

Construction of microfluidic chips using polydimethylsiloxane for adhesive bonding†

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Received 22nd July 2005, Accepted 7th October 2005

First published as an Advance Article on the web 17th October 2005

DOI: 10.1039/b510494g

A thin layer of polydimethylsiloxane (PDMS) prepolymer, which is coated on a glass slide, is transferred onto the embossed area surfaces of a patterned substrate. This coated substrate is brought into contact with a flat plate, and the two structures are permanently bonded to form a sealed fluidic system by thermocuring (60 °C for 30 min) the prepolymer. The PDMS exists *only* at the contact area of the two surfaces with a negligible portion exposed to the microfluidic channel. This method is demonstrated by bonding microfluidic channels of two representative soft materials (PDMS substrate on a PDMS plate), and two representative hard materials (glass substrate on a glass plate). The effects of the adhesive layer on the electroosmotic flow (EOF) in glass channels are calculated and compared with the experimental results of a CE separation. For a channel with a size of approximately 10 to 500 μm , a ~ 200 –500 nm thick adhesive layer creates a bond without voids or excess material and has little effect on the EOF rate. The major advantages of this bonding method are its generality and its ease of use.

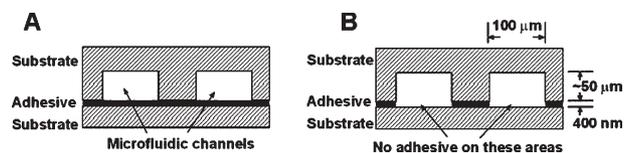
Introduction

Increasingly, microfluidic chips^{1–4} are being used to perform fast separations. This technique has made significant advances with the introduction of soft lithography^{3–7} for creating the microfluidic channels and with the introduction of different components, such as valves and pumps.^{8–11} A major stumbling block remains the rapid fabrication of microfluidic platforms. This paper describes a simple, convenient bonding method for the formation of microfluidic devices of various materials, including glass and polymers such as PDMS, at nearly ambient conditions. This method is based on selectively coating only the embossed surfaces of a patterned substrate with a thin adhesive layer of polydimethylsiloxane (PDMS) prepolymer. Subsequent thermocuring of the adhesive between the substrate and a flat plate forms enclosed channels in which the adhesive does not interfere with the functions of the formed channels.

The fabrication of a microfluidic device involves two major steps: (1) the formation of open channels in substrates, and (2) the bonding of the substrates to form enclosed microfluidic networks. The open channels can be etched in glass and silicon with standard MEMS procedures^{1,12} or formed in polymers with convenient techniques such as replica molding, stamping, and other soft lithographic techniques.^{6,7,13,14} The bonding step, in contrast, usually is time-consuming and fault-prone, requiring skill and careful control. Bonding of glass and silicon substrates needs very high temperatures (normally above 500 °C) with certain pressures and/or voltages (usually many

hours to a day) and thermoplastic polymers require milder conditions with temperatures above their glass transition point under careful control of the pressure.¹⁴ To improve this procedure, several methods that can bond microfluidic substrates in mild conditions have been proposed.^{15–19} Among these, bonding with an adhesive layer is particularly appealing. For example, a thin layer of epoxy has been used to glue glass substrates¹⁷ or additional channels are used to accommodate the adhesive.¹⁹ This type of method can be represented as shown in Scheme 1A. In spite of its simplicity, the glue that covers the flat substrates can be annoying in some common applications. During an on-chip capillary electrophoretic separation, the electroosmotic difference between this glue and the channel material could greatly impair the separation efficiency. Therefore, channels bound in this way cannot be used (unless the glue is the same material as the channel itself) in applications that require uniformity of its wall materials.

To retain the simplicity of this method and also to create bonded channels with uniform walls, we present here an improved method with an adhesive layer that leaves all the channel walls in its original state except for a negligible ($\sim 0.2\%$) portion of exposed adhesive (Scheme 1B). Our



Scheme 1 Schematic illustration of the cross sections of bonded channels from (A) adhesive bonding method where the adhesive covers the whole area of the bottom substrate, and (B) bonding method where the adhesive exists only on the contact areas of the substrates. The dimensions shown in the scheme represent some typical numbers of the dimensions in a microfluidic device.

