Anal. Chem. **2008,** *80,* 2311−2318

Accelerated Articles

Fabrication of a Microfluidic System for Capillary Electrophoresis Using a Two-Stage Embossing Technique and Solvent Welding on Poly(methyl methacrylate) with Water as a Sacrificial Layer

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Methods for fabricating poly(methyl methacrylate) microchips using a novel two-stage embossing technique and solvent welding to form microchannels in microfluidic devices are presented. The hot embossing method involves a two-stage process to create the final microchip design. In its simplest form, a mold made of aluminum is fabricated using CNC machining to create the desired microchannel design. In this work, two polymer substrates with different glass transition temperatures (T_g), polyetherimide (PEI) and poly(methyl methacrylate) (PMMA), were used to make the reusable secondary master and the final chip. First, the aluminum mold was used to emboss the PEI, a polymeric substrate with $T_g \sim 216$ °C. The embossed PEI was then used as a secondary mold for embossing PMMA, a polymeric substrate with a lower T_g (∼105 °C). The resulting PMMA substrate possessed the same features as those of the aluminum mold. Successful feature transfer from the aluminum mold to the PMMA substrate was verified by profilometry. Bonding of the embossed layer and a blank PMMA layer to generate the microchip was achieved by solvent welding. The embossed piece was first filled with water that formed a solid sacrificial layer when frozen. The ice layer prevented channel deformation when the welding solvent (dichloroethane) was applied between the two chips during bonding. Electrophoretic separations of fluorescent dyes, rhodamine B (Rh B) and fluorescein (FL), were performed on PMMA microchips to demonstrate the feasibility of the fabrication process for microreplication of useful devices for separations. The PMMA microchip was tested under an electric field strength of 705 V cm^{-1}. Separations of the test mixture of Rh B and FL generated 55 500 and 66 300 theoretical plates/meter, respectively.

Significant progress has been made in the development of microfluidic devices since the concept was initially introduced 15 years ago.[1] Since the introduction of the first commercial microfluidic lab-on-a-chip-based systems for life science applications, the field has grown immensely, as can be seen from the growing numbers of microfluidics companies, microfluidic-based products, and publications in the past few years. Microfluidic devices have become increasingly popular due to their ability to analyze minute quantities of samples and their high-throughput sampling capabilities. These devices offer unique advantages, such as decreased consumption of reagents and sample; reduced analysis time; more portable instrumentation; and in some instances, lower limits of detection. The miniaturization of microfluidic devices has also made possible the development of portable analytical instrumentation for which lower applied potentials and smaller power supplies are required.

Miniaturized devices can be interfaced serially and ganged into highly integrated parallel systems that allow for the development of micro total analysis systems (μTAS). This integration can make complex analyses simpler to perform. In μTAS devices, all the steps in the analysis, including sample processing, separation, and detection, can be performed on one chip. In addition, μTAS devices can reduce the chance of human error, such as mislabeling and contamination, by decreasing the number of times a sample is handled.

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10.1021/ac7021647 CCC: $40.75
Published on Web 02/28/2008

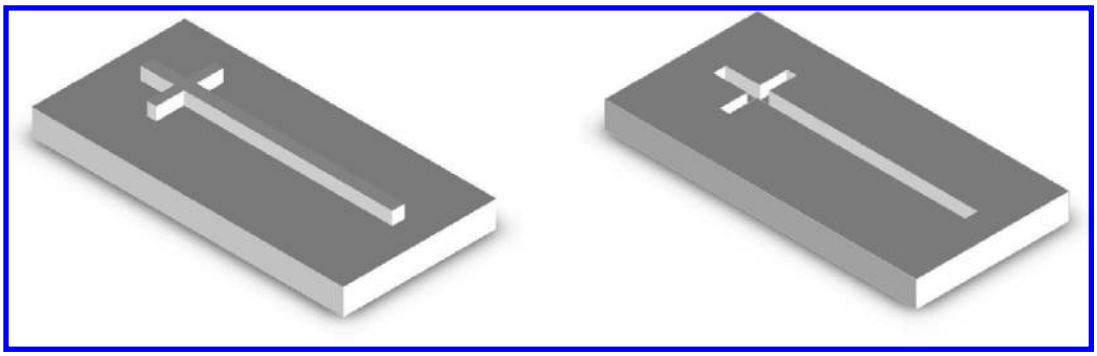

Figure 1. Master designs produced by micromachining. Recessed features (right) created by cutting into the metal stock and raised features (left) created by removing the excess metal around the desired feature areas result in molds to be used for feature replication.

Since the introduction of μTAS, microchip capillary electrophoresis (CE) has been explored as an attractive separation technique. Microchip CE has been applied to a wide range of applications, including environmental monitoring, biomedical and pharmaceutical analysis, clinical diagnostics, and forensic investigations.[2−5] Numerous reports have been published regarding the successful transition of CE systems to the microchip platform,[6−9] although much optimization remains to be realized in both system performance and ease of manufacture.

