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Phase-Changing Sacrificial Materials for Solvent Bonding of High-Performance Polymeric Capillary Electrophoresis Microchips

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A new method for solvent bonding polymeric substrates to form microfluidic systems has been demonstrated. Prior to device sealing, channels in an embossed poly(methyl methacrylate) (PMMA) piece are filled with a heated liquid (paraffin wax) that forms a solid sacrificial layer at room temperature. The sacrificial material prevents the bonding solvent (acetonitrile) and softened PMMA from filling the channels. Once the sealing step is complete, the sacrificial layer is melted and removed, leaving enclosed microfluidic channels. We found that PMMA substrates welded together using this method could withstand internal pressures of >2250 psi, more than 1 order of magnitude higher than their thermally bonded counterparts. To demonstrate the usefulness of this method, microchip capillary electrophoresis (CE) devices in PMMA were created and tested. Amino acid and peptide mixtures were separated in <15 s, with >40 000 theoretical plates in a 2.5-cm separation distance. Electric fields as high as 1.5 kV/cm were applied in these microchips, and >300 CE runs were performed on a single device with no degradation of separation performance. The simplicity of the methods presented here and the improved robustness of the resulting devices should facilitate the broader implementation of polymer microchips in microfluidic analyses.

As the lab-on-a-chip field has developed, polymers such as poly(dimethylsiloxane) (PDMS), poly(methyl methacrylate) (PMMA), and polycarbonate (PC) have been employed increasingly as device substrates[1−3] in favor of glass, which was originally used almost exclusively.[4] This shift toward polymeric substrates has likely occurred because of two factors. First, the templated procedures used to create microchips in polymers allow a single photolithographically defined master to be used to pattern numerous devices,[5,6] thus decreasing the need for cleanrooms and other costly instrumentation. Second, the polymeric materials themselves are typically less expensive than microchip-quality glass, and lower costs per device should facilitate the development of disposable microfluidic systems.[3]

Despite these attractive features of polymeric materials, glass remains the substrate of choice for very fast[7] or high-performance[8,9] microchip CE. This performance gap is due in part to the convenience of adapting the well-characterized chemistry of fused-silica capillaries for surface modification in a wide array of glass microchip applications. Also, the thermal conductivity of glass is higher than that of commonly used polymers (e.g., PMMA, PC, and PDMS),[10] which provides better dissipation of Joule heating and enables higher electric fields in microchannels in glass substrates.

Unfortunately, glass microchips must be patterned and etched individually in a cleanroom, and the thermal annealing of glass substrates to enclose microcapillaries generally takes place in a furnace at >400 °C for several hours.[1] Moreover, special care must be taken to ensure that the bonded surfaces are extremely clean and lacking even small particulates, or thermal bonding will not be successful.[11] Low- and room-temperature glass bonding approaches that avoid high-temperature processing have been reported,[12−15] but the resulting adhesion is weaker than in thermally sealed devices, and even greater care must be taken to ensure that the surfaces are extremely clean and flat.

To avoid a sealing step for microcapillary enclosure and to create submicrometer features, sacrificial techniques have been explored. In these methods, a channel design is patterned on top of a bulk substrate, and a thin film of a different material is deposited over the entire surface, covering the patterned design. Next, the sacrificial material under the deposited layer is etched

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(1) de Mello, A. J. *Lab Chip* **2002**, *2*, 31N−36N.
(2) Becker, H.; Locascio, L. E. *Talanta* **2002**, *56*, 267−287.
(3) Boone, T. D.; Fan, Z. H.; Hooper, H. H.; Ricco, A. J.; Tan, H.; Williams, S. J. *Anal. Chem.* **2002**, *74*, 78A−86A.
(4) Reyes, D. R.; Iossifidis, D.; Auroux, P.-A.; Manz, A. *Anal. Chem.* **2002**, *74*, 2623−2636.
(5) Martynova, L.; Locascio, L. E.; Gaitan, M.; Kramer, G. W.; Christensen, R. G.; MacCrehan, W. A. *Anal. Chem.* **1997**, *69*, 4783−4789.
(6) Duffy, D. C.; McDonald, J. C.; Schueller, O. J. A.; Whitesides, G. M. *Anal. Chem.* **1998**, *70*, 4974−4984.
(7) Jacobson, S. C.; Culbertson, C. T.; Daler, J. E.; Ramsey, J. M. *Anal. Chem.* **1998**, *70*, 3476−3480.
(8) Culbertson, C. T.; Jacobson, S. C.; Ramsey, J. M. *Anal. Chem.* **2000**, *72*, 5814−5819.
(9) Paegel, B. M.; Emrich, C. A.; Wedemayer, G. J.; Scherer, J. R.; Mathies, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 574−579.
(10) MatWeb Material Property Data. http://www.matweb.com (accessed Jan 2005).
(11) Simpson, P. C.; Woolley, A. T.; Mathies, R. A. *J. Biomed. Microdevices* **1998**, *1*, 7−26.
(12) Wang, H. Y.; Foote, R. S.; Jacobson, S. C.; Schneibel, J. H.; Ramsey, J. M. *Sens. Actuators, B* **1997**, *45*, 199−207.
(13) Chiem, N.; Lockyear-Shultz, L.; Andersson, P.; Skinner, C.; Harrison, D. J. *Sens. Actuators, B* **2000**, *63*, 147−152.
(14) Ito, T.; Sobue, K.; Ohya, S. *Sens. Actuators, B* **2002**, *81*, 187−195.
(15) Jia, Z.-J.; Fang, Q.; Fang, Z.-L. *Anal. Chem.* **2004**, *76*, 5597−5602.

