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Phase-Changing Sacrificial Materials for Interfacing Microfluidics with Ion-Permeable Membranes To Create On-Chip Preconcentrators and Electric Field Gradient Focusing Microchips

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We have developed a novel approach for interfacing ionically conductive membranes with microfluidic systems using phase-changing sacrificial layers. Imprinted microchannels in a polymer substrate are filled with a heated liquid that solidifies at room temperature, a monomer solution is placed over the protected channels and polymerized to form a rigid semipermeable copolymer, and then the protective layer is melted and removed, leaving an open microchannel interfaced with a polymer membrane. We have applied this method in miniaturizing electric field gradient focusing (EFGF) and carrying out on-chip protein preconcentration. A semipermeable copolymer in the EFGF microchips fills a region of changing cross-sectional area, which allows a gradient in electric field to be established when an electrical potential is applied. Our technique provides microchip EFGF devices that offer 3-fold improved resolution in protein focusing compared with capillary-based systems. In addition, these EFGF microchips can separate peptide samples with resolution similar to what is obtained in capillary electrophoresis microdevices, and the micro-EFGF systems enrich analytes by a factor of >150. Finally, we have fabricated membrane-integrated microfluidic devices that can concentrate protein samples (R-phycoerythrin) over 10 000-fold to facilitate microchip capillary electrophoresis. Interfacing microchannels with ion-permeable membranes has great potential to enhance microchip analysis of biomolecules.

Microfluidic devices have made possible extremely fast[1] and high-performance[2−4] chemical separations, but perhaps the most significant promise of lab-on-a-chip technology is in the ability to combine multiple sample handling and analysis steps onto a single miniaturized platform.[5−8] Such integration can decrease the total analysis time significantly, especially with complex samples that require extensive pretreatment. For example, microfluidic mixers and reactors have been combined on microchip capillary electrophoresis (μ-CE) devices having six parallel separation lanes to perform multiple immunoassays in ~1 min.[9] In another case, μ-CE systems with polymerase chain reaction chambers having on-chip heaters, temperature sensors, and valves facilitated genotyping from whole bacterial cells in <10 min.[10] However, one challenge associated with μ-CE is concentration sensitivity, since small sample volumes are loaded in these systems typically. Thus, it can be advantageous to integrate sample cleanup and preconcentration on-chip to enhance the signal intensity from dilute specimens.

As size-selective membranes can facilitate many sample preparation and manipulation steps, researchers have focused on interfacing membranes with microfluidics. For example, Smith and co-workers[11] created a microdialysis system that sandwiched commercially available sheet membranes between microchannel-containing substrates, allowing samples to be purified from interfering high- and low-molecular weight species prior to being introduced into a mass spectrometer. In a similar setup, affinity microdialysis was performed on-chip; antigen−antibody complexes were retained by a sheet membrane while smaller, unbound components were removed.[12] Then, the purified complexes were exposed to counterflowing air through a second membrane, which concentrated the sample through solution evaporation. Nanoporous track-etched polycarbonate membranes were utilized in interfacing microchannels on different substrates.[13−15] Analyte

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(1) Jacobson, S. C.; Culbertson, C. T.; Daler, J. E.; Ramsey, J. M. *Anal. Chem.* **1998**, *70*, 3476−3480.
(2) Culbertson, C. T.; Jacobson, S. C.; Ramsey, J. M. *Anal. Chem.* **2000**, *72*, 5814−5819.
(3) Emrich, C. A.; Tian, H.; Medintz, I. L.; Mathies, R. A. *Anal. Chem.* **2002**, *74*, 5076−5083.
(4) 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.
(5) Liu, Y.; Garcia, C. D.; Henry, C. S. *Analyst* **2003**, *128*, 1002−1008.
(6) Vilkner, T.; Janasek, D.; Manz, A. *Anal. Chem.* **2004**, *76*, 3373−3386.
(7) Erickson, D.; Li, D. *Anal. Chim. Acta* **2004**, *507*, 11−26.
(8) Lagally, E. T.; Mathies, R. A. *J. Phys. D* **2004**, *37*, R245−R261.
(9) Cheng, S. B.; Skinner, C. D.; Taylor, J.; Attiya, S.; Lee, W. E.; Picelli, G.; Harrison, D. J. *Anal. Chem.* **2001**, *73*, 1472−1479.
(10) Lagally, E. T.; Scherer, J. R.; Blazej, R. G.; Toriello, N. M.; Diep, B. A.; Ramchandani, M.; Sensabaugh, G. F.; Riley, L. W.; Mathies, R. A. *Anal. Chem.* **2004**, *76*, 3162−3170.
(11) Xiang, F.; Lin, Y.; Wen, J.; Matson, D. W.; Smith, R. D. *Anal. Chem.* **1999**, *71*, 1485−1490.
(12) Jiang, Y.; Wang, P.-C.; Locascio, L. E.; Lee, C. S. *Anal. Chem.* **2001**, *73*, 2048−2053.
(13) Kuo, T.-C.; Cannon, D. M., Jr.; Chen, Y. N.; Tulock, J. J.; Shannon, M. A.; Sweedler, J. V.; Bohn, P. W. *Anal. Chem.* **2003**, *75*, 1861−1867.