Glass has been the most widely used substrate for production of microfluidic systems[10,11] owing to many of its characteristics, such as high thermal stability and biocompatibility, resistance to many chemicals, optical transparency, and surface properties that are well suited for use in μTAS. Moreover, pre-existing microfabrication techniques, i.e., photolithography and chemical etching for glass substrates, have been well-established in the microelectronics industry. In addition to their advantages, chips with glass substrates also have some limitations. The fabrication of glass microchips is often expensive and time-consuming, and it involves processing procedures using hydrofluoric acid. Glass is fragile and can often break during the fabrication process. These disadvantages have made polymers attractive materials for μTAS fabrication. There are a large variety of polymer materials, including poly(methyl methacrylate) (PMMA),[12,13] polycarbonate, polyethylene terephthalate, and polydimethylsiloxane (PDMS),[14] that are available with different physical and chemical characteristics that make them suitable for different kinds of microfluidic applications.

Polymers such as PMMA have great potential to be used as alternate materials for microfluidic systems. PMMA offers advantages such as low cost, ease of fabrication, biocompatibility, and greater flexibility over silicon and glass. The expensive microfabrication step is required only to make the master structure, which then can be replicated many times into the polymer substrate. The cost-effective and relatively rapid fabrication procedures possible with polymeric materials for microfluidic devices have generated much attention as the need has started to grow for single-use, disposable microchips for chemical and biochemical analyses.

The conventional hot embossing technique typically is performed in a single step using a mold with negative features. The process results in a feature transfer of the mold into a thermoplastic substrate creating what is referred as "positive" features. The resulting features of the plastic substrates are complementary to that of the features on the mold. In this work, a two-stage embossing technique was employed[15,16] to fabricate the microfluidic chip. The method involves a two-step procedure to produce the final product. In the first step, a primary mold with positive features was embossed into a thermoplastic substrate to create a secondary mold with negative features. In the second step, the secondary mold is used to emboss positive features into another polymeric substrate to form the final product. It is essential for the secondary mold to have a much higher glass transition temperature (T_g) than the final product to avoid feature deformation of the secondary mold during the second embossing step.

The motivation for using the two-stage embossing method instead of the conventional one-step embossing technique is the need for coplanarity of bonding surfaces at the interface between substrates in a microfluidic chip.[15] Another major difference between the single-step approach and this new two-stage method is the process of creating the primary master, which is usually performed using a CNC machining tool on an aluminum stock. As illustrated in Figure 1 (left), fabrication of a mold for one-step embossing requires milling a positive (raised) feature into an aluminum substrate. On the other hand, the fabrication of a mold with negative (recessed) features (Figure 1; right) for use in the two stage method is considerably easier and faster. Moreover, the CNC programming needed to perform the machining is much

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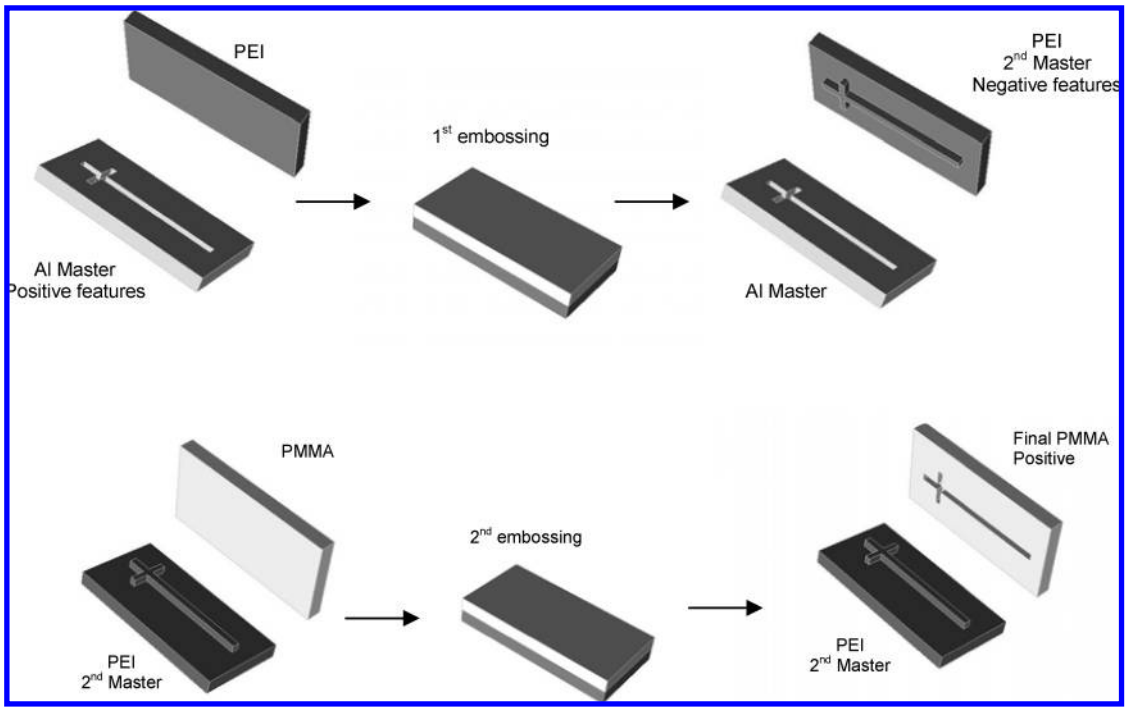

Figure 2. The two-stage embossing process, from primary aluminum master to final PMMA chip. The aluminum master serves as the primary mold in the embossing process. In the first embossing step, the positive features of the aluminum master are imprinted as negative features into a PEI substrate to produce the secondary mold. In the second embossing step, the negative features of the secondary mold are imprinted as positive features into a PMMA substrate to produce the final chip.