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away[16,17] or thermally decomposed,[18−21] leaving microcapillaries defined by the cover layer and the base substrate. While these sacrificial methods have successfully created devices without a thermal bonding step, the fabrication protocols are involved, and templated procedures are not possible because each device is patterned individually.

Phase-changing materials (typically waxes) have been incorporated into fluidic microchips to create micropumps,[22] membrane actuators,[23] and valves,[24,25] but these materials have not been used as sacrificial layers in microdevice fabrication. In addition, Liu et al.[24] recently used a solvent-assisted thermal bonding method to seal PC substrates at ~200 °C, but the large feature dimensions (>300 μm deep) made it unnecessary to protect the channels from the bonding solvent.

Here we report a general approach for solvent bonding of polymeric substrates using a phase-changing sacrificial material. Microchannels are imprinted in PMMA with a silicon template and filled with a liquid, which forms the sacrificial layer upon solidification. After solvent bonding, the device is heated above the melting temperature of the sacrificial material to enable its facile removal as a liquid. These solvent-bonded substrates can withstand internal pressures of >2250 psi, much higher than thermally bonded PMMA. We demonstrate the functionality of solvent-bonded microfluidic systems by performing rapid and high-resolution CE separations of fluorescently labeled amino acids and peptides. These separations compare favorably with glass microchip CE of peptide mixtures[26−28] and surpass those previously done on polymer microchips[29−32] in terms of both speed and efficiency. We have performed separations in electric fields as high as 1500 V/cm, the highest reported to date for polymer microchips. Finally, a single device was used for more than 300 runs over a three-month period without a decrease in separation performance, demonstrating long device lifetimes.

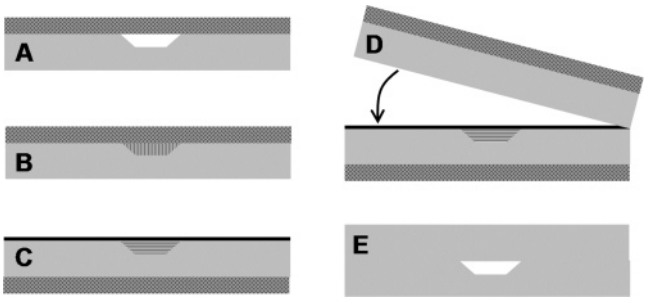

Figure 1. Solvent bonding to create microfluidic systems in polymers. (A) A PDMS slab (dark gray with white crosshatching) is sealed to an imprinted PMMA substrate (gray), temporarily forming enclosed microchannels. (B) The assembly is heated, and liquid paraffin wax (gray with vertical lines) fills the microchannels. (C) The device is cooled to solidify the wax (gray with horizontal lines), and the PDMS slab is removed and placed on the opposite side of the PMMA to protect the device exterior. The patterned side of the PMMA is then coated with acetonitrile (black). (D) A second, blank PMMA piece, which also has PDMS protecting its exterior, is pressed against the acetonitrile-coated PMMA for 2 min to effect bonding. (E) The device is heated to melt the sacrificial layer, which is removed by a combination of applied vacuum and dissolution in cyclohexane.

