Published in final edited form as:

Anal Chem. 2007 August 15; 79(16): 6249–6254.

Integrated Membrane Filters for Minimizing Hydrodynamic Flow and Filtering in Microfluidic Devices

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Abstract

Microfluidic devices have gained significant scientific interest due to the potential to develop portable, inexpensive analytical tools capable of quick analyses with low sample consumption. These qualities make microfluidic devices attractive for point-of-use measurements where traditional techniques have limited functionality. Many samples of interest in biological and environmental analysis, however, contain insoluble particles that can block microchannels, and manual filtration prior to analysis is not desirable for point-of-use applications. Similarly, some situations involve limited control of the sample volume, potentially causing unwanted hydrodynamic flow due to differential fluid heads. Here, we present the successful inclusion of track-etched polycarbonate membrane filters into the reservoirs of poly(dimethylsiloxane) capillary electrophoresis microchips. The membranes were shown to filter insoluble particles with selectivity based on the membrane pore diameter. Electrophoretic separations with membrane-containing microchips were performed on cations, anions, and amino acids and monitored using conductivity and fluorescence detection. The dependence of peak areas on head pressure in gated injection was shown to be reduced by up to 92%. Results indicate that separation performance is not hindered by the addition of membranes. Incorporating membranes into the reservoirs of microfluidic devices will allow for improved analysis of complex solutions and samples with poorly controlled volume.

The field of microfluidics has grown rapidly in diversity during the past decade. Modern microfluidic chips are capable of capillary electrophoresis (CE), liquid chromatography, derivatization, immunoassays, enzymatic digestions, polymerase chain reaction, coupling to mass spectrometry, microdialysis, valving and pumping, and liquid–liquid extractions and have been reviewed extensively.¹ Combining several of these techniques onto a single, concerted microchip is done with the goal of developing miniaturized total analysis systems (μ TAS). Such integrated systems exhibit increased functionality and should have a comparable increase in performance.¹

One area requiring improvement in the μ TAS field is the ability to directly analyze complex liquid samples without concern over sample volume or suspended particulate matter. One approach to managing unknown sample size and particulate matter is to incorporate nanoporous membranes in the microfluidic chip to retard hydrodynamic flow and filter the sample. Several groups have already incorporated membranes into microchips. Applications include

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microdialysis,^{2–9} filtering of cells or blood,^{9–13} protein digestion,^{14,15} membrane chromatography,^{15,16} pumping,¹⁷ desalting of proteins prior to MS analysis,^{18,19} gated injection and construction of multilayer microchips,^{20–28} gas sensing,²⁹ creating an electrospray ionization interface,³⁰ establishing pH gradients,²⁷ liquid–liquid extraction,³¹ and electrophoretic stacking.³² Sweedler, Bohn, and co-workers have characterized flow properties through nanoporous membranes incorporated in microfluidic channels.^{33,34} They found that fluid flow through nanofluidic membranes was dependent on ionic strength, pore diameter, pH, and relative hydrophilicity of the membrane material. Membrane materials commonly used in microchips include polycarbonate (PC)^{7,10–13,20–28} and poly(vinylidene fluoride),^{14–16,18,19} although other materials can be used as well.¹² A wide variety of microchip substrate materials have been used for incorporating membranes including PC,^{3,30} poly(methyl methacrylate),^{27,28,35} poly(dimethylsiloxane) (PD-MS),^{6,11–16,20–27,36,37} glass,^{7,30,31} and polyimide.³⁶ Two reviews cover the developments of membranes in microfluidic devices in more detail.^{38,39}

In the current literature, most membranes are placed between microfluidic channels at junctions, although Thorslund et al. placed a membrane between the sample reservoir and a network of microchannels.¹² There are several advantages to placing the membrane at the reservoir. For instance, filtering the sample prior to entrance into any microfluidic channels prevents clogging due to particulates. During electrophoresis, the membrane does not contact the separation channel, avoiding band broadening due to differences in surface composition and charge. One important aspect of membranes in microfluidics that has been mostly ignored is the ability of membranes to reduce hydrodynamic flow because of the small pore size. In microchip CE, reservoir heights must be optimized to prevent unwanted hydrodynamic flow.⁴⁰ Pressure heads from solution reservoirs can change injection volume, affect migration times, and decrease separation efficiency.