Table 1. Thermal Embossing Conditions Used for Imprinting PEI Substrate (top) and PMMA (bottom)[a]

temperatures (°C)	pressures (psi)	time (s)
Embossing Program for PEI		
80	0	60
160	0	60
220	0	150
220	600	150
Embossing program for PMMA		
75	0	60
90	0	60
120	0	150
120	600	150

[a] Constant pressure at 600 psi was applied at the end of the program until the substrate reached a demolding temperature of 50 °C.

simpler as compared to the other method. This technique is especially well suited for fabrication of a primary mold that requires the removal of a large amount of metal to create the design. With this technique, tedious procedures of fabricating the primary mold can be avoided, thus decreasing the time needed for mold fabrication.

Various bonding techniques have been reported for polymeric substrates. Thermal press-bonding is the most commonly used method for sealing plastic microfluidic chips.[17,18] Other bonding methods include vacuum-assisted thermal bonding,[19] microwave bonding,[20] hot water bath embossing,[21] lamination,[22] plasma oxidation,[23] and the use of adhesives.[24,25] However, the bond strength created by the thermal bonding method is usually much lower than that for the solvent-bonded chips. Recently, Lin et al. compared bonding strengths of various bonding procedure and showed that solvent bonded PMMA chips showed a much higher bonding strength (more than 17 times as strong) as compared to the thermally bonded chips.[26] Moreover, the heating process during thermal bonding in plastic chips may lead to channel deformation.

Chlorocarbon solvents such as chloroform, chlorormethane, and dichloromethane are effective organic solvents for bonding PMMA chips.[26] The use of solvent welding, however, has some disadvantages: it may lead to clogging or channel deformation during sealing. One way to resolve this issue is by using a sacrificial layer to protect the channels from the bonding solvent. This layer prevents the channel from deforming on exposure to the solvent during bonding.[26] Sacrificial materials such as waxes or low melting temperature alloys have been used in sealing microdevices during fabrication steps. Kelly et al. showed successful bonding of PMMA chips by using paraffin wax as the

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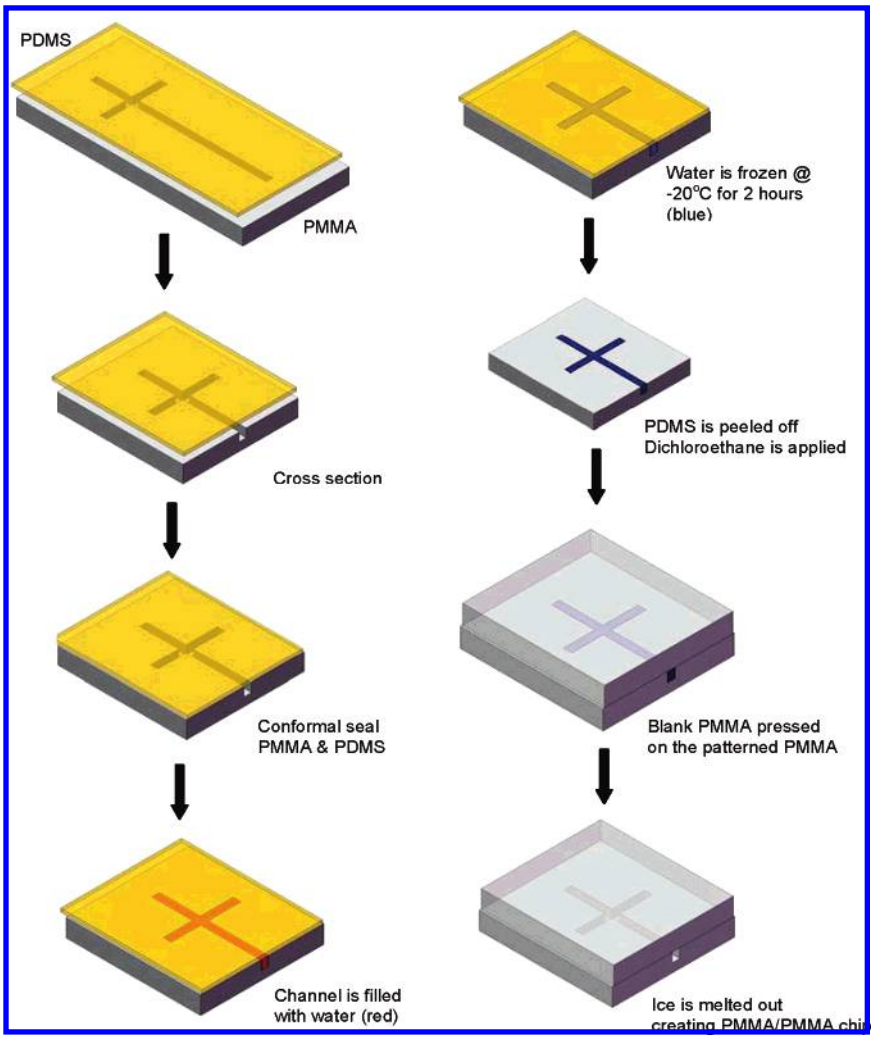