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transport between channels was controlled by applying an electric field across the membrane, enabling selected fractions from one channel network to be driven electrokinetically through the nanopores and introduced into the opposing channel structure. Samples were injected and fractions were collected across a membrane using this approach, showing considerable control in analyte manipulation. Ramsey et al.[16,17] demonstrated size-selective barriers for DNA concentration prior to electrophoretic separation. Channels in a μ-CE injector were connected electrically through small pores in a thin sodium silicate layer. DNA molecules that were driven electrokinetically to the sodium silicate membrane were too large to pass through, and over time the concentration of the trapped DNA increased ~100-fold; the enriched sample plugs were then separated electrophoretically. More recently, a similar system was developed for coupling protein preconcentration with microchip CE;[18] however, greater sample enrichment than the ~600-fold observed would be beneficial. Zhang and Timperman[19] employed a conceptually similar preconcentration system that had a sandwiched, track-etched membrane. Rather than pore size, charge played the dominant role in analyte trapping, as the 10−50-nm through holes were much larger than the molecules that were enriched. While these examples demonstrate the broad applicability of membrane-based microsystems to various modes of sample pretreatment and manipulation, most utilized commercial sheet membranes sandwiched between microfluidic device substrates. Such configurations have limited device geometries and are constrained by the properties of available materials.

The ability to polymerize semipermeable barriers in situ in microfluidic networks adds design flexibility and enables membranes with a variety of properties to be explored. Recently, a dialysis system that incorporated an in situ-polymerized membrane was reported by Kirby and co-workers.[20] A microchannel was filled with a prepolymer solution having an appropriate photoinitiator, and a laser beam was focused into a plane to effect spatially controlled polymerization. This produced a membrane that divided the channel in two along its length, allowing dialysis to take place between countercurrent flows. Membrane properties could be altered by tailoring the prepolymer composition, but a complicated optical setup was required, and repeated laser exposures with fresh monomer solution in the channels were necessary to complete polymerization. Thus, improved methods are still needed for the convenient creation of polymer membranes in microfluidic networks.

Electric field gradient focusing (EFGF) is an analytical technique that is facilitated by having a semipermeable membrane interfaced with a separation column.[21−26] Briefly, a gradient in electric field, combined with a constant-velocity pressure-driven flow in the opposite direction, causes charged analytes to focus into stationary bands along the column according to electrophoretic mobility. A capillary-based EFGF design that interfaced an in situ-polymerized semipermeable copolymer of changing cross-sectional area with a ~100-μm-diameter focusing column has been reported.[25] The copolymer permitted current-carrying buffer ions to pass through, but the bulk fluid and protein analytes could not. The focusing column was formed by polymerizing the semipermeable copolymer around a wire in a well of changing cross-sectional area. After polymerization, the wire was pulled out from one of the ends of the microchip, leaving an open cylindrical column connected to capillaries at both sides of the membrane. Although this approach allowed for smaller-dimension devices than previous membrane-incorporating EFGF designs,[21−23,27] several limitations were also apparent. For example, further column miniaturization was impractical, as thinner wires were more fragile and difficult to use. In addition, a diameter mismatch between the focusing channel and the capillaries would reduce resolution if analytes were eluted from the column. Improved EFGF device fabrication methods that avoid these challenges, while enabling smaller channel dimensions, would be valuable.

We recently described a procedure for solvent bonding of polymer microdevice substrates.[28] Imprinted microchannels in poly(methyl methacrylate) (PMMA) were filled with a phase-changing sacrificial layer (PCSL), after which solvent was applied to the surface. A cover plate was placed in contact with the patterned substrate to allow a robust seal to form, and then the PCSL, which prevented solvent from filling the microchannels during bonding, was melted and removed. More generally, PCSL placeholders could be applied in other microfluidics applications, such as integrating membranes with microchannels.