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† Electronic supplementary information (ESI) available: Calculation of electroosmotic flow field and other experimental details. See DOI: 10.1039/b510494g

method starts with selectively coating a patterned substrate with a very thin layer of adhesive. After two substrates are brought into contact, the adhesive is cured either with heat or radiation to bond the substrates permanently together. Because the adhesive layer is very thin (hundreds of nanometers) compared to the dimensions of the microchannels and is only coated on the embossed surfaces (*not* the channel area) of the substrates, the channels retain the walls of their original material and can maintain their desired functions essentially unaffected by the adhesive. Two model substrates—PDMS (an example for soft materials) and glass (an example for hard materials)—are chosen to demonstrate the simplicity and versatility of this technique.

The bonded channels have been tested for their bonding strengths and their separation abilities in capillary electrophoresis (CE). This bonding method is fully compatible with common materials that are used in microfluidics; besides glass and PDMS, we also tested this bonding method to make microfluidic channels of PMMA with success. It is also easy to incorporate components of different materials into a microfluidic device. Using this bonding technique, we have integrated polymeric filter membranes into microfluidic systems.

Results and discussion

Bonding of PDMS substrates to form closed channels

We demonstrated the bonding method with PDMS microfluidic networks. Fig. 1 schematically illustrates the bonding

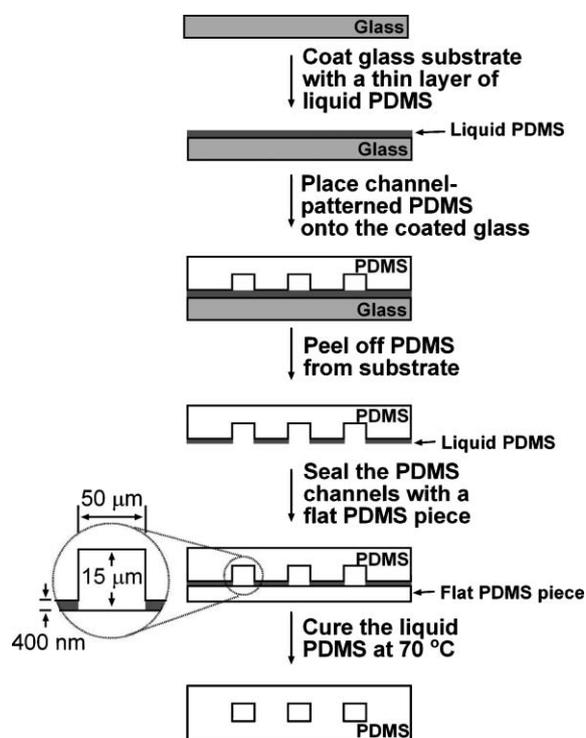


Fig. 1 Schematic illustration of the bonding procedure with a thin glue layer of PDMS prepolymer. The inset shows the dimensions of a normal microfluidic channel with the glue layer whose dimension has been enlarged on purpose.

procedure of a patterned PDMS layer to a flat PDMS plate to form a sealed fluidic system with a thin layer of liquid PDMS prepolymer as glue. A toluene solution of PDMS prepolymer was spin-coated onto the surface of a glass slide to cover the slide with a thin layer of glue. A PDMS substrate with channels patterned on its surface by soft lithography was placed onto the coated glass slide. All flat areas on the embossed PDMS substrate surface were placed in contact with the thin layer of glue; the recessed areas of the channels, however, were kept a distance from the glue. During the subsequent step of removing the PDMS substrate from the glass, part of the thin adhesive was transferred from the glass slide to the substrate. It is important to keep the channel area free of the glue because the channels and the glue may be made of different materials and the channel shape can be altered. This PDMS substrate with glue-coated surface was ultimately placed onto a flat PDMS plate. After these two pieces were brought into good contact, they were placed into an oven at 60 °C for half an hour to cure the PDMS prepolymer. Fig. 2 shows optical images of two typical microchannels formed in this manner. A razor blade was used to cut through the channels so that their cross section could be examined under a microscope. Because both the channels and adhesive layers were made of PDMS, the whole device was bonded into a homogeneous structure. Consequently, the bonding interface between the substrates was invisible. The image on the right in Fig. 2 shows that PDMS channels with an aspect ratio down to $\sim 1:7$ can be formed with this bonding method.