Figure 3. A schematic of the bonding procedure used to create PMMA/PMMA microchips. The PMMA chip is first conformally sealed with a piece of PDMS. The channel is filled with water and the PMMA/PDMS assembly is placed in a freezer at −20 °C for 2 h until the water inside the channel solidifies, forming a sacrificial layer for solvent welding. The PMMA/PDMS assembly is then removed from the freezer and placed on a cooling block (−20 °C). The PDMS layer is removed, and a uniform layer of dichlororethane (∼300 μL) is applied onto the PMMA chip. A blank piece of PMMA is pressed on top of the patterned PMMA chip with an applied pressure of ∼5 psi for 2 min for bonding. After 2 min, the applied pressure is released, and the PMMA chip is removed from the cooling block to allow the ice to melt off at room temperature.

sacrificial layer. Following the bonding process, the sacrificial layer must be removed from the microchannel. With paraffin, this was done by heating the assembly to melt the sacrificial layer. Removal of the sacrificial materials might be incomplete, causing some residual material to be trapped inside the channel. This may present a challenge during CE analyses as the excess sacrificial material trapped inside the channel may affect the electroosmotic flow. As a consequence, the separation and the reproducibility of the analysis are compromised.

To eliminate this problem, in this work, a solvent bonding method using 1,2-dichloroethane (DCE) for welding and ice as a sacrificial layer is reported for sealing two-layer PMMA-based microchips made using a novel two-stage embossing technique. The high solubility of DCE in PMMA substrates can create a major challenge that causes clogging or channel deformation during sealing; thus, water was chosen as the sacrificial layer because it is readily available, nontoxic, has a low evaporation rate, a high freezing point relative to DCE, and a low melting point that makes it easier to flush out after sealing, as compared to using other sacrificial media. By freezing the water, removal of the sacrificial layer can be done by simply letting the ice melt from the channel at room temperature. This makes water one of the most compatible sacrificial media in the application of microfluidic devices.

The transparency of the PMMA microchips made it possible to employ fluorescence detection. Only a small quantity of sample is required, and the low detection limit of fluorescence detection will make it possible to use the chips for disposable, single use purposes, such as biomedical applications. For our preliminary studies, electrophoretic separations of fluorescent dyes, rhodamine B and fluorescein, were performed to demonstrate the utility of a PMMA microchip fabricated via this new approach.

EXPERIMENTAL

Microfabrication. The primary master was made by micromachining aluminum stock using a standard G-code CNC program on a Tool Crafter mill (CMS, CNC, Laguna Hills, CA). In the first step of the embossing process, the aluminum master was hot-embossed on a PEI substrate (McMaster-Carr, Santa Fe Springs, CA) according to a program shown in Table 1.

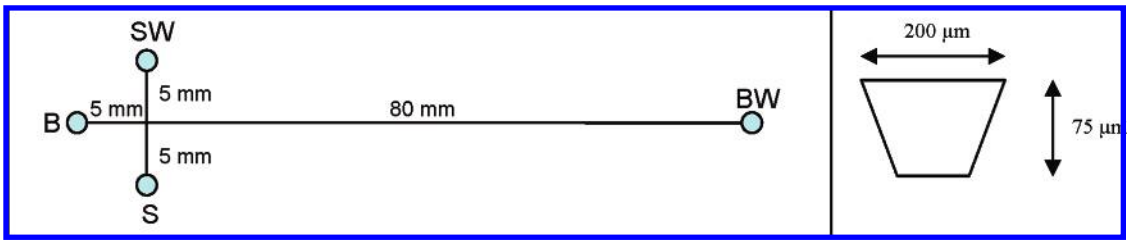

Figure 4. Schematic of the microchip layout showing the channel lengths and the approximate cross-sectional dimensions. Each channel has a 1.5-mm-diameter well at each end; the wells are labeled buffer reservoir (B), buffer waste (BW), sample reservoir (S), and sample waste (SW).

The resulting imprint produced negative features into the PEI substrates that served as the secondary mold in the process. In the second imprinting process, features on the PEI were embossed into a PMMA substrate (McMaster-Carr, Santa Fe Springs, CA). As shown in Figure 2, the final PMMA product then contains the same features as the primary master. The process used for two-stage embossing is illustrated in Figure 2.[15] The channel features were measured using a profilometer (Dektak 3 Surface Profile Measuring System, Veeco Instrument Inc, Santa Barbara, CA).