Here we demonstrate a simple technique for in situ polymerization of membranes interfaced with microfluidic networks, based on the PCSL approach developed for making solvent-bonded microchips.[28] The procedure involves placing a prepolymer solution over PCSL-filled microchannels, hydrogel polymerization, and then sacrificial material removal. We have studied two different applications of this fabrication technique: microchip EFGF (μ-EFGF) and protein preconcentration in microchannels. The smaller dimensions of the μ-EFGF systems relative to other changing cross-sectional area-based EFGF platforms result in decreased laminar flow dispersion and narrower analyte bands. The membrane-based protein preconcentration microdevices have imprinted channels in PMMA, which are interfaced with a semipermeable copolymer using a PCSL in a manner similar to that for μ-EFGF systems. Importantly, we have achieved greater than 10 000-fold protein sample enrichment in these membrane-integrated PMMA microchips.

(14) Cannon, D. M., Jr.; Kuo, T.-C.; Bohn, P. W.; Sweedler, J. V. *Anal. Chem.* **2003**, *75*, 2224−2230.
(15) Kuo, T.-C.; Cannon, D. M., Jr.; Shannon, M. A.; Bohn, P. W.; Sweedler, J. V. *Sens. Actuators, A* **2003**, *102*, 223−233.
(16) Khandurina, J.; Jacobson, S. C.; Waters, L. C.; Foote, R. S.; Ramsey, J. M. *Anal. Chem.* **1999**, *71*, 1815−1819.
(17) Khandurina, J.; McKnight, T. E.; Jacobson, S. C.; Waters, L. C.; Foote, R. S.; Ramsey, J. M. *Anal. Chem.* **2000**, *72*, 2995−3000.
(18) Foote, R. S.; Khandurina, J.; Jacobson, S. C.; Ramsey, J. M. *Anal. Chem.* **2005**, *77*, 57−63.
(19) Zhang, Y.; Timperman, A. T. *Analyst* **2003**, *128*, 537−542.
(20) Song, S.; Singh, A. K.; Shepodd, T. J.; Kirby, B. J. *Anal. Chem.* **2004**, *76*, 2367−2373.
(21) Koegler, W. S.; Ivory, C. F. *Biotechnol. Prog.* **1996**, *12*, 822−836.
(22) Greenlee, R. D.; Ivory, C. F. *Biotechnol. Prog.* **1998**, *14*, 300−309.
(23) Huang, Z.; Ivory, C. F. *Anal. Chem.* **1999**, *71*, 1628−1632.
(24) Wang, Q.; Lin, S.-L.; Warnick, K. F.; Tolley, H. D.; Lee, M. L. *J. Chromatogr., A* **2003**, *985*, 455−462.
(25) Humble, P. H.; Kelly, R. T.; Woolley, A. T.; Tolley, H. D.; Lee, M. L. *Anal. Chem.* **2004**, *76*, 5641−5648.
(26) Kelly, R. T.; Woolley, A. T. *J. Sep. Sci.* **2005**, *28*, 1985−1993.
(27) Myers, P.; Bartle, K. D. *J. Chromatogr., A* **2004**, *1044*, 253−258.
(28) Kelly, R. T.; Pan, T.; Woolley, A. T. *Anal. Chem.* **2005**, *77*, 3536−3541.