In this paper, we present the successful incorporation of track-etched polycarbonate membrane filters between the reservoirs and microchannels in PDMS microchips. These chips are shown to successfully remove insoluble particles before they can enter the channel. Separations of cations, anions, and fluorescently labeled amino acids show the compatibility of the membrane method with different analyte types, background electrolytes, electroosmotic flows, detection methods, and injection methods. The separation performance with a range of reservoir heights is characterized for microchips without membranes, with a membrane on only the sample reservoir, and membrane inclusion on all four reservoirs. The results show the potential of the membrane filters to improve analyses for a variety of applications where suspended particles and unequal reservoir heights can be problematic.

MATERIALS AND METHODS

Materials

Potassium chloride, magnesium sulfate heptahydrate, sodium nitrate, sodium bicarbonate, sodium tetraborate, sodium dodecyl sulfate (SDS), boric acid, glutamic acid, L-histidine (HIS), dimethyl sulfoxide (DMSO) toluene, ethyl acetate, and acetone were purchased from Fisher Scientific. Oxalic acid, 1,2-diaminocyclohexane (DACH, mixture of cis and trans), and propylene glycol methyl ether acetate were obtained from Aldrich. *N*-Dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate (DDAPS), 3-morpholino-2-hydroxypropanesulfonic acid (MOPSO), and L-arginine were purchased from Sigma. Fluorescein-5-isothiocyanate (FITC isomer I) was purchased from Invitrogen. Piperazine was obtained from Acros Organics. Amino acids used for fluorescent labeling were obtained from Fluka. All chemicals were used without further purification. Solutions were prepared in 18.2 MΩ water from a Milli-Q purification system. Gold microwires (25-μm diameter) were obtained from GoodFellow Corp. Track-etched polycarbonate membranes with pore sizes of

200 and 800 nm were purchased from Whatman. Fluorescent particles were obtained from Duke Scientific.

Microchip Construction

Construction of PDMS microchips,⁴¹ inclusion of microwires for electrochemical detection,⁴² and the extraction of PDMS oligomers from the bulk polymer⁴³ were performed as described previously and are summarized below. The 100-mm silicon wafers (Silicon Inc.) were cleaned with acetone and then spin-coated with SU-8 (Microchem) photoresist at 2100 rpm. A negative mask was placed on the wafer, exposed to ultraviolet light, and developed in propylene glycol methyl ether acetate to give a positive relief of the microchannels on the wafer surface. Sylgard 184 (Dow Corning) elastomer was mixed with cross-linking agent (10:1 ratio), degassed under vacuum, poured onto the wafer, and cured at 60 °C for at least 2 h. Reservoirs were cut with 3- or 4-mm biopsy punches (Robbins Instruments). Irreversible sealing of the PDMS was accomplished with a 45-s air plasma exposure at 18 W (Harrick Scientific PDC-32G) followed by conformal contact of the oxidized pieces. For extracted chips, oligomers were removed by submerging cured PDMS chips in toluene, ethyl acetate, and acetone, respectively, for at least 2 h each. Extracted chips were sealed with a 2-min plasma exposure according to previously published work.⁴³

For microchips utilizing conductivity detection, gated injection was used.⁴⁴ Channel widths were 76 μm , injection arms were 7 mm in length, separation channel length was 4 cm, and detection-to-waste spacing was 3 mm. Microwire spacing was 150 μm center-to-center. For microchips using fluorescence detection, a double-T intersection was used for pinched injection. Channel widths were 50 μm , while injection arms were 7 mm long with a 250- μm offset. Fluorescence detection was performed 1.5 cm downchannel from the double-T intersection.

Inclusion of a membrane in a microfluidic device requires formation of a good seal to prevent leakage and dead volume. PDMS prepolymer has been shown to be effective in aiding sealing when used as a glue.^{20,28,44} Figure 1 schematically shows the inclusion of a membrane into a microfluidic device. Uncured PDMS was spin-coated (1500 rpm) onto a blank piece of cured PDMS. Reservoir holes were cut in the desired locations using biopsy punches (3 or 4 mm, depending on experiment). The membrane was cut to the desired size (slightly larger than the reservoir) and shape with scissors and then placed over the reservoir. The uncured PDMS soaked into the pores of the membrane, helping to flatten the membrane to the chip. No significant lateral diffusion of the polymer into the reservoir area of the membrane was observed. This half of the microchip was cured at 60 °C for at least 40 min. After curing, the membrane side of the chip was plasma sealed to the channel side to form an irreversible seal. Poor sealing of the membrane could result in leakage zones at the membrane edge where bubbles of unsealed PDMS could be present (see Supporting Information Figure S-1). Occurrences of this poor seal were reduced by using a relatively thin (<2 mm) blank piece of PDMS for the membrane side of the chip and applying additional pressure in the membrane region of chip during the sealing process. Problems could also be avoided by rolling (Figure 1f) the PDMS during sealing to ensure any air bubbles avoided the microchannel, similar to the approach of Hediger et al.¹⁰ with paraffin foil and glue.