Solvent Welding. Four 1.5-mm-diameter reservoir holes were drilled on the PMMA chip. To fill the channel with water, the patterned side of the PMMA chip was conformally sealed with a flat piece of PDMS, forming a temporary enclosed channel. The PDMS (Sylgard 184, Dow Corning, NC) was mixed in a 10:1 ratio of monomer to the curing agent, poured onto a clean unpatterned glass slide, and thermally cured for 3 h at 60 °C.

The PMMA/PDMS enclosed channel was then filled with water through the buffer waste reservoir hole using a Microport Interface (Cascade Microtech, Inc., Beaverton, OR). All of the reservoir holes and channels were completely filled with water to ensure no air bubbles were present. The PMMA/PDMS assembly was placed in a freezer at −20 °C for 2 h. Water inside the channel solidified with room for expansion through the filling ports, forming a solid sacrificial layer to enable solvent welding with protected channels.

After the water solidified, the PMMA/PDMS assembly was removed from the freezer onto a cooled block (−20 °C). The PDMS piece was then peeled off the PMMA, and a uniform layer of dichlororethane (∼300 μL) was applied onto the PMMA chip. A blank piece of PMMA was pressed on top of the patterned PMMA chip with an applied pressure of ∼3 psi for 2 min for bonding. After 2 min, the applied pressure was released, and the PMMA chip was removed from the cooled block to allow the ice to melt off at room temperature. Finally, the channel was flushed with water using a microport for at least 15 min before use. A schematic of the bonding procedure is shown in Figure 3.

The morphology of the solvent-bonded microchips was studied using an Amray (Bedford, MA) scanning electron microscope (SEM) operated at 10 kV. The microchip was frozen in liquid nitrogen and fractured to facilitate obtaining cross-sectional images of the microchip.

Separations. Each channel had a 1.5-mm-diameter well at each end; the wells were labeled buffer reservoir (B), buffer waste (BW), sample reservoir (S), and sample waste (SW), as shown in Figure 4. The distance to the B, S, and SW reservoirs from the cross section was 5 mm each, and the length of the separation channel from the cross section to BW was 80 mm. To run an on-chip separation, reservoirs B, BW, and SW were filled with 5 μL of 10 mM, pH 9 sodium borate buffer, and reservoir S was filled with 5 μL of sample. For injection, the SW reservoir was grounded; potentials of 1500, 1100, and 2000 V were applied at reservoir S, B, and BW, respectively, for 20 s. During separation, reservoir BW was grounded, and a potential of 4000 V was applied at reservoir B while 3600 V was applied at reservoirs S and SW. Table 2 shows the on-chip injection and separation voltage program.

Table 2. Voltage Program Used for Electrophoretic Injection and Separation on the PMMA Microchip[a]

	Reservoir Potential (kV)				
step	sample (S)	buffer (B)	sample waste (SW)	buffer waste (BW)	duration (s)
injection	1.5	1.1	GND	2.0	20
separation	3.6	4.0	3.6	GND	150

[a] GND indicates ground.

The compounds used for the experiments were rhodamine B (Lambda Physik, Acton, MA) and fluorescein (Sigma, St Louis, MO). Each sample was individually diluted with 10 mM sodium borate (Integra, Renton, WA), pH 9 buffer solution. The instrument used to operate the microchip was the Micralyne Microfluidic Tool Kit μTK (Micralyne Inc., Edmonton, Alberta, Canada). The system provides electrophoretic on-chip operation that consists of high voltage power supplies coupled with a green laser induced fluorescence detection system containing a 532-nm long-pass filter, a 568.2-nm bandpass filter, and a PMT detector. The μTK system was controlled by LabView software (National Instruments, Austin, TX).

RESULTS AND DISCUSSION

A photomicrograph of a PMMA chip produced using the two-stage embossing technique is shown in Figure 5. Cross sections of the microchannels at 6× and 40× magnification are shown. Dimensions of the microchannels were measured by profilometry and were 200 μm in width and 75 μm in depth.

The PEI/PMMA thermal embossing procedures were performed 20 times to demonstrate the reproducibility of the technique. Microfluidic chips produced using these techniques were shown to have smooth and reproducible features. The channels were characterized with a surface profilometer to determine the depth and width of the channels. The results show that the profile of the channel on PMMA corresponds well to that