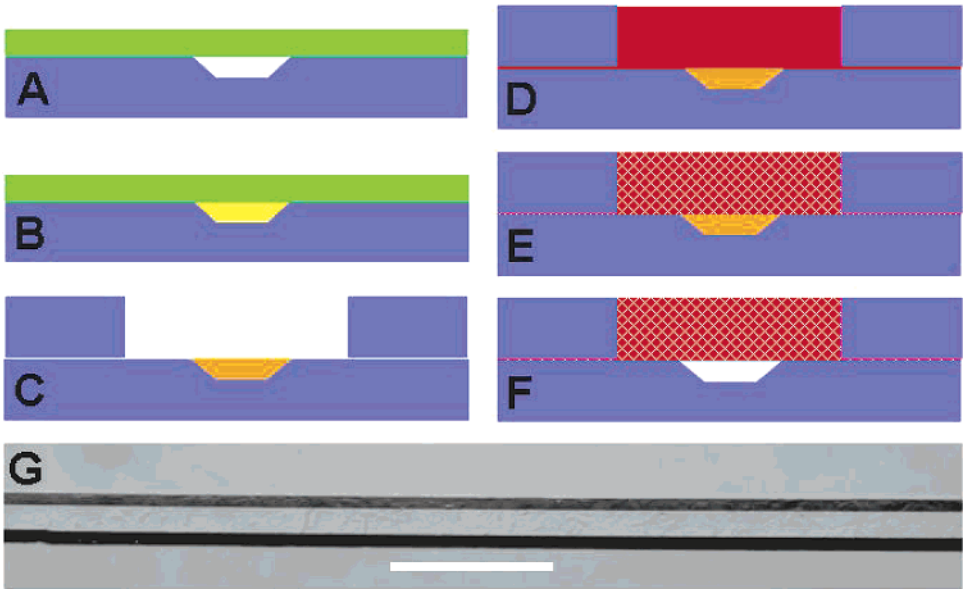

Figure 1. PCSL fabrication procedure for in situ polymerization of a semipermeable copolymer interfaced with a microfluidic system. (A) PDMS (green) is placed on an imprinted PMMA substrate (blue), forming enclosed microchannels. (B) The assembly is heated, and liquid PCSL (yellow) fills the microchannels. (C) The device is cooled to solidify the PCSL (orange), the PDMS is removed, and a PMMA substrate with an opening is placed on top. (D) Prepolymer solution (red) is loaded into the well. (E) The ion-permeable hydrogel (red with white crosshatching) is photopolymerized. (F) The PCSL is melted and removed. (G) Photomicrograph of a microchannel interfaced with a semipermeable copolymer following the above procedure. The scale bar is 250 μm.

EXPERIMENTAL SECTION

Sample Preparation and Materials. All buffer solutions were made using purified water from a Barnstead EasyPure UV/UF system (Dubuque, IA) and passed through a 0.2-μm filter (Pall, East Hills, NY) prior to use. Peptide standards (Sigma-Aldrich, St. Louis, MO) were labeled fluorescently[29] by combining 200 μL of a 2 mM solution of each peptide in 10 mM, pH 9.2 carbonate buffer with 50 μL of 6 mM fluorescein isothiocyanate (FITC; Molecular Probes, Eugene, OR) in dimethyl sulfoxide. The mixture was allowed to react at room temperature in the dark for at least 3 days prior to use. R-phycoerythrin (R-PE; Polysciences, Warrington, PA) and recombinant enhanced green fluorescent protein (GFP; Clontech, Palo Alto, CA) were used after dilution in run buffer. Diluted R-PE solutions for the calibration curve, ranging in concentration from 40 ng/mL to 500 μg/mL, were prepared in 20 mM, pH 8.0 Tris buffer.

The in situ-polymerized semipermeable copolymers for EFGF and protein preconcentration microdevices were similar to those employed for capillary-based EFGF.[25] The prepolymer solution consisted of 34 wt % hydroxyethyl methacrylate, 24 wt % methyl methacrylate, 17 wt % 100 mM Tris buffer (pH 8), 21 wt % poly(ethylene glycol) acrylate, 3 wt % ethylene glycol dimethacrylate, and 1 wt % 2,2-dimethoxy-2-phenylacetophenone (photoinitiator). All reagents for the copolymer were obtained from Sigma-Aldrich and used as received. The PMMA for device substrates was Acrylite OP-3 (Cyro, Rockaway, NJ), and the paraffin wax PCSL (melting point, 65 °C) was from Service Assets (Newport Beach, CA).

Microdevice Fabrication. Silicon wafers were patterned photolithographically and wet etched; these substrates served as templates for hot embossing PMMA as described in an earlier report.[30] Figure 1 illustrates the different device fabrication steps for interfacing a semipermeable copolymer with microchannels. In brief, this procedure involves filling a microchannel with PCSL, placing a PMMA piece with an opening on top of the imprinted substrate, filling the well with prepolymer solution, UV photopolymerization, and then PCSL melting and removal.

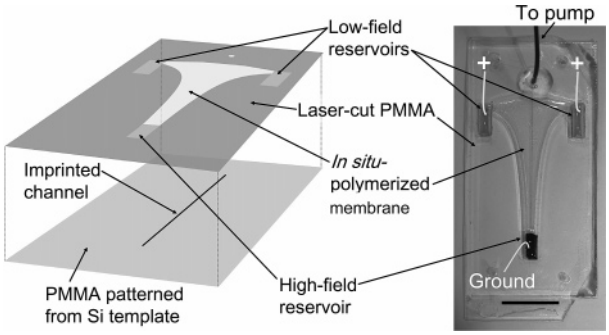

Figure 2. Schematic depiction (left, exploded view) and photograph (right) of an EFGF microchip. In the photograph, buffer reservoirs and the microchannel were filled with colored solution for enhanced visualization. The scale bar on the photograph is 1 cm. Additional description is in the text.