EXPERIMENTAL SECTION

Microfabrication. Microchips were made by imprinting raised features from photolithographically patterned and anisotropically etched silicon templates into PMMA substrates using previously described methods.[33] Imprinted PMMA substrates (Acrylite OP-3, Cyro, Rockaway, NJ) were 1.75 in. × 1 in. × $^1/_8$ in. and had 3-mm-diameter reservoir holes aligned with the channel ends. A CO_2 laser cutter (C-200, Universal Laser Systems, Scottsdale, AZ) was used to excise the PMMA substrates from larger sheets and create the reservoir holes. A clean, flat, $^1/_{16}$-in.-thick piece of PDMS (Sylgard 184, Dow Corning, Midland, MI), which had been cured according to the manufacturer's specifications, was sealed to the patterned side of a PMMA substrate (Figure 1A), temporarily forming enclosed channels. The PMMA/PDMS assembly was mounted on a glass microscope slide and placed on a heating block at 85 °C (above the melting temperature of the paraffin wax) for 30 s. A pipet was used to quickly transfer melted paraffin wax (melting point, 65 °C; Service Assets, Newport Beach, CA) from a heated vial to three of the reservoirs before the melted wax could cool and solidify. After three reservoirs were filled with melted wax, vacuum was applied for 1−2 s at the fourth reservoir to ensure that all channels were filled (Figure 1B) and no air pockets were present, after which the PMMA/PDMS assembly was removed from the hot plate and cooled to room temperature. Next, the channels were inspected under a microscope. If a small amount of paraffin wax had solidified beyond the channels, the imprinted PMMA substrate was left in contact with the PDMS until the paraffin wax outside the channels had dissolved in the PDMS, which was then removed. PDMS was sealed to the nonimprinted side of the PMMA and to a blank piece of $^1/_{16}$-in.-thick PMMA to prevent the solvent from contacting the device exterior. Aliquots of 200−400 μL of acetonitrile were pipetted directly onto the channel-containing substrate (Figure 1C) to cover the entire surface, and the unpatterned PMMA was placed in contact with the solvent-coated substrate and held together with

(16) Turner, S. W.; Perez, A. M.; Lopez, A.; Craighead, H. G. *J. Vac. Sci. Technol. B* **1998**, *16*, 3835−3840.
(17) Foquet, M.; Korlach, J.; Zipfel, W.; Webb, W. W.; Craighead, H. G. *Anal. Chem.* **2002**, *74*, 1415−1422.
(18) Suh, H.-J.; Bharathi, P.; Beebe, D. J.; Moore, J. S. *J. Microelectromech. Syst.* **2000**, *9*, 198−205.
(19) Harnett, C. K.; Coates, G. W.; Craighead, H. G. *J. Vac. Sci. Technol. B* **2001**, *19*, 2842−2845.
(20) Jayachandran, J. P.; Reed, H. A.; Zhen, H.; Rhodes, L. F.; Henderson, C. L.; Allen, S. A. B.; Kohl, P. A. *J. Microelectromech. Syst.* **2003**, *12*, 147−159.
(21) Metz, S.; Jiguet, S.; Bertsch, A.; Renaud, P. *Lab Chip* **2004**, *4*, 114−120.
(22) Sethu, P.; Mastrangelo, C. H. *Sens. Actuators, A* **2003**, *104*, 283−289.
(23) Klintberg, L.; Svedberg, M.; Nikolajeff, F.; Thornell, G. *Sens. Actuators, A* **2003**, *103*, 307−316.
(24) Liu, R. H.; Yang, J.; Lenigk, R.; Bonanno, J.; Grodzinski, P. *Anal. Chem.* **2004**, *76*, 1824−1831.
(25) Pal, R.; Yang, M.; Johnson, B. N.; Burke, D. T.; Burns, M. A. *Anal. Chem.* **2004**, *76*, 3740−3748.
(26) Rocklin, R. D.; Ramsey, R. S.; Ramsey, J. M. *Anal. Chem.* **2000**, *72*, 5244−5249.
(27) Gottschlich, N.; Jacobson, S. C.; Culbertson, C. T.; Ramsey, J. M. *Anal. Chem.* **2001**, *73*, 2669−2674.
(28) Ramsey, J. D.; Jacobson, S. C.; Culbertson, C. T.; Ramsey, J. M. *Anal. Chem.* **2003**, *75*, 3758−3764.
(29) Dou, Y.-H.; Bao, N.; Xu, J.-J.; Chen, H.-Y. *Electrophoresis* **2002**, *23*, 3558−3566.
(30) Lacher, N. A.; de Rooij, N. F.; Verpoorte, E.; Lunte, S. M. *J. Chromatogr., A* **2003**, *1004*, 225−235.
(31) Hu, S.; Ren, X.; Bachman, M.; Sims, C. E.; Li, G. P.; Allbritton, N. *Electrophoresis* **2003**, *24*, 3679−3688.
(32) Kato, M.; Gyoten, Y.; Sakai-Kato, K.; Nakajima, T.; Toyo'oka, T. *Anal. Chem.* **2004**, *76*, 6792−6796.
(33) Kelly, R. T.; Woolley, A. T. *Anal. Chem.* **2003**, *75*, 1941−1945.