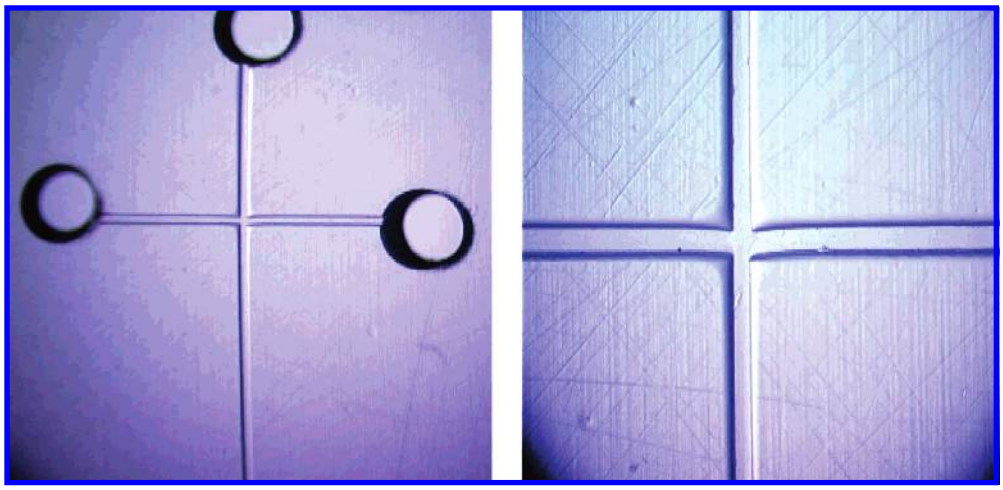

Figure 5. A cruciform PMMA microchip at 6× (left) and 40× (right) magnification.

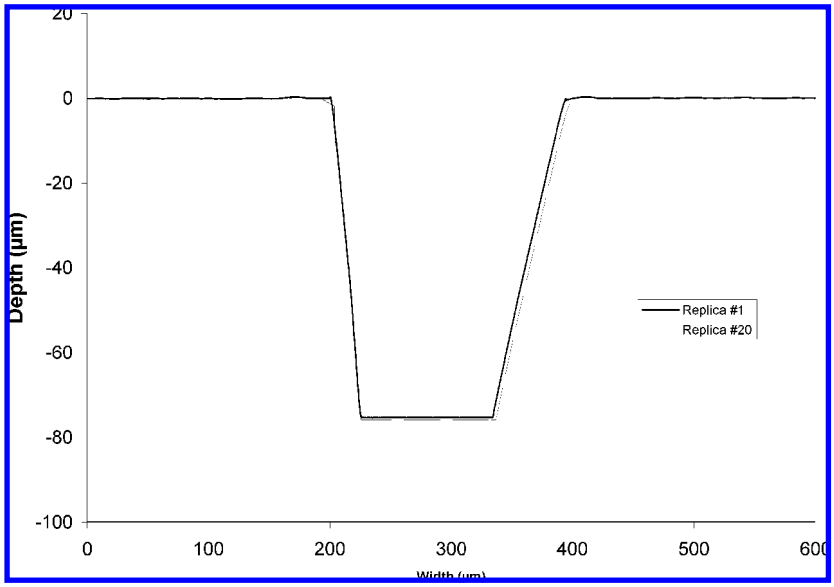

Figure 6. Measurements of the microchannel features obtained by surface profilometry. Depths and widths of the main channel in PMMA replica no. 1 (solid line) vs PMMA replica no. 20 (broken line) are compared.

of the primary mold and indicates good homogeneity of the imprinting process.

Temperature and pressure are critical aspects in this two-stage thermal embossing process. Embossing performed at temperatures below or above T_g of the substrate to be imprinted showed rough, uneven features on the chip surface. Results showed that embossing performed at temperatures much higher than the T_g of PMMA affected the smoothness of the surface. Pockets of bubbles appearing on the PMMA surface and channel deformity were also observed. These findings suggest that at temperatures much beyond T_g, the PMMA becomes too soft during the imprinting process and is unable to retain "clean" features from the PEI master. Embossing at T_g allowed the PMMA to soften enough to be imprinted without cracking. The embossing cycle was repeated 20 times with the program shown in Table 1, and profilometry results demonstrate that we can obtain excellent feature reproduction in the PMMA chips (Figure 7). Figure 6 shows an overlay of profiles of the first PMMA replica and the 20th embossed PMMA replica. The results clearly show that the profiles of the channels in both replicas correspond well to one another and that the features can still be preserved after 20 embossing cycles. The channel features of the primary aluminum mold and the features of the 20th PMMA substrate produced were compared, and the results show that there is a slight increase in the height (1 μm; 1%) and an increase in width (2 μm; 1%) in the PMMA substrate as compared to the primary mold. The PMMA channels appear to be a bit asymmetrical according to the profilometer. It was determined that this apparent asymmetry is an artifact of the profilometer used to obtain the scans: SEM images reveal symmetrical channel features for the aluminum master and the PMMA replicas.