EFGF Microdevices. The imprinted PMMA had straight microchannels that were 3 cm long (Figure 2) with trapezoidal cross sections that were 30 μm deep, having a width that increased from 40 to 80 μm from bottom to top. A flat, 2-mm-thick piece of poly(dimethylsiloxane) (PDMS; Sylgard 184, Dow Corning, Midland, MI) had two 500-μm-diameter through holes set 3 cm apart. The PDMS was sealed reversibly to the imprinted PMMA piece (Figure 1A) such that the drilled holes aligned with the channel ends. The temperature of the PDMS/PMMA assembly was raised

(29) Monnig, C. A.; Jorgenson, J. W. *Anal. Chem.* **1991**, *63*, 802−807.

(30) Kelly, R. T.; Woolley, A. T. *Anal. Chem.* **2003**, *75*, 1941−1945.

to 85 °C on a heating block, and 10 μL of melted paraffin wax PCSL was transferred quickly from a heated vial to one of the holes in the PDMS piece. Vacuum was applied to the other opening to fill the channel with melted PCSL (Figure 1B). The assembly was transferred to a heating block at 35 °C for 3 min to solidify the PCSL (Figure 1C), and then the system was cooled to room temperature. Lowering the device temperature in two steps prevented the deposition of solid PCSL in regions beyond the microchannels.[28] Next, the PDMS was peeled from the surface, and a PMMA cover plate having a region of changing cross-sectional area cut from its center with a CO_2 laser cutter (C-200, Universal Laser Systems, Scottsdale, AZ) was aligned with the imprinted, PCSL-protected PMMA as shown in Figures 1C and 2 (left). In addition to the changing cross-sectional area pattern, rectangular buffer reservoirs and a 0.9-mm-diameter hole for connecting tubing to provide counterflow were cut from the PMMA cover plate (see Figure 2). The substrates were clamped together, and epoxy (No. 14250, Devcon, Danvers, MA) was applied around the perimeter of the assembly. Four holes, one at each of the device corners, were drilled through the cover plate to allow air pockets to escape when the prepolymer solution was added. Melted paraffin wax was pipetted and then solidified in the pump access hole and the high-field reservoir, and rectangular PDMS plugs were inserted into the low-field reservoirs (Figure 2). Approximately 400 μL of prepolymer solution was pipetted into the changing cross-sectional area region (Figure 1D), also filling the interstitial space between the two PMMA substrates. The prepolymer-containing device was mounted on a copper block maintained at 4 °C and placed under a 320-W Hg arc lamp (model 5000, Dymax, Torrington, CT) for 5 min to polymerize the semipermeable copolymer (Figure 1E). Cooling during polymerization prevented the PCSL from melting. Next, the device was heated to 85 °C to liquefy the PCSL, which was removed from the channel and reservoirs by applying vacuum (Figure 1F). Once the EFGF microchip had returned to room temperature, the channels were flushed with hexanes (EM Science, Darmstadt, Germany) to dissolve residual PCSL, and the PDMS plugs that defined the low-field buffer reservoirs were removed. Finally, a 20-cm-long piece of flexible tubing (0.9-mm o.d.) was inserted into the pump access hole and sealed in place with epoxy (Figure 2, right). A photomicrograph of a completed EFGF microchannel is presented in Figure 1G, and an image of an entire μ-EFGF device is shown in Figure 2, right.

Microchip Preconcentration Systems. Imprinted PMMA microchannels had the same dimensions as those in EFGF microdevices and were filled with PCSL as described above. A PMMA cover plate, which had two 3-mm-diameter through holes set 3 cm apart and one 5-mm-diameter opening centered between the two smaller holes, was aligned with the PCSL-filled PMMA bottom piece (Figure 3). The two PMMA substrates were clamped together, and the two smaller holes were filled with PCSL. Then, 200 μL of prepolymer solution was added to the membrane reservoir. Photopolymerization and PCSL removal were carried out as described above for EFGF microchip fabrication.

EFGF Microdevice Operation. A 100-μL gastight syringe (Hamilton, Reno, NV) having run buffer of either 20 or 100 mM Tris (pH 8) with 0.5% w/v hydroxypropyl cellulose (HPC) was connected to the flexible tubing and placed in a syringe pump

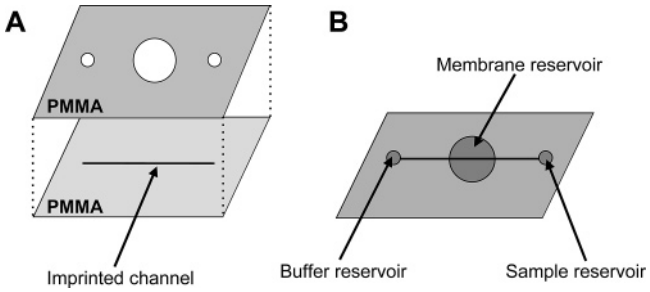

Figure 3. Schematic depiction of a membrane-based preconcentration microdevice. (A) Exploded view. (B) Assembled device view. Additional description is in the text.