For membrane incorporation into extracted PDMS, the above procedure was modified since the spin-coated PDMS is unextracted. A sacrificial piece of PDMS was spin-coated with uncured PDMS followed by punching reservoirs using the biopsy punch. The membrane was applied to the sacrificial piece and prepolymer allowed to soak into the pores. The membrane was then transferred to an extracted PDMS piece, placed over the desired reservoir, and cured. The remainder of the sealing process remained the same, and no difference in sealing success rate was observed between the two methods.

FITC Labeling of Amino Acids

Stock solutions of amino acids (10 mM) were prepared in 10 mM sodium bicarbonate, pH 9.0. Solutions of 1 mM FITC I isomer were prepared fresh daily in DMSO. Each amino acid was labeled with FITC individually by combining 90 μ L of 10 mM amino acid solution and 10 μ L of 1 mM FITC and then reacted in the dark for 2 h with gentle mixing. Mixtures of derivatized amino acids were prepared in the 10 mM borate, 1 mM SDS, pH 9.0 (BGE) and diluted to 5 μ M prior to injection.

Instrumentation

The high-voltage power supplies used for capillary electrophoresis were described previously.⁴⁵ Fluorescence data were acquired with a Nikon Eclipse TE2000-U microscope and Photometrics Cool Snap HQ² camera at 20 Hz. Electropherograms were generated using Metamorph software and filtered with a three-point median filter. Conductivity detection was accomplished by connecting the leads of a Dionex CD20 to the detection microwires. The CD20 was set to monitor the 0–200 μ S range and output 0–1 V. Output from the CD20 was monitored with a National Instruments USB-6210 DAQ and LabView 8.0 software. Data were collected at 20 kHz, and every 2000 points boxcar averaged to give an effective collection rate of 10 Hz. These data were smoothed with a rectangular half-width of two points.

RESULTS AND DISCUSSION

Filtering of Particulate Matter

Membrane performance was first measured by testing the ability of the membranes to pass small molecules while retaining larger objects. Microchips were constructed with one straight channel connecting a membrane-containing reservoir to a reservoir without a membrane. The fluorescence of the solution in the channel at the membrane edge could then be monitored to see whether fluorescent components passed through the membrane and down the channel. This measurement was performed both before and after voltage application. When a solution of fluorescein was tested, fluorescence was observed to travel down the channel just as it would without a membrane, confirming the membranes permit small molecules to migrate into the channel. To test membrane performance for larger components, 500-nm rhodamine-containing polystyrene particles were used. The results are shown in Figure 2. Prior to applying a voltage to the reservoir, no particles were observed in the channel. When a voltage was applied to the channel, particles were observed to pass through a 800-nm-pore membrane and down the microchannel. The same experiment with a 200-nm-pore membrane showed no particles entering the channel. The results confirm that the membranes can selectively dictate what components enter the microfluidic channel based on size. One benefit of filtering at the reservoir appears in applications involving particulate-containing samples. With on-chip filtering, channel clogging is prevented.

Electrophoretic Separations

The compatibility of membrane incorporation with capillary electrophoresis was confirmed next. A separation of the anions chloride, nitrate, sulfate, and oxalate is shown in Figure 3a, while an example cation separation of potassium, sodium, piperazine, and arginine is shown in Figure 3b. These two separations exhibit the compatibility of the membranes with hydrophilic compounds and gated injection. No change in migration order, relative peak height, or band broadening was observed between membrane chips and nonmembrane chips, showing the advantage of placing the membrane away from the separation channel. For instance, chips without membranes gave a sulfate (50 μ M) peak area of 241 ± 31 (arbitrary units), whereas chips with membranes on all four reservoirs showed a peak area of 255 ± 37 , which is not statistically different (see Supporting Information Figure S-2). Since no significant effect was