Requirements for the bonding layer

There are two requirements on the choice of the adhesive material. First, the bonding material must form a thin, smooth layer (less than 0.5 μm thick) on the glass slide and be transferred without beading to the substrate. Stated another way, this material should have proper viscosity and it must wet the glass slide and the substrate. PDMS prepolymer is an excellent choice because it wets most materials and it can be spin coated into layers down to 100 nm thick. Fig. 3 shows a plot of layer thickness *versus* PDMS prepolymer concentration

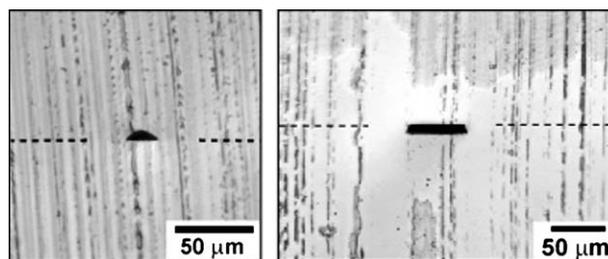


Fig. 2 Optical microscopic images of the cross sections of two PDMS fluidic channels that are formed with PDMS bonding. The channels were not filled with liquid while the images were taken. The dashed line on each picture indicates the interface between the two substrates of the channel. The streaks on the images are caused by the mechanical cutting from a razor blade. It has been suggested that clearer breaks can be obtained by freezing the samples in liquid nitrogen followed by breaking it.

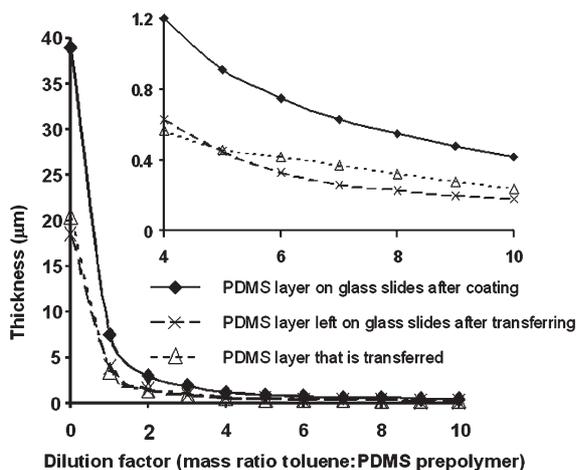


Fig. 3 Spin curves of PDMS prepolymer. Each curve represents the thickness of cured PDMS layer *versus* the dilution mass ratio of PDMS prepolymer from toluene. The solid line is for the PDMS on a glass slide after spin coating, the dashed line for the layer left on the slide after transferring, and the dotted line for the layer that has been transferred to a patterned PDMS substrate. The spin condition for all samples was 500 RPM for 3 s followed by 1500 RPM for 60 s. The inset shows an expanded view about the right portion of the figure.

in toluene. Fig. 3 also presents the thickness of PDMS prepolymer on the substrate after transferring. The data show that the PDMS prepolymer partitions approximately equally between the glass slide and the substrate after the transfer step. A layer of liquid no more than 0.5 μm thick ensures that no glue will spread into the channels.

Second, the adhesive must be easily cured to achieve permanent bonding of the substrates. UV-curable polymers can be used if one of the substrates (*e.g.*, glass and plastics) is transparent. An example of such an adhesive is SU-8 photoresist, which can be diluted with cyclopentanone and cured with UV light. More generally, thermocurable polymers can be used for almost all the microfluidic materials, including glass, plastics, and silicon. This paper focuses on the use of the PDMS prepolymer; the application of other suitable bonding materials is believed to be similar.