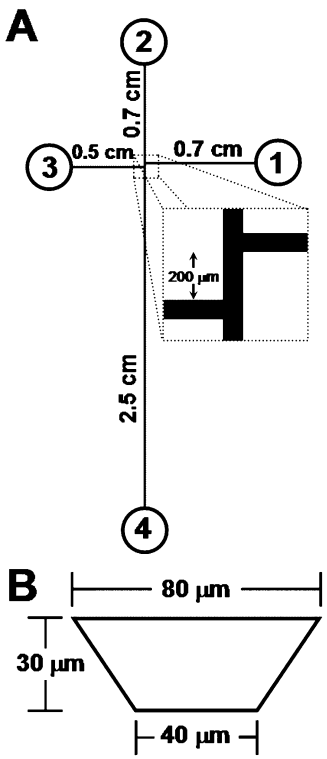

Figure 2. Schematic of microchip layout, showing (A) channel lengths and reservoir numbers and (B) approximate cross-sectional dimensions.

an applied pressure of 2 psi for 2 min to effect bonding (Figure 1D). Effective bonding was feasible with as little as 3 μL of acetonitrile/cm^2 (~35 μL/device); with volumes over 400 μL/device, excess solvent sometimes flowed from the bonding interface and impaired the optical clarity of the surface. The PMMA pieces were brought together at an angle as shown in Figure 1D to allow any air bubbles to escape out the side. After the designated time, the applied pressure was released, and the pieces of PDMS were peeled from the device. To remove the sacrificial layer from the microchannels (Figure 1E), 10 μL of cyclohexane was pipetted into each of the reservoirs, and the device was placed on the heating block until the paraffin wax melted. Vacuum was applied at one of the reservoirs to begin removing paraffin wax from the channels, after which that reservoir was refilled with cyclohexane. The same procedure was repeated at each of the reservoirs, and the device was cooled to room temperature. To ensure that all residual sacrificial material was removed, the channels were soaked in cyclohexane for >5 min before vacuuming all liquid from the device. Complete removal of the paraffin wax was verified by the absence of air bubbles upon filling the channels with water, as air bubbles typically became trapped at any points in the channel where the hydrophobic wax was still present. A schematic of the microchip layout and channel dimensions is shown in Figure 2.

Bond Strength Determination. To measure the internal pressure that could be applied to solvent-bonded substrates, we threaded a hole to accept a $^5/_{16}$-in. −24 brass fitting in a 1 in. × 1 in. × $^1/_2$ in. piece of PMMA. The threaded piece was solvent bonded to a 1 in. × 1 in. × $^1/_4$ in. PMMA substrate using the same solvent, applied pressure, and time as for microchips. For comparison, we also thermally bonded substrates of the same dimensions by clamping the pieces together and placing them in an oven at 107 °C. After $^1/_2$ h, the thermally bonded PMMA was cooled and evaluated to ensure bonding completeness. If voids were found, the substrates were reclamped, and the bonding procedure was repeated. The brass fitting, which connected the PMMA to a N_2 gas cylinder via $^1/_{16}$-in. copper tubing, was threaded into the bonded assemblies. The copper tubing was branched to allow a pressure transducer (MSP-300, Measurement Specialties, Fairfield, NJ) with a linear response between 0 and 2500 psi to be connected. The regulator on the gas cylinder was opened gradually, increasing the internal pressure in the bonded substrate until either the pieces separated or the maximum pressure of 2250 psi was reached.

Separation and Detection of Amino Acids and Peptides. The amino acids were from ICN Biomedicals (Aurora, OH), and the peptides were from Sigma-Aldrich (St. Louis, MO). Each analyte was individually diluted in pH 9.2, 10 mM carbonate buffer, which was passed through a 0.2-μm filter (Pall, East Hills, NY) prior to use. The amino acids and peptides in each solution were labeled fluorescently using fluorescein-5-isothiocyanate (FITC; Molecular Probes, Eugene, OR).[34] For amino acids, 200 μL of 6 mM FITC in dimethyl sulfoxide (DMSO) was combined with 600 μL of a 3 mM solution of each amino acid. For peptides, 200 μL of a 2 mM solution of each peptide was combined with 50 μL of 6 mM FITC in DMSO. All solutions were allowed to react at room temperature in the dark for at least 24 h; longer times (up to 5 days) enabled the reaction to go to completion such that the unreacted FITC peak was eliminated.

Prior to use, microchip channels were filled with 10 mM carbonate buffer, pH 9.2, having 0.5% (w/v) hydroxypropyl cellulose (HPC; average MW 100 000; Sigma-Aldrich). The HPC served to minimize electroosmotic flow and analyte adsorption to the channel walls.[35−37] Channels were filled by micropipetting 16 μL of the buffer into reservoirs 1, 2, and 3 (Figure 2) and applying vacuum to reservoir 4, after which reservoir 4 was also filled with 16 μL of buffer. To load samples in the injection well (reservoir 1) when HPC-containing buffer was used as run buffer, vacuum was applied to reservoir 1 to remove its contents, and the well was filled with 16 μL of amino acid or peptide sample in 10 mM carbonate, pH 9.2. To run a separation without HPC in the buffer, the HPC-containing buffer was vacuumed from the device; the channels and reservoirs 2−4 were filled with pH 9.2, 10 mM carbonate buffer; and reservoir 1 was filled with 16 μL of sample. For injection, reservoirs 1, 2, and 4 (Figure 2) were grounded, and reservoir 3 was maintained at an injection voltage ranging from +300 to +1250 V (depending on the separation voltage used) for at least 20 s. During separation, reservoirs 1 and 3 were held at the injection voltage, reservoir 2 was grounded, and a potential between +1.0 and +4.5 kV was applied at reservoir 4. The injection and separation voltages for each run are indicated in the corresponding figure legends. All peaks were identified by spiking.