(PHD 2000, Harvard Apparatus, Holliston, MA), enabling counterflow as low as 0.4 nL/min in the channel. Two Pt electrodes were connected to a high-voltage power supply and inserted into the low-field reservoirs, which were filled with run buffer, and a grounded Pt electrode was placed in the high-field reservoir (Figure 2). For analyte introduction, the counterflow was stopped, and the high-field reservoir was filled with sample dissolved in buffer. The mixture was injected electrokinetically for 30 s at 500 V, after which the power supply was turned off. Modifying the injection time or voltage would accommodate a range of sample concentrations or analyte electrophoretic mobilities. After injection, the sample was pipetted from the high-field reservoir, and the well was rinsed and refilled with run buffer. The applied potential and counterflow were then adjusted to focus the proteins or peptides into discrete bands.

Microchip Capillary Electrophoresis. CE experiments were performed in solvent-bonded PMMA microchips. Device fabrication, channel dimensions, and operating procedures have been described previously.[28] The separation distance was 2.5 cm, and the channel cross-sectional dimensions were the same as for EFGF microchips. The run buffer was 100 mM Tris (pH 8.1) with 0.5% w/v HPC, the injection potential was +300 V, and the separation potential was +1.0 kV.

Protein Preconcentration Microchip Operation. To concentrate proteins, the microchannel was filled with 20 mM Tris pH 8.0 buffer containing 0.5% HPC; buffer was placed in the buffer and membrane reservoirs, and R-PE solution was loaded in the sample reservoir (see Figure 3B). R-PE was concentrated at the membrane when 500 V were applied between the sample and membrane reservoirs.

Fluorescence Instrumentation. Detection of focused analytes in μ-EFGF devices was accomplished as described previously.[25] Briefly, micrographs were obtained by passing the 488-nm line of an Ar ion laser into a 4×, 0.12 NA objective on an inverted microscope (TE300, Nikon, Tokyo, Japan) and imaging the resulting fluorescence with a digital camera (Coolpix 995, Nikon). Photomicrographs were converted to electropherograms by averaging the fluorescence intensity across the channel at each point along the focusing column using the image processing program ImageJ 1.34s (National Institutes of Health). Noise due to laser speckle and reflections from the semipermeable copolymer was filtered from the electropherograms by boxcar averaging. When all focused bands could not be probed in a single image, the column was scanned through a confocal detection point using a translation stage. For scanning detection, the laser was passed

through a 10× beam expander prior to being focused with a 20×, 0.45 NA objective. The collected fluorescence was filtered spatially with a 200-μm-diameter pinhole and detected at a photomultiplier tube (HC 120-05, Hamamatsu, Bridgewater, NJ).

For microchip preconcentration experiments, the digital camera was replaced with a cooled CCD camera (CoolSnapHQ, Roper Scientific, Tucson, AZ). A 200-ms exposure time was selected, and the average intensity in the channel was determined for each CCD image. A calibration curve was generated from the average fluorescence signals of standard R-PE solutions flowing through the channel. The R-PE concentration factor at the membrane was obtained from the fluorescence signal and the calibration curve.

RESULTS AND DISCUSSION

For the PCSL approach to be effective for in situ membrane incorporation, the solid sacrificial material must not be soluble in either the monomer solution or the polymerized hydrogel. The prepolymer used in these studies could be placed in contact with solid paraffin wax in a microchannel for >20 min without any observable dissolution taking place at the microscopic level. In contrast, another potential PCSL, poly(ethylene glycol), dissolved readily in the monomer mixture and was not suitable. While paraffin wax and the semipermeable copolymer used here made an appropriate combination for our experiments, other PCSLs could be explored for interfacing different materials with microchannels.

Initial experiments that applied solvent bonding[28] to affix the PMMA substrates together prior to adding the prepolymer solution frequently resulted in air pockets forming at the PMMA–membrane junction at the low-field end of the EFGF microchips. While these bubbles did not form in every device, the fabrication yield was sufficiently low that alternatives were pursued. We found that when the semipermeable copolymer served both as the ionically conductive membrane to provide the electric field gradient and as the adhesive to bond the cover plate to the patterned substrate, air pockets were not observed at the PMMA–membrane junction. In this design, the thin membrane layer extending beyond the changing cross-sectional area region made current leakage a possible concern. However, the semipermeable copolymer thickness in the adhesive region (<10 μm) was much less than in the electric field gradient formation area (>1 mm), and the copolymer was ~100 times less conductive than the run buffer solution in the channel.[25] As such, we did not observe any problems with current or analyte leakage in membrane-bonded devices. Also, the size-selective properties of the membrane allowed complete exclusion of the molecules tested, such that there was no residual fluorescent staining of the channels after analytes were flushed from the devices. If the microchips were stored in a humid environment when not in use, which avoided stresses on the membrane associated with dehydration, a single device could be used reliably for more than 1 week, and no leakage was observed between the membrane and PMMA.