Bonding of glass substrates to form enclosed channels

If the patterned substrates are hard materials such as glass, silicon, and hard plastics, it is difficult to peel the substrate from glue-coated glass slides to coat only the embossed areas of the substrate. One approach to overcome this problem is to spin coat the PDMS prepolymer onto a flat surface of PDMS prior to transferring it to the hard substrate. We found, however, that this approach suffered from the problem that application of toluene caused swelling of the PDMS flat, thus making it difficult to spin-coat smoothly the flat. Therefore, we developed an alternative approach (Fig. 4A). After a thin layer of PDMS prepolymer was coated onto a glass slide, a PDMS flat (~ 2 mm thick) was placed onto the slide and peeled off from the slide; a thin layer of PDMS prepolymer was transferred onto the PDMS flat. The patterned substrate was then contacted with this coated PDMS flat, and after the flat was peeled off, a thin layer of PDMS prepolymer was left

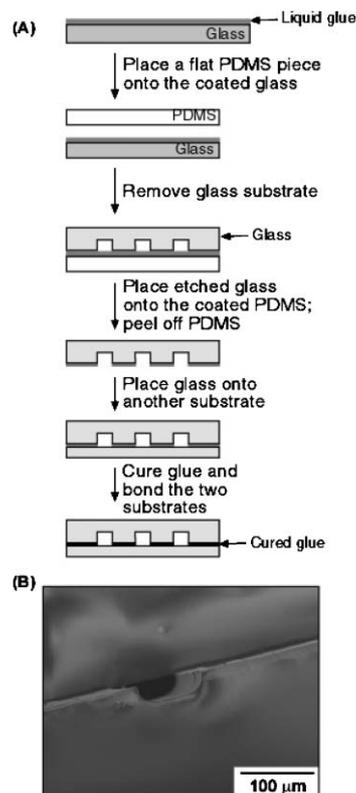


Fig. 4 Bonding of hard substrates with PDMS prepolymer: (A) schematic of the procedure, which is slightly altered from the bonding of soft substrates as shown in Fig. 1; and (B) an SEM image of the PDMS-bonded glass fluidic channel. The contrast of the image is caused by a charging effect during SEM scanning on the native glass surface.

on the embossed areas of the patterned hard substrate. This coated substrate was placed onto a flat plate and pressed together by finger pressure to form a closed microfluidic chip. Fig. 4B shows an SEM image of the cross section of a PDMS-bonded glass channel.

Improved design for preventing air trapping

Because PDMS is elastomeric and gas-permeable, the formation of air bubbles can be avoided if either of the bonding materials is made of PDMS. But when both substrates are hard materials (for example, glass, silicon, or hard plastic), air cannot leak through the bulk material and it is possible for air bubbles to become trapped between the substrates, especially in large contacting areas. These air bubbles can change the wanted shape of the device and can impair its function. To avoid the formation of air bubbles between the substrates, a grid of channels is incorporated into the design (Fig. 5), which helps release air. Because the patterning and bonding steps of the fabrication are parallel in nature, the addition of these grid channels in the design costs almost no extra time or effort. Each one of these channels has two outlets that are connected to the open air, but they are isolated from any channel on the device and do not interfere with the function of the chip. The space between two neighboring channels in the grid is ~ 500 μm .

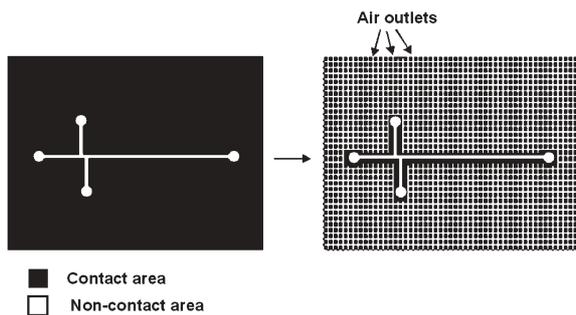


Fig. 5 Improved design for avoiding air trapping during the bonding process. The pattern on the left is the original design for a “double-T” injection CE separation channel, and the pattern on the right is the improved design for the same device by adding air-escaping channels.