Channel widths (W) and heights (H) were measured and plotted for each of the PMMA replicas (Figure 7a and b). In no instance did channel widths or heights in the PMMA replicas exceed those of the primary mold by more than 1% and 4%, respectively. The W/H aspect ratios of the 20 PMMA replicas were calculated and plotted, and they range between 2.58 and 2.74; the W/H aspect ratio of the primary mold was 2.67 (Figure 7c). The successful transfer of features from the primary mold to the final substrate demonstrate that the two stage embossing tech-

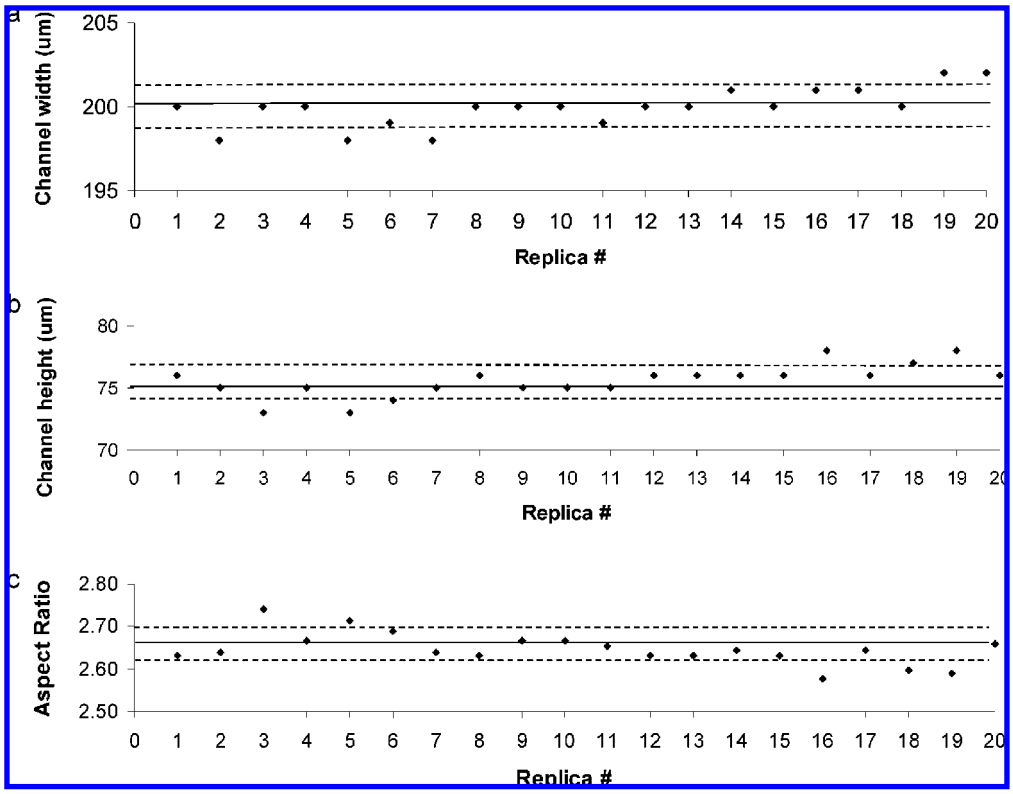

Figure 7. Comparison of the channel width (a), height (b), and the aspect ratio (c) of the 20 PMMA embossed replicas. The solid line represents the mean, and the dotted lines represent ±1 standard deviation of the mean.

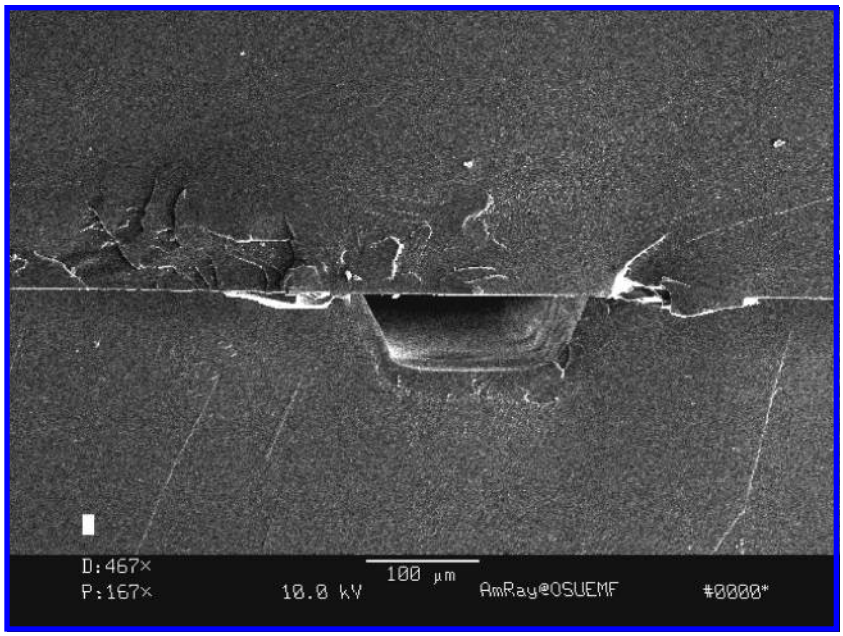

Figure 8. SEM image of a PMMA microchip showing the channel cross section. For imaging, the chip was frozen in liquid nitrogen and fractured to provide a cross section for imaging.

nique is reproducible and efficient and has great potential in the area of microfluidic fabrication.