Figure 4A shows the separation of two natively fluorescent proteins, R-PE and GFP, in an EFGF microchip. These same species had been analyzed previously in a capillary-based EFGF device (Figure 4B),[25] which allowed the performance to be compared. Average peak widths in the microchip separation were over 4-fold narrower than those in the capillary-based device, and

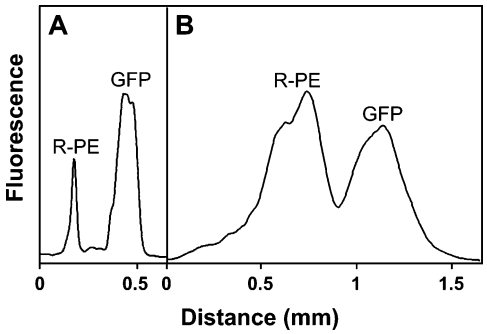

Figure 4. Separation of R-PE and GFP in (A) a μ-EFGF device and (B) a capillary-based EFGF system. In (A), 20 mM Tris, pH 8.7 was used, and the counterflow rate and applied potential were 20 nL/min and +1000 V, respectively. For (B), the run buffer was 5 mM Tris, pH 8.7, the counterflow rate was 30 nL/min, and the applied potential was +2000 V. Maximum fluorescence intensities were normalized to be the same in (A–B).

the resolution increased 3-fold. Although the comparison between the two platforms is not perfect because the buffer composition and run conditions differed somewhat, the decreased peak widths in the μ-EFGF experiment in the presence of an electric field gradient comparable to that in the capillary device (based on similar peak spacing) indicate that band broadening is reduced in μ-EFGF systems. This observation is consistent with the expectation that as channel cross-sectional dimensions shrink, Taylor dispersion decreases.[26]

With a lower applied voltage that created a shallower gradient in a μ-EFGF device, it was possible to focus a mixture of fluorescently labeled peptides that had electrophoretic mobilities spaced more closely than R-PE and GFP. For comparison, the peptides were analyzed by μ-EFGF (Figure 5A) and μ-CE (Figure 5B) using the same initial concentrations and run buffer. Peak resolution calculations for the two analyses indicate comparable separation between peak pairs (a–b) and (b–c); for (a–b), resolutions for μ-EFGF and μ-CE were 4.3 and 5.1, respectively; for (b–c) the respective resolutions were 1.3 and 1.2. The resolution for peaks (c–d) was considerably higher in the μ-EFGF study (7.9 compared with 1.4 for μ-CE), presumably due to a shallower electric field gradient near the high-field end of the device. While the buffer conductivity precluded the use of electric fields above ~300 V/cm in μ-CE, we have shown that higher-resolution separations of these peptides are possible in 10 mM carbonate buffer with an electric field of 1000 V/cm.[28] Importantly, the μ-EFGF experiments demonstrate an improvement in resolution over capillary-based EFGF as a result of decreased dispersion in the smaller channels, and the separation performance is comparable to μ-CE.

The ability of μ-EFGF to concentrate analytes is also evident in Figure 5. The normalized, background-subtracted fluorescence signal for FITC-FLEEI (peak a) was 6.2 for μ-EFGF and 0.04 for μ-CE, showing a concentration enhancement of >150-fold in μ-EFGF, which is especially notable given the <10 min total analysis time. In these experiments, the gain in the signal-to-noise ratio was less than 150-fold, due to the higher noise levels in on-column scanning detection in μ-EFGF compared to stationary point detection in μ-CE. To reduce noise in the scanning setup, improved spatial filtering could be used to avoid detection of