The laser-induced fluorescence system has been described previously.[33] Briefly, excitation of the fluorescently labeled amino

(34) Monnig, C. A.; Jorgenson, J. W. *Anal. Chem.* **1991**, *63*, 802−807.
(35) Shen, Y.; Xiang, F.; Veenstra, T. D.; Fung, E. N.; Smith, R. D. *Anal. Chem.* **1999**, *71*, 5348−5353.
(36) Shen, Y.; Smith, R. D. *J. Microcolumn Sep.* **2000**, *12*, 135−141.
(37) Sanders, J. C.; Breadmore, M. C.; Kwok, Y. C.; Horsman, K. M.; Landers, J. P. *Anal. Chem.* **2003**, *75*, 986−994.

acids and peptides was achieved with the 488-nm line from an air-cooled Ar ion laser, which was focused ~500 μm from the end of the separation channel using a 20×, 0.45 NA objective. Fluorescence was collected with the same objective, and stray light was removed by confocal spatial filtering with a 200-μm-diameter pinhole. A photomultiplier tube detected photons passing through the pinhole, and the detector output was recorded on a computer at 100 Hz.

Safety Information. The 40% aqueous KOH and 10% buffered HF used as etchants to micromachine the Si templates are both corrosive. To avoid skin or eye contact with these solutions, safety goggles, a face shield, and elbow-length nitrile gloves should be worn. The voltages used for electrophoretic injection and separation can cause electric shock, so appropriate precautions such as current-limiting settings on power supplies and isolation of electrical leads should be taken. Solvents for bonding PMMA and dissolving paraffin should be used in a fume hood. The high pressures used to test bond strengths pose a projectile hazard. A face shield should be worn, and the bonded substrates should be secured inside a metal container during testing.

RESULTS AND DISCUSSION

After filling the microchannels with the sacrificial layer (see Experimental Section and Figure 1), we sometimes observed that a small amount of paraffin wax had solidified outside of the imprinted channels (Figure 3A). This was likely due to the hydrophobic interaction between the paraffin wax and the PDMS, and bonded devices made directly from these substrates typically had poorly defined channels (Figure 3B) that led to reduced separation efficiency. This problem was largely eliminated by having the liquid sacrificial material in contact with PDMS for as little time as possible (removing substrates from the heating block 1−2 s after first introducing the liquid paraffin wax). Furthermore, residual paraffin wax outside the channel regions dissolved[38] into the PDMS within 1−2 h, leaving well-defined channels filled with the sacrificial layer. Figure 3C shows the same channel region presented in Figure 3A, after 80 min in contact with the PDMS; essentially all the undesired sacrificial material had dissolved in the PDMS. Alternatively, the accumulation of sacrificial material outside of the channels could likely be eliminated by employing a less hydrophobic sealing material than native PDMS, such as O_2 plasma-oxidized PDMS.[6] Figure 3D shows the channel intersection region of a solvent-bonded CE microchip made from the imprinted substrate from Figure 3A and C. The small amount of topography visible in the channels, caused by the granularity of the solidified wax, did not affect performance, as CE separations in such devices had symmetric peaks with reproducible theoretical plate counts. We are currently exploring alternate sacrificial materials that have less granularity upon solidification.

Figure 4 shows cross sections of patterned features at various stages of microchip fabrication. A small indentation in the top of the sacrificial layer, which is caused by paraffin shrinkage upon solidification, is visible in Figure 4B. Profilometry indicates that the magnitude of shrinkage is less than ~10% of the channel cross-sectional area; these smaller dimensions are retained in the final bonded devices (Figure 4C). Additionally, if the PDMS is left in contact with the wax-filled substrate at room temperature, wax from the channel dissolves into the PDMS at a rate of ~1 μm/h. Importantly, we have not observed the total collapse of channel features during solvent bonding; such channel deformation occurs more commonly when thermally bonding polymers. We have also found the phase-changing sacrificial layer and solvent bonding approach to be successful with much shallower, 7-μm-deep channels.

