (ALAL), and Gly-Gly-Tyr-Arg (GGTA), was completed within 15 s (Fig. 5B). The resolution was 1.0 between PA and FLEEI, 1.5 between FLEEI and ALAL and 4.3 between ALAL and GGTA, indicating that the ~ 3 cm channel length is good enough for rapid separation with acceptable resolution. We anticipate that our microdevices might also find application in protein CE.

Our results clearly demonstrate that PMMA microdevices made by solvent imprinting and bonding can be used for microchip electrophoresis. Importantly, the microstructures can be formed in just 15 min. This combination of solvent imprinting and bonding offers a new and simple way to fabricate microfluidic structures in polymers, with bonding robustness similar to that in our previous solvent-sealed devices [18]. Moreover, with appropriate selection of imprinting and bonding solvent, this method can be adapted readily to other polymeric materials, offering great potential for polymer microchip fabrication.

4. Conclusions

We have shown that PMMA microstructures can be created by imprinting SU-8 features into acetonitrile-softened surfaces, and the resulting channels can be enclosed readily using solvent bonding. This procedure is rapid (~ 15 min), easy to implement, and offers good separation performance. The speed, simplicity, versatility, and low cost of this new

fabrication route make it extremely attractive for the widespread production of hard polymer microchips.

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Figure 1.

PMMA microchip fabrication process. (A, B) PMMA substrates have a layer of acetonitrile deposited on the surface. (C) A patterned SU-8 template on glass is pressed down on a solvent-softened PMMA surface. (D) An unpatterned glass slide is contacted to the other acetonitrile-coated PMMA substrate. (E) The SU-8 patterned template is removed, leaving a PMMA piece with imprinted microchannels, (F) and the glass slide is removed from the PMMA cover plate. (G) The cover plate is placed atop the imprinted substrate and held with applied pressure for 30 s to seal the channels. (H) Microchip design; the injector offset is not drawn to scale.

Figure 2.

SEM images depicting the effect of imprinting time on the channel structure. Imprinting times were (A) 6, (B) 10, (C) 20, (D) 30, and (E) 60 min. (F) Schematic of imprinted dimensions: channel height (h), channel edge ridge height (h'), channel width at the bottom (w), and channel width at the top (w'). Substrates are inverted relative to their orientation in bonded devices.

Figure 3.
Top-view SEM of an imprinted channel injection region.

Figure 4. Effect of solvent exposure time on bonded channel structures. SEM images of bonded channel cross sections for acetonitrile exposure times of (A) 0.5 and (B) 2 min.

Figure 5.

Electrophoretic separation of FITC-labeled (A) amino acids and (B) peptides in rapidly prototyped PMMA microchips, using 10 mM Tris buffer (pH 8.5) with 0.5% (w/v) HPC. Peaks in both separations were identified by spiking; no FITC peak was observed in (B) because the labeling reaction was allowed to proceed to completion [18].

Table 1

Channel structure dimensions for different imprinting times; see the schematic in Fig. 2F for definitions of h , h' , w , and w' .

Imprinting time (min)	h (μm)	h' (μm)	w (μm)	w' (μm)
6	13	3.4	55	74
10	16	2.3	54	70
20	16	2.0	55	66
30	16	1.7	54	62
60	16	~0	57	55