In this work, 1,2-dichloroethane was used as the solvent for bonding two PMMA chips together. A careful approach must be taken during the bonding procedure, since PMMA has a high solubility in DCE. The solvent can serve as the agent for bonding, but it can also create problems, such as clogging or channel deformation during sealing. One way to resolve this issue is by using a sacrificial layer to protect the channels from the bonding solvent. The patterned side of the PMMA chip was conformally sealed with a piece of PDMS forming a temporary enclosed channel. Prior to bonding, the PMMA microchannel was filled with water through one of the reservoir holes. After freezing the PMMA/PDMS assembly for 2 h, the water solidified, forming a solid sacrificial layer. This layer prevented the channel deformation from the solvent during bonding, which otherwise would fill and

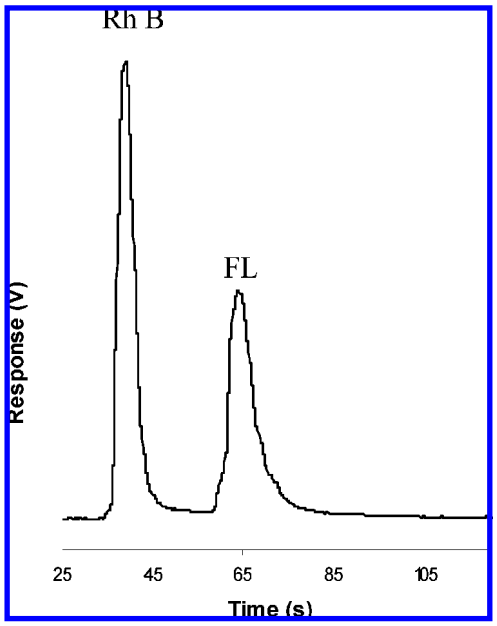

Figure 9. Electropherograms showing an on-chip electrophoretic separation of a 10^{-3} M RhB/10^{-3} M FL test mixture.

soften the channel.[27] After the completion of the bonding process, the sacrificial layer was removed by simply letting the ice melt from the channel.

Solvent welding performance was evaluated by testing the bonding strength of the PMMA chips. Other studies have shown that solvent welds yield a much higher bonding strength than other methods, such as thermal bonding. Results on solvent-bonded PMMA chips conducted by Lin et al. indicated a bonding strength of 3.8 MPa (551 psi) using DCE.[26] Our solvent-bonded PMMA chips using water as the sacrificial layer were tested under a pressure of 2000 psi. The chips were able to withstand the pressure without breaking or leaking. This suggests excellent performance potential for packing sorbent materials inside these chips for applications in CEC or micro-LC. Figure 8 shows SEM images of the PMMA solvent bonded microchips. As can be seen from the images, clean-cut channels were obtained without channel deformation. The water sacrificial layer allowed the channels to retain their shape.

The microchip was not an optimized device, and the primary objective of this work was to develop a new and more efficient means of producing microfluidic devices. To that end, it is important to demonstrate that these two-stage embossed, solvent-welded chips can be used to perform an electrophoretic separation. The potential of the fabricated chips for microfluidic CE was evaluated using the florescent dyes Rh B and FL as simple test probes. The sample, a mixture containing 10^{-3}M rhodamine B (Rh B) and 10^{-3}M fluorescein (FL) in 10 mM, pH 9 sodium borate buffer, was injected on the PMMA microchip according to the program shown in Table 2. Electrophoretic separation of the two compounds was achieved on the PMMA chip, as illustrated in Figure 9. The migration times of the two peaks (~40 and 62 s) were consistent with the Rh B and FL peaks run individually on the PMMA chip under the same experimental conditions. In the pH 9 sodium borate buffer solution, Rh B is neutral and FL is negatively charged.[28] Rh B is thus a good neutral marker for electroosmotic flow, and the negatively charged FL peak appeared later than the uncharged Rh B peak. The PMMA microchip was tested under a maximum applied voltage of 6 kV (field strength 705 V cm^{-1}). Separations of the test mixture were achieved with a separation field strength of 470 V cm^{-1}. The Rh B and FL peaks have theoretical plate numbers of 55 500/m and 66 300/m, respectively.

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CONCLUSIONS

This paper presents a novel method for fabrication of PMMA microdevices via a two-stage embossing procedure and by using a sacrificial-layer-protected solvent bonding technique. This method of fabrication provides a rapid, cost-effective, simple, and versatile approach of utilizing and producing PMMA microdevices. The PEI secondary mold can be used repeatedly as a tool for microfluidic chip fabrication on other lower T_g polymer substrates. The experimental results show that the PMMA chips can be used for separations of fluorescent dyes successfully and reproducibly in a microchip capillary electrophoresis analysis. This work indicates that the fabricated PMMA microchip electrophoresis devices can provide alternative solutions to overcome some of the limitations in fabricating polymer-based microchip devices, making chemical and biomedical analyses on them more attractive.

ACKNOWLEDGMENT

The authors acknowledge Dana Hutanu for assistance in obtaining SEM images and Corey Koch and Ted Hinke for their technical assistance with the fabrication of the microchips.

Received for review October 19, 2007. Accepted November 27, 2007.

AC7021647