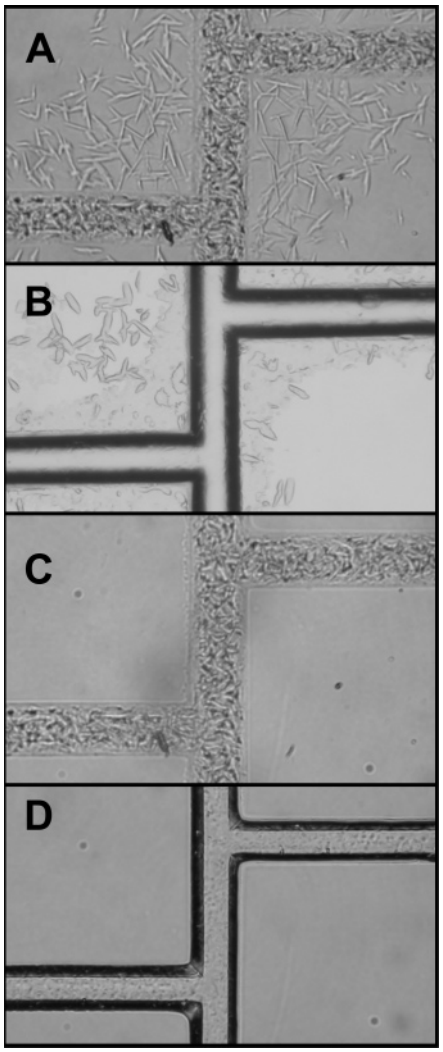

Figure 3. Photographs of the double-T injector region of a device. (A) PMMA/PDMS assembly just after filling the channels with sacrificial material; some paraffin wax had solidified outside the channels. (B) A poorly defined microchannel resulting from solvent bonding without removing excess wax. (C) The same PMMA/PDMS assembly as (A), but 80 min later when excess sacrificial material had dissolved in the PDMS, leaving well-defined channels. (D) A completed microchip made from the PMMA substrate in (A, C) after solvent bonding and sacrificial layer removal.

Our comparison of the bond strength in thermally and solvent-bonded substrates yielded the following results. For solvent-bonded PMMA, all three test devices withstood the maximum tank pressure (2250 psi) without separating. In contrast, three thermally bonded PMMA devices failed at 145, 232, and 222 psi, giving an average failure pressure of ~200 psi. These tests show that solvent-bonded PMMA can withstand at least 1 order of magnitude higher internal pressure than thermally bonded PMMA. The ability to withstand pressures of >200 psi is valuable

(38) Lee, J. N.; Park, C.; Whitesides, G. M. *Anal. Chem.* **2003**, *75*, 6544−6554.

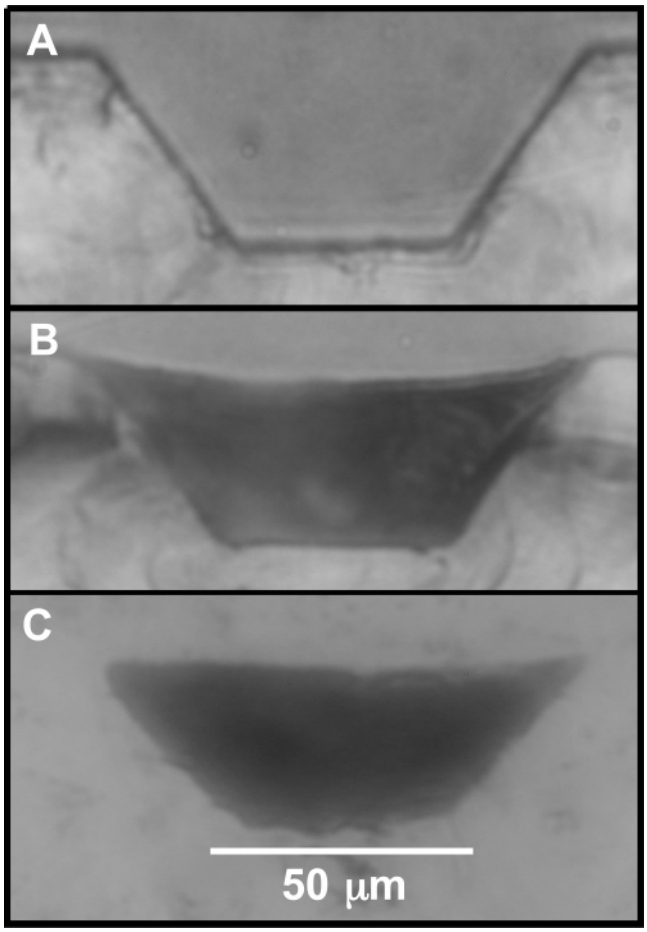

Figure 4. Channel cross-sectional photomicrographs at various fabrication stages. (A) Imprinted channel. (B) Imprinted channel filled with wax and having the PDMS removed. (A, B) were obtained by scoring and then fracturing the substrates, which led to some roughness in the surrounding bulk PMMA. (C) Bonded device after wax removal; the slightly roughened appearance around the channel perimeter was the result of using a diamond-tipped circular saw to obtain the cross section. The scale bar in (C) applies to all images.

