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Size selective DNA transport through a nanoporous membrane in a PDMS microfluidic device

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A microfluidic counter current dialysis device for size based purification of DNA is described. The device consists of two polydimethylsiloxane (PDMS) channels separated by a track etched polycarbonate membrane with a 50 nm pore size. Recovery of fluorescein across the membrane was compared with 10 and 80 nucleotide (nt) ssDNA to characterize the device. Recovery of all three analytes improved with decreasing flow rate. Size selectivity was observed. Greater than 2-fold selectivity between 10 nt and 80 nt ssDNA was observed at linear velocities less than 3mm/s. Increasing the ionic strength of the buffer increased transport across the membrane. Recovery of 80 nt ssDNA increased over 4-fold by adding 30mM NaCl to the buffer. The effect was size dependent as 10 nt showed a smaller increase while the recovery of fluorescein was largely unaffected by increasing the ionic strength of the buffer.

There is considerable interest in integrating nanoscale structures into microfluidic devices^{1, 2}. Macromolecules behave differently as the dimensions of surrounding structures approach the size of the molecule itself³. Both preparative and analytical applications that take advantage of these differential properties have been proposed^{1–5}.

Membranes have been integrated to add functionality and flexibility to microfluidic devices^{6, 7}. Fabrication of 3D fluidic networks by integrating membranes containing monodisperse distributions of nanopores into microfluidic devices is relatively straightforward^{8–10}. The nanopores of the membrane controls fluidic transport between different planes in the device¹¹. The large surface-to-volume ratio maximizes analyte separations based on physical size. Multilayer devices with membranes have great potential for removing impurities and pre-concentrating samples before further analysis in microfluidic devices^{12–14}, dramatically reducing offline process time and increasing sensitivity when coupled with mass spectrometry¹⁵ or capillary isoelectric focusing (CIEF)¹⁶. Membranes have also been used in cell-related research^{17, 18}, protein digests^{19, 20} and bioreactors^{20, 21}. Membranes are used widely in chemical analysis²², especially when the sample is mass-limited^{14, 23}.

Many groups have studied methods for controlling molecular transport through nanopores^{24–32}. Sweedler *et al.* manipulated transport electrokinetically taking advantage of electroosmotic flow and high field strengths in the pores.^{9, 24, 30, 33} Kovarik and Jacobson sandwiched a polycarbonate membrane between two PDMS channels to trap particles using electrophoretic and dielectrophoretic forces²⁸. Nishizawa *et al.* prepared ion selective membranes which rejected ions of the same polarity as the excess charge on the inner walls of the metal tubules by imposing an electrical bias potential on the membrane²⁵. Hou *et al.* modified the surface of gold coated pore walls in polycarbonate membranes to achieve pH-responsive transport of ions due to electrostatic opening and closing of membrane pores²⁷. Jirage *et al.* explored the use of gold nanotubules with inner diameters of less than 1 nm to separate small molecules³⁴. Schmuhl *et al.* used surfactants to physically open or close

nanometer sized channels and pores to gate molecular transport³⁵. The Yager group generated laminar fluid interfaces in microfluidic devices for diffusion based sample extraction, separation and detection without using a membrane^{36–40}.

Short chain nucleic acids are commonly purified using electrophoretic separation on agarose or polyacrylamide gels followed by subsequent recovery of from the gel using electroelution, adsorption, diffusion or gel liquification⁴¹. It is a slow process not amenable to automation^{41, 42}. A nanopore based microfluidic purification device could potentially resolve these issues. The current paper describes our initial work in developing such a device. Figure 1 shows a schematic of the device. A 6 μm thick polycarbonate membrane was sandwiched between two 250 μm wide channels. A simple counter current diffusion based design was chosen to make fabrication and operation of the device as simple as possible.