Strength of bonded microfluidic chips

To test the bonding strength of our microfluidic chip, two plates of various materials were bonded together with a thin layer of PDMS ($\sim 0.4 \mu\text{m}$ thick). A small hole (1 mm in diameter) was drilled through the top plate for connection to a gas tank that acted as an external pressure source. Both PDMS and glass were tested. Curing PDMS prepolymer on top of PDMS substrates that are surface-modified or plasma-oxidized does not ensure strong bonding. To avoid this problem, all PDMS substrates are used without any surface modification.

We found that both chips remained as one integral unit when the pressure was increased up to 400 kPa. For comparison, two PDMS flat pieces with conformal contact started to separate from each other when the pressure was ~ 20 kPa. We have expected that the identical material of the glue and the channel in the PDMS case would create the same bonding strength as a uniform PDMS piece. The experiment showed differently. If the PDMS of the channel was cured for more than 30 min at 70°C before bonding, the final channel could be peeled and separated along the bonding surface with help of a scalpel; if the curing time was ~ 20 min (the PDMS was cured in its very early stage with a sticky surface), the bonded PDMS pieces formed an integral unit and could not be separated along the bonded surface. The glass pieces that were formed with PDMS bonding (~ 400 nm thick bonding layer) could not be separated without breaking the glass.

Capillary electrophoretic separations

The difference of the bulk material and the bonding layer of a microfluidic device in their zeta potential could potentially degrade its capability in CE separation. The reliability and performance of a glass microfluidic chip bonded with a thin glue layer were tested in the CE separation of a mixture of two NDA-derivatized (naphthalene-2,3-dicarboxaldehyde) amino acids under an applied voltage of 0.29 kV cm^{-1} . Fig. 6A shows the dimensions of a glass chip (its cross section of $\sim 33 \mu\text{m} \times 80 \mu\text{m}$ shown in Fig. 4B) that was bonded with a PDMS layer of ~ 400 nm thickness. A typical electropherogram obtained from this chip is shown in Fig. 6B, which is compared to that obtained using a glass chip with identical dimensions formed with conventional high-temperature thermal bonding (Fig. 6C).

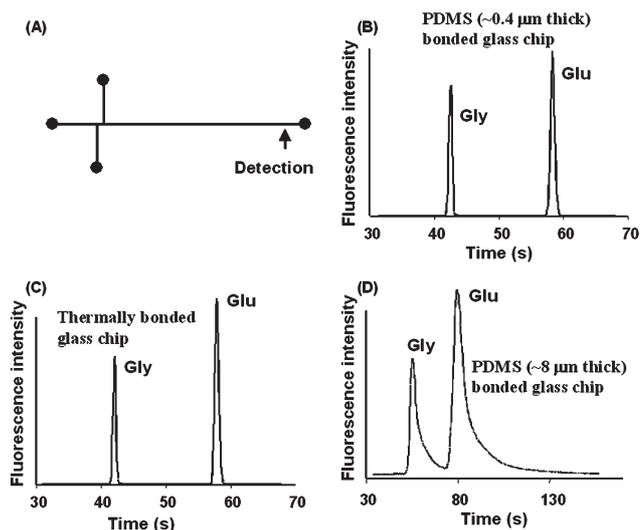


Fig. 6 CE separations in glass channels. (A) Diagram showing the dimensions of the glass chip. (B) Electropherogram from a channel bonded with 400 nm thick PDMS layer. (C) Electropherogram from a channel bonded with the conventional method. (D) Electropherogram from a channel bonded with an $8 \mu\text{m}$ thick PDMS layer. A plug of solution containing a mixture of NDA-derivatized glycine and glutamic acid (with a mole ratio of 2:3) was injected into a 7 cm long channel for separation on each chip. The running buffer is 50 mM borate at pH ~ 9.2 and the separation voltage is 0.29 kV cm^{-1} .