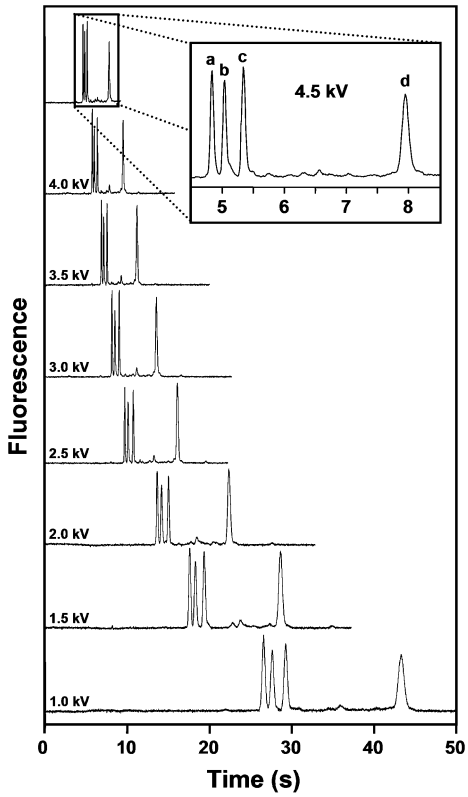

Figure 5. Electropherograms of a mixture of FITC-amino acids at different separation voltages (shown on figure). Injection voltages from bottom to top: +300, +450, +600, +750, +900, +1050, +1150, and +1250 V. Amino acid concentrations were 75 nM, and the run buffer was 10 mM carbonate, pH 9.2, with 0.5% (w/v) HPC. Peaks: (a) Gly, (b) Asn, (c) Phe, and (d) Arg.

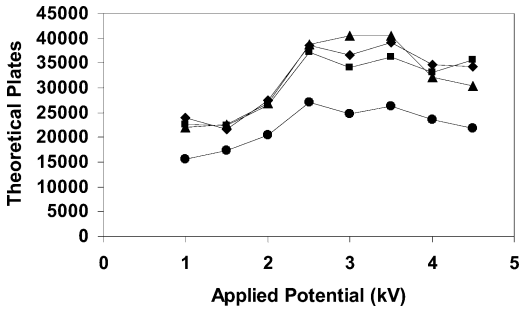

Figure 6. Theoretical plates versus applied voltage for the amino acid separations shown in Figure 5. Legend: Gly (◆), Asn (■), Phe (▲), Arg (●).

for the replacement of viscous sieving media[39] commonly used in capillary gel electrophoresis of DNA and proteins.

We tested the solvent-bonded microchips by separating a mixture of amino acids at different applied voltages to find the range that provided the highest theoretical plate numbers. Figure 5 shows the separation of FITC-labeled glycine, asparagine, phenylalanine, and arginine at potentials ranging from 1.0 to 4.5 kV. At 4.5 kV, the four peaks are baseline resolved, and the separation is completed in just 8 s. The highest theoretical plate numbers were obtained between 2.5 and 3.5 kV, as shown in Figure 6. The plateauing of theoretical plate numbers and their eventual decrease at higher potentials may be largely due to the 280-μm offset (center-to-center) in the double-T injector rather than Joule heating or other fundamental limits. For example, the width at half-height of the glycine peak in the 4.5-kV separation in Figure 5 is only 320 μm, making the injected sample plug length the most significant contributor to peak breadth.

To minimize electroosmotic flow and prevent analyte adsorption,[35−37] the channels were filled with buffer containing 0.5% (w/v) HPC prior to each run. In some cases, the run buffer also contained 0.5% HPC (e.g., Figure 5). We also evaluated the migration time reproducibility for CE in HPC-free buffer by running 10 replicate injections of the amino acid mixture at 1-min intervals. The migration time for FITC−Arg had a relative standard deviation (RSD) of 0.9% for 10 consecutive runs, indicating that the adsorbed polymer coating was stable over that time. Furthermore, over 2 days with the channels flushed and refilled multiple times, the RSD was 1.5% for 25 runs. A representative separation performed in a channel that was treated with HPC, but filled with HPC-free buffer, is shown in Figure 7. For comparison, a separation of the same mixture run in HPC-containing buffer is also shown. Theoretical plate numbers were not significantly different for the separations in the two solutions,

(39) Buchholz, B. A.; Shi, W.; Barron, A. E. *Electrophoresis* **2002,** *23,* 1398−1409.