EXPERIMENTAL

Reagents and Chemicals

PDMS was made using Sylgard 184 silicone elastomer kit (Dow Corning, Midland, MI). Nuclease free water (Integrated DNA technologies, Coralville, IA) was used to prepare all buffer and sample solutions. Fluorescein was purchased from ACROS organics (New Jersey, USA). FAM labelled ssDNA sequences (10 nt: 5'FAM-AGC AGC ACA G and 80 nt: 5'FAM-AGC AGC ACA GAG GTC AGA TGG GAA GCC CGC TGT GAC ATC TGG AGC CGG TTC CCG GAG CCA CCT ATG CGT GCT ACC GTG AA) were synthesized by Integrated DNA technologies (Coralville, IA). Buffers consisted of 2mM NaH_2PO_4 , 300 μM Triton X-100 with various concentrations of NaCl (0, 5mM, 10mM, 30mM, 50mM, 100mM, 200mM), adjusted to pH 7.3 using 100mM NaOH (Mallinckrodt, Paris, KY). Fluorescein and ssDNA solutions were all diluted to 1 μM using in these buffers. All buffer and sample solutions were degassed before use. Piranha solution (3:1 H_2SO_4 : H_2O_2 , Ashland Chemical, Dublin, OH) was used to clean silicon wafers. Diluted HCl was used to reactivate the PDMS surface. SU8–2050 (Microchem Corp., Newton, MA) was used to fabricate the master. All other reagents and chemicals are from Sigma-Aldrich (St. Louis, MO).

Fabrication

The fabrication process is outlined in Figure 2. A two step soft lithography process was used.⁴³ An SU8 master was fabricated and multiple PDMS replicas were then cast from this master. The chip geometry was designed using CAD software which was then used to print a transparency film mask. Standard photolithography procedures were used to make the SU8 master. A new silicon wafer was cleaned in piranha solution for 10 min followed by acetone, methanol, isopropanol and deionized water rinses. The negative photoresist SU8 2050 was spin coated on the dried wafer using the manufacture's recommended procedure. The film mask was used to expose the photoresist in a MABA6 aligner (Karl Suss, Munich, Germany). developing in propylene glycol monomethyl ether acetate (P.M. Acetate) for 6 min yielded a master with the desired geometry. The master was pre-treated with fluorinated silanes (UCT, Bristol, PA). Degassed PDMS prepolymer (10:1 ratio) was then poured onto the master and baked at 70°C for 3 hours. The cured PDMS was removed from the master. A second PDMS layer was made using the same process. The resulting channels were 250 μm wide and 110 μm deep. The total channel length was 14.8cm with an active length where the channels overlapped of 12.8 cm. Inlet and outlet holes were punched into one PDMS layer using a piece of 20 gage stainless steel tubing (McMaster-Carr, Chicago, IL). Both PDMS layers were sonicated in dilute HCl for 10 minutes to reactivate prior to bonding⁴⁴. A 10mm $\times$ 15mm $\times$ 6 μm polycarbonate membrane with 50nm pore diameter (Sterlitech Corp., Kent, WA) was placed on one layer of PDMS over the transport area of

the channel. The PDMS layers were O₂ plasma treated at 600 mTorr and 75 W for 10 s. The two layers were then quickly aligned and bonded irreversibly. The entire assembly was placed on a hotplate at 70 °C for 1 hour to complete the bonding process. Two glass substrates were used to support the device and four screws on the corners of the substrates were finger tightened to seal the membrane and the PDMS slabs (see Figure 2). 0.5" long pieces of stainless steel tubing with the same inner and outside diameter as a 21 gage needle (New England Small Tube Corp., Litchfield, NH) were inserted into the inlet and outlet holes of the device allowing connections to be made with Tygon tubing (0.03 ID, 0.09 OD, Small parts, Inc, Miramar, FL). Buffer was pumped into the device using a syringe pump.

Instrumentation and Data Collection

An AZ 100 stereomicroscope (Nikon Corp., Tokyo, Japan) mounted with a 120W X-cite metal halide lamp (EXFO Photonic Solutions Inc., Ontario, Canada) and a Cascade 512B CCD camera (Photometrics, Tucson, AZ) was used for fluorescence imaging. Fluorescence was collected through a 1.6× objective with a 3× zoom and filtered using an Endow GFP bandpass filter set (450–490 nm excitation and 500–550nm emission) and a dichroic mirror (495nm cutoff). Images were recorded and processed using MetaVue software (Downingtown, PA).