The elution times of the peaks on the electropherograms are slightly shifted, which was probably caused by the difficulty of positioning the detection point at exactly the same location along the separation channels for these two experiments. Other differences between these two electropherograms are within the experimental uncertainty caused by variations of CE separations on the same glass chip.

Increasing the thickness of the PDMS further gradually damages the separation, both slowing down the EOF in the channel (thus longer elution time) and broadening each of the analyte elution peaks. Fig. 6D shows an example with a CE electropherogram obtained from the same glass substrates bonded with a PDMS layer of $\sim 7 \mu\text{m}$ thickness.

We have also calculated the influence on the electroosmotic flow from a PDMS bonding layer (see ESI†). The dimension of the rectangular glass channel is $10 \mu\text{m} \times 10 \mu\text{m}$, and it is bonded with a $1 \mu\text{m}$ thick PDMS layer. Fig. 7 shows the results of the calculation. We find that only the flow field within $\sim 1\text{--}2 \mu\text{m}^2$ cross-sectional area on each side of the channel is altered. Thus, the PDMS layer makes a negligible influence on the total flow for devices having $100 \mu\text{m}^2$ cross sectional area of the channels. We conclude that a bonding layer that is thinner than one tenth of the channel height should be acceptable for most CE applications.

Experimental section

Chemicals and materials

Amino acids were from Sigma. Sodium borate, sodium phosphate (dibasic, heptahydrate), potassium phosphate (monobasic), sodium cyanide, crystal violet, concentrated

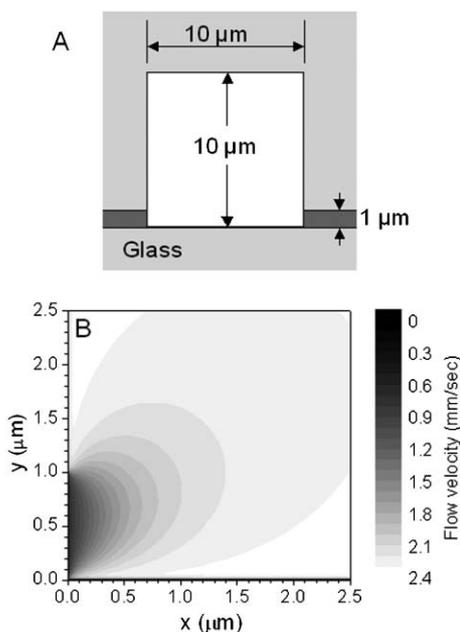


Fig. 7 Computed EOF in a $10\ \mu\text{m} \times 10\ \mu\text{m}$ glass channel bonded with $1\ \mu\text{m}$ of PDMS layer. (A) Schematic of the channel. (B) The flow field in a $2.5\ \mu\text{m} \times 2.5\ \mu\text{m}$ region at the lower left corner of the channel cross section. Parameters for the computation are: zeta potential for glass = $-100\ \text{mV}$; zeta potential for PDMS = $-30\ \text{mV}$; electrolyte concentration = $1\ \text{mM}$ (monovalent salt); electric field strength = $300\ \text{V cm}^{-1}$; viscosity of water = $0.00089\ \text{N s m}^{-2}$; grid size for computation = $2.44\ \text{nm}$.

hydrochloric acid (HCl) and dimethyl sulfoxide (DMSO) were obtained from Aldrich. Naphthalene-2,3-dicarboxaldehyde (NDA) was purchased from Molecular Probes (Eugene, OR), poly(dimethylsiloxane) (PDMS) prepolymer (RTV 615A and 615B) was purchased from General Electric (Waterford, NY), photoresists (SU-8 50 and SPR 220-7) and SU-8 developer were purchased from MicroChem (Newton, MA), and silicon wafers were purchased from Silicon Sense (Nashua, NH).