observed on the electrophoretic separation, membranes can be added to many existing analyses without modifying separation protocols. To test membranes with relatively hydrophobic compounds, pinched injection, and extracted PDMS, fluorescently labeled amino acids were separated. Figure 4 shows separations of FITC-labeled histidine, glycine, and valine with both native PDMS and extracted PDMS surfaces. Extracted PDMS exhibits a faster EOF, as expected. Again, the presence of the membrane has no significant effect on the separation chemistry, showing that the membrane does not inhibit the migration of the analytes of interest. It should be noted that although each example separation utilized background electrolytes containing surfactants, surfactants are not required to permit analyte flow through the membranes, and successful separations were performed without surfactants (data not shown).

Dampening Hydrodynamic Flow

Hydrodynamic flow in microchannels caused by differences in fluid pressure heads can be detrimental to separations. The ability of membranes to retard hydrodynamic flow in the microchannels was characterized in gated injection mode by monitoring the peak area of sulfate ($50\ \mu\text{M}$) while varying reservoir heights (Figure 5). The three reservoirs containing buffer had equal heights, and their heights were varied with respect to the sample reservoir. For systems without hydrodynamic flow restriction, peak area should scale proportionally to the height difference of the sample and buffer reservoirs due to the effect of hydrodynamic flow on the injected sample size. Microchips without membranes are sensitive to this trend, showing peak area changes of 20%/mL of reservoir height. For instance, when the sample height was 1.1 mm above that of the buffers, peak area increased by 21%. This height difference would require only a 7.8- μL discrepancy between reservoirs when using 3-mm-diameter reservoirs. However, peak areas for chips containing membranes show significantly less change with respect to reservoir height differences, even at larger height discrepancies. As expected, smaller pore membranes perform better than those with larger pores. The 800-nm membranes reduced hydrodynamic dependence of peak area by 55%, while 200-nm membranes reduced peak area dependence by 86%. Placing 200-nm membranes on every reservoir dampened hydrodynamic flow even more effectively than using only a single membrane. Placing 200-nm membranes on every reservoir reduced the peak area dependence by 92%. At the same 1.1-mm height difference mentioned earlier for nonmembrane chips, a peak area increase of only 2% was observed, ~10 times less than the effect seen in chips without membranes. Similar trials with pinched injection and fluorescent compounds also showed benefit from the membranes. When the sample was 1 mm below the buffer, the injection arm would not fill with analyte on microchips lacking membranes, so no separations could be performed. When a membrane was included on the sample reservoir, this problem was not encountered. Additionally, analyte peak areas were less sensitive to reservoir height when a membrane was included on the sample reservoir than when no membrane was used. Sensitivity to the pressure head from the reservoirs was decreased by ~60% when a 200-nm-pore membrane was placed on the sample reservoir in pinched injection (see Supporting Information Figure S-2). The ability of membranes to reduce hydrodynamic flow allows the use of different solution volumes in different reservoirs without losing quantitative accuracy. Some potential applications include the analysis of small samples where buffer reservoirs have fluid heights above the sample, situations where sample volume cannot be strictly controlled, three-dimensional microfluidic chips with reservoirs located on different chip levels, and adaptation to automatic sampling interfaces that may operate at pressures slightly above or below atmospheric pressure.

Reproducibility and Durability

Although the construction of membrane-containing microchips does require some degree of technique, the inclusion of the membranes onto the reservoir is fairly robust. We found that following the construction scheme shown in Figure 1 yielded 40 successful chips in 41 attempts (98%), where success was considered a membrane that had a good seal with the channel and

no leakage of solution at the membrane interface. For best dampening of hydrodynamic flow, we observed that the membrane area under the reservoir must be free of dead volume and therefore the membrane should have no creases or wrinkles. With this stipulation, 34 successful chips were prepared in 41 attempts (83%). Separation performance reproducibility was similar to that of nonmembrane chips as measured by peak areas in electropherograms (see Supporting Information Figure S-2). This indicates that variability in other aspects of microchip construction and performance dominates over variability in membrane performance.