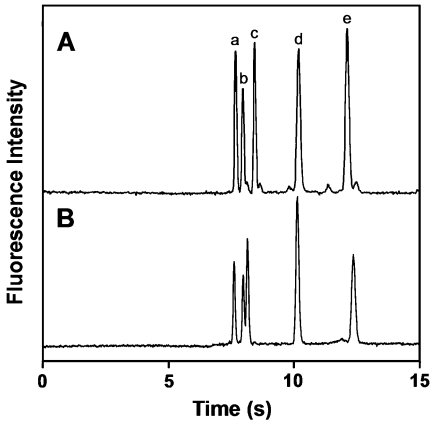

Figure 7. Separation of FITC-labeled amino acids in 10 mM carbonate buffer, pH 9.2 with (A) and without (B) 0.5% (w/v) HPC in the run buffer. Peaks: (a) Gly, (b) Asn, (c) Phe, (d) FITC, and (e) Arg. The injection voltage was +800 V, and the separation voltage was +3.0 kV.

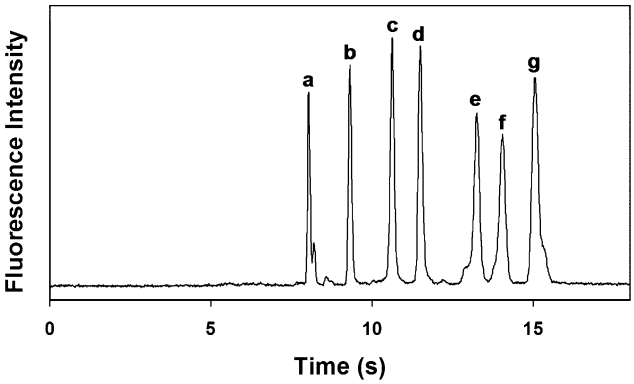

Figure 8. Separation of FITC-labeled peptides. Peaks: (a) FLEEI; (b) FA; (c) FGGF; (d) Leu enkephalin; (e) angiotensin II, fragment 3−8; (f) angiotensin II; and (g) GGYR. The buffer composition and voltages were the same as in Figure 7A, and the concentration of all peptides was 110 nM.

but the selectivity changed slightly for some analytes, most likely due to increased buffer viscosity when HPC was present. For this reason, FITC−Asn and FITC−Phe were fully resolved when HPC was added to the run buffer (Figure 7A) but not when HPC was absent (Figure 7B). Passivating channel walls with an additive and then running the separation without that additive present in the run buffer should be useful where such buffer components would interfere with detection (e.g., mass spectrometry).

To further demonstrate the suitability of solvent-bonded CE microchips for high-performance biological analyses, we separated FITC-labeled peptides in HPC-containing buffer (Figure 8). The separation performance was similar for the peptides and amino acids; peak a in Figure 8 has a theoretical plate number of 43 000, corresponding to 1.7×10^6 plates/m. We also separated the peptides in buffer lacking HPC (not shown), and the plate numbers and resolution were similar to those in Figure 8.

The excellent performance of these PMMA microchips in CE clearly demonstrates the usefulness of the solvent bonding technique. We applied electric fields nearly twice as high as those previously reported in PMMA microchips,[40] which enabled separations with >40 000 theoretical plates in ~10 s. We believe that higher fields are possible in our devices because the robust bonding is more resistant to dielectric breakdown at elevated voltages. Furthermore, a single device was used for >300 separations over the course of 3 months with no degradation of separation performance. These results demonstrate a significant advance in fabrication technology that should make polymer substrates more attractive for a broad range of microchip analyses.

A key advantage of the phase-changing sacrificial layer solvent bonding approach is that it should be generalizable to other combinations of polymeric substrates, sacrificial materials, and bonding solvents. The requirements for application to new systems are (1) a sacrificial material that has a melting temperature below the glass transition temperature of the chosen polymer and (2) a bonding solvent that can dissolve the polymer substrate but not the sacrificial material. We are currently exploring appropriate combinations of solvents and sacrificial materials to create microfluidics in other polymeric materials, which should broaden the application of this technique.

CONCLUSION

We have shown that polymeric microchips can be created by using a sacrificial material to protect channel integrity during solvent bonding. This phase-changing sacrificial layer fabrication method is simple to implement, and tests show that solvent-bonded devices can withstand >10-fold higher internal pressures than thermally bonded substrates. CE separations of FITC-labeled amino acids and peptides were successfully carried out on solvent-bonded devices in as little as 8 and 15 s, respectively, with theoretical plate numbers exceeding 40 000 for both analyses. Finally, devices can be operated at electric fields of >1500 V/cm and can be used for hundreds of electrophoretic separations without any change in performance. Solvent bonding with phase-changing sacrificial layers should help to overcome some of the previous limitations of polymer microfluidic devices and make them more attractive for chemical analyses.

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(40) Pumera, M.; Wang, J.; Opekar, F.; Jelinek, I.; Feldman, J.; Lowe, H.; Hardt, S. *Anal. Chem.* **2002**, *74*, 1968−1971.