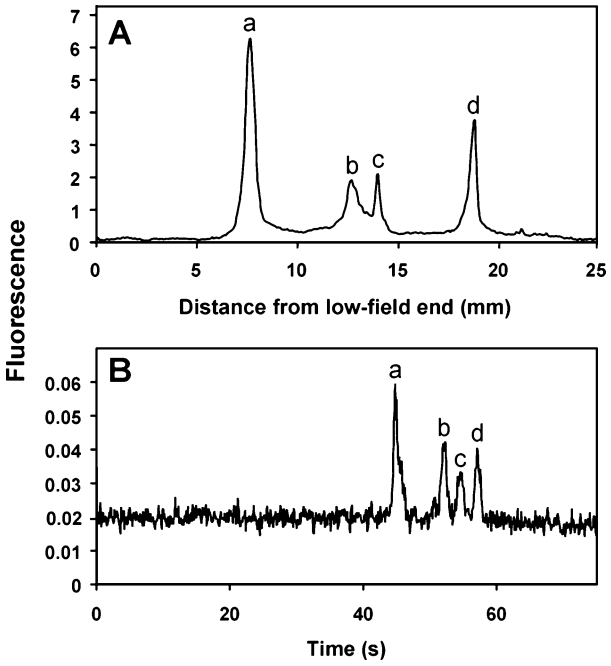

Figure 5. Separation of fluorescently labeled peptides by (A) μ-EFGF and (B) μ-CE. Peaks are (a) FLEEI, (b) FGGF, (c) angiotensin II, fragment 3−8, and (d) GGYR. Initial peptide concentrations were 50 nM, and the run buffer was 100 mM Tris (pH 8.1) with 0.5% HPC.[28] The separation potential was 200 V in (A) and 1000 V in (B). The counterflow rate in (A) was 5 nL/min.

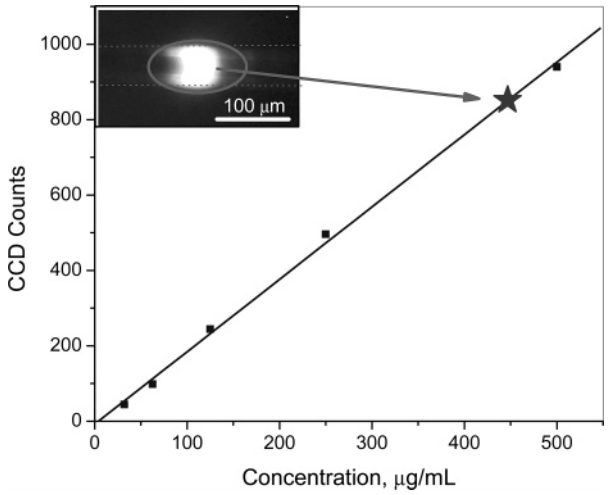

Figure 6. Calibration curve for CCD signal as a function of R-PE concentration in standard solutions. (Inset) CCD image of 40 ng/mL R-PE concentrated at the membrane reservoir for 40 min; the signal from this image corresponds to the star on the calibration curve.

semipermeable copolymer background fluorescence; alternatively, focused peaks could be eluted past a point detector.[24]

To quantify the enrichment factor in microchip membrane-based protein preconcentration, a calibration curve was generated from the fluorescence signal from flowing standard R-PE solutions (Figure 6). Linear regression yielded a slope of 1.92, an intercept of −7.51, and an R^2 value of 0.9984. When 40 ng/mL R-PE was transported electrokinetically to accumulate at the membrane for 40 min, a CCD signal of 850 was obtained, corresponding to a concentration of 450 μg/mL (Figure 6), a >10 000-fold enrichment factor. For higher-concentration R-PE samples, the fluorescence signal exceeded the range of the calibration curve after 30 min of loading. With shorter concentration times, we were also able to achieve significant enrichment factors. For example, 100 ng/mL R-PE was concentrated ~4000-fold in 15 min. These results illustrate the power of interfacing ion-permeable membranes with microfluidics to preconcentrate samples.

CONCLUSIONS

We have reported a simple method for the in situ polymerization of semipermeable membranes in microfluidic devices. Channels are first filled with a liquid, which becomes a protecting sacrificial material upon solidification. A monomer solution is then poured over the filled microchannels and UV polymerized to form an ion-permeable hydrogel. Finally, the PCSL is melted and removed, leaving a microfluidic network interfaced with a polymer membrane.

We have applied this fabrication approach in making μ-EFGF devices and membrane-based protein preconcentration microchips. Because the μ-EFGF channel cross-sectional dimensions were smaller than those of previous membrane-based EFGF setups, Taylor dispersion was reduced, resulting in narrower focused bands. EFGF of natively fluorescent proteins was demonstrated with improved resolution compared to earlier work. Moreover, fluorescently labeled peptides were focused with >150-fold sample enrichment and comparable resolution to μ-CE. Membrane-based protein preconcentration microchips were also shown to provide sample enrichment factors of >10 000, significantly increasing the potential concentration range of biological specimens that can be analyzed by μ-CE. Finally, this general fabrication approach should be adaptable to other applications that require an ion-permeable hydrogel to be interfaced with microchannel networks and, as such, should provide a useful tool for the development of integrated microfluidic systems.

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