Polycarbonate membranes are relatively fragile and their incorporation into microfluidics increases operational complexity, so some handling precautions are warranted. The membrane can be creased either by flexing the PDMS at the membrane location or by applying considerable pressure (no exact value measured, but at least several PSI) to the microfluidic system. To prevent tearing, physical contact between the electrophoresis electrodes and the membrane was avoided, and plastic pipets were used instead of glass ones. To ensure adequate cleaning of the sample reservoir, a rinsing step was performed between samples. The channel under the membrane represents the only dead volume, and the solution in it was not found to be problematic between samples since it was immediately flushed to the sample waste reservoir. For the most rigorous applications, an electrophoretic flushing step could be added to ensure the sample channel and membrane were completely rinsed. When the aforementioned precautions are taken, the membranes maintain their performance for extended periods. When the appropriate precautions were taken, the analysis of inorganic anions (see Figure 3) could be carried out over a period of weeks and dozens of injections on the same chip with no discernible decrease in performance.

CONCLUSIONS

The successful incorporation of polycarbonate track-etched membranes between the reservoirs and microchannels of PDMS microfluidic devices was demonstrated. Membranes were shown to filter polystyrene microparticles, preventing them from entering the microchannel underneath the reservoir. Filtering was shown to be size selective and dependent on membrane pore diameter. Separations of cations, anions, and fluorescently labeled amino acids demonstrated the compatibility of the membranes with both gated and pinched injections and fluorescence and conductivity detection. The monitoring of analyte peak area at various sample reservoir volumes showed the ability of membranes to dampen hydrodynamic flow in microfluidic chips. Results indicate that including membranes into the reservoirs of microfluidic chips will protect the microchannels from particulate matter and help to increase the ruggedness and applicability of microchip capillary electrophoresis analyses in situations of limited or uncontrolled sample volume and high-sample complexity.

Acknowledgements

The authors thank Marshall Wilkinson of Colorado State University for his assistance in the preparation of photomasks. Funding for this work was provided by the U.S. Department of Energy STTR Phase II Grant DE-FG02-04ER-86179, the U.S. Environmental Protection Agency SBIR Phase II Project EP-D-05-058, and the U.S. National Institute of Health grant EB 004876-01A1.

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Figure 1. Construction steps for membrane inclusion

(A) Start with a PDMS blank, (B) spin coat a layer of uncured PDMS on the blank substrate, (C) punch reservoir holes, (D) place membrane over reservoir, (E) cure PDMS in oven at 60 °C, (F) use a rolling motion to help ensure air bubbles avoid microchannels during sealing, and (G) top-down view of constructed microchip with membrane on sample reservoir.

Figure 2.

(a) Membrane chip and voltage configuration, (b) 800-nm-pore membrane edge prior to voltage application shows no particles, (c) 800-nm-pore membrane edge after applying voltage showing 500-nm particles have passed through membrane and into channel. (d) The 200-nm-pore membrane edge prior to voltage application shows no particles. (e) The 200-nm-pore membrane edge after applying voltage shows no particles, indicating successful filtration.

Figure 3. Separation of anions

(a) and cations (b) using gated injection and conductivity detection. Peak identities are (1) chloride, (2) nitrate, (3) sulfate, (4) oxalate, (5) potassium, (6) sodium, (7) piperazine, and (8) L-arginine. Separation electric field of -150 V/cm used for anions and $+150$ V/cm for cations. Anion BGE: pH 3.9; 10 mM glutamic acid; 1.2 mM DACH; 10 mM DDAPS; 0.2 mM EDTA. Cation BGE: pH 6.4; 15 mM MOPSO; 15 mM HIS; 5 mM DDAPS.

Figure 4.

Separation of FITC-labeled amino acids with pinched injection and fluorescent detection on native PDMS (A) and extracted PDMS (B). Mixtures of 5 μ M histidine (1), valine (2), and glycine (3) were injected for 30 s at 292 V/cm and separated at 267 V/cm. Fluorescence was detected 1.5 cm from injection. BGE was 10 mM borate, 1 mM SDS, pH 9.0.

Figure 5.

(a) Relative peak area of sulfate with changing sample–buffer reservoir heights for a microchip without a membrane and one with membranes on all four reservoirs. Peak areas are normalized to the values obtained at equal reservoir heights. Conditions are the same as those used for the anions in Figure 3. Error bars are standard deviations of three chips. (b) Slopes of the plot shown in (a) for several membrane configurations. Error bars are standard error from the regression analysis.