Formation of PDMS molds

To form a master for molding into PDMS, the surface of a patterned silicon wafer was made more hydrophobic by exposing it to perfluoro-1,1,2,2-tetrahydrooctyltrichlorosilane vapor (United Chemical Technologies, Inc., Bristol, PA) in a vacuum desiccator to prevent adhesion of the elastomer to the wafer or the photoresist structures during the curing step.

Well-mixed liquid PDMS prepolymer (RTV 615 A:B with mass ratio of 10:1) was poured onto the master, degassed, and cured in an oven ($60\ ^\circ\text{C}$, 0.5–1 h). The cured PDMS layer ($\sim 4\ \text{mm}$ thick) was peeled from the master. For microfluidic channels, holes were punched through it for reservoirs. Flat PDMS plates were obtained by curing liquid PDMS prepolymer against bare silicon wafers.

The thickness of PDMS films in our experiments was determined by the following steps: (1) cut the PDMS film with a scalpel and remove one side of the film from the silicon substrate to create an edge; (2) add UV-curable epoxy (UVO

114, Epoxy Technology, Billerica, MA) to the edge and cure it to create a matching step with the same thickness; and (3) measure the height of the epoxy step by a profilometer (Sloan Technology Corp., Santa Barbara, CA).

Experimental setup for CE separations

Microfluidic experiments were carried out on an Axiovert 135 (Carl Zeiss, Thornwood, NY) inverted microscope having long working distance objectives (Zeiss LD Achromat 20 \times and 40 \times). The CE separation uses a home-built high voltage power supplier. A continuous wave violet diode laser (5 mW, 405 nm, Nichia, Mountville, PA) aligned through the back port of the microscope (with condenser removed) was used for fluorescence excitation. The emitted light was filtered spectroscopically (dielectric band pass, Chroma Technology, Rockingham, VT, and holographic notch filters, Kaiser Optical Systems, Ann Arbor, MI) and spatially (1000 μm pinhole in the back parafocal plane) before being imaged onto a photomultiplier tube (PMT, Hamamatsu, Bridgewater, NJ) biased at $-1\ \text{kV}$. PMT signals were converted to voltage with a picoammeter (Keithley Instruments, Cleveland, OH), and collected with a data acquisition card (National Instruments, Austin, TX) in a PC running custom software created with LabView (National Instruments).

EOF computation

The electroosmotic flow field in a rectangular channel is computed numerically using the method described by Andreev *et al.*²⁰ Briefly, the charge density in the channel is first calculated by solving the Poisson–Boltzmann equation using the zeta potentials of the sidewalls as the boundary condition. The flow field is then calculated by solving the Poisson equation when an external electric field is applied along the channel. Details of the computation procedure are described in the ESI.†

Other experimental details including the transferring patterns with photolithography, etching channels on patterned glass, and fluorescent derivatization are given in the ESI.

Conclusions

We have demonstrated a convenient, general method that can be used to bond substrates of various materials to form enclosed microfluidic channels, by transferring a thin layer of adhesive to a patterned substrate and sealing the substrate to a flat plate to form a microchip. This method is an improvement on previous methods using adhesives in that our method can selectively apply the adhesive only to the contact surfaces of two microfluidic substrates. In this way, all applied adhesives are fully used so that almost all the bonded channels remain in their original state with essentially no interference to the desired functions of the channels. This method is expected to be applicable to most microfluidic materials. For example, the SU-8 photoresist can be used to form microfluidic chips that involve organic solvents such as ethanol. In addition, the bonding layer can be removed (*e.g.*, PDMS layer can be dissolved in tetrabutylammonium fluoride solution) to release the substrates without damage for reuse (data not shown here),

thus saving efforts for the nontrivial processes of remaking glass substrates. We believe this improved bonding method with adhesives facilitates the fabrication of microfluidic devices.

Acknowledgements

We are grateful to Dunwei Wang for helping with the SEM imaging. This material is based upon work supported by the National Science Foundation under Grant BES-0508531-47.041.

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