Size Discrimination of the Membrane

Buffer solution (2mM NaH₂PO₄, 300μM Triton X-100, 50mM NaCl) and 1μM fluorescein or fluorescently labelled DNA, dissolved in the same buffer, were simultaneously pumped into inlets #1 and #2 (see Figure 1) generating counter current flow in the device. The flow rate was varied from 0.05 ml/hr to 1 ml/hr, corresponding to linear velocities ranging from 0.51 mm/s to 10.1 mm/s. Approximately 10 channel volumes of solution were pumped through the device before data was recorded to ensure stability of the signal. 20 ul solution was collected from outlet #1 to determine the amount of fluorescent analyte that crossed the membrane. This was compared with the intensity of the fluorescent solution pumped into the device (at inlet #2). All fluorescence measurements were made using a Synergy2 plate reader (BioTek Instruments, Winooski, VT) with $\lambda_{ex} = 485\text{nm}$ and $\lambda_{em} = 528\text{nm}$. Analyte recovery (R) was determined according to:

$$R = \frac{I_{outlet}}{I_{inlet}} \quad (1)$$

where I_{outlet} is the fluorescence intensity of the solution exiting outlet #1 and I_{inlet} is the fluorescence intensity of the solution entering the device at inlet #2.

RESULTS AND DISCUSSION

Design Considerations

The active region of the device consisted of two channels, with dimensions of 12.8 cm long × 250 μm wide × 110 μm deep, separated by a polycarbonate track etched membrane. Polycarbonate membranes were chosen due to their mechanical robustness, stability under fabrication conditions and the precisely controlled cylindrical pores typical of these materials. The membrane was 6 μm thick with 50 nm diameter pores. The active region defined by overlapping channels had a total area of 0.32 cm² with approximately 1.92×10⁸ pores randomly distributed across this area. The membrane was thin enough to allow PDMS to form a good seal during the fabrication procedure.

A counter flow dialysis strategy was adopted to maximize recovery across the membrane. Sample solution and buffer were pumped into each channel in opposite directions. In this

device, diffusion is considered to be the major mechanism contributing to transport across the membrane. According to Fick's first law, the rate of diffusion flux is determined by the concentration difference at the boundary⁴⁵. If a cocurrent flow is adopted, transport takes place until equilibrium is reached and the concentration is equal on either side of the membrane. Assuming equal flow rates, recovery in a cocurrent arrangement is therefore limited to 50% since there is no net transport at equilibrium. However, counter flow dialysis has no such limitation. At every position along the length of the channel, the analyte concentration in the buffer solution is lower than that in the sample solution. The system does not reach equilibrium and net transport occurs along the entire length of the channel with the maximum recovery approaching 100% if the equilibration time is long enough^{46–48}.

A potential drawback of the counter current approach used here is the generation of pressure differences across the membrane. The nanopores have much higher resistance than the microfluidic channels minimizing flow through the pores at low flow rates. Thus diffusion is the dominant transport mechanism and convection through the nanopores can be considered minimal. However, if bubbles or other obstructions block the channels more significant pressure differences and unpredictable flow patterns can occur. It is therefore important to degas all the solutions. Addition of Triton X-100, a non-ionic surfactant, also decreases bubble size and facilitates their clearance from the device, even at low flow rates⁴⁹.

Characterization of the Device

Fluorescein and fluorescently labelled ssDNA of two different lengths (10 and 80 bases) were used to characterize the fully fabricated device. The molecular weights for fluorescein, 10 nt DNA and 80 nt DNA were 332 Da, 3583.5 Da and 25278.4 Da, respectively, providing a series of analytes with a wide size range distribution. Figure 3 shows fluorescence images of the dialysis channels when the device is perfused with fluorescein (A), 10 nt ssDNA (B) and 80 nt ssDNA (C). The sample solution entered the device from the lower left corner and exits through the channel at the top right corner. The buffer solution, on the opposite side of the membrane, enters from the lower right and exits from the upper left channel. Fluorescence intensity decreased along the length of the channel as analyte crossed the membrane and was carried back to the left in the counter flow. This is especially evident for fluorescein and to a lesser extent 10 nt and 80 nt DNA. Figure 4 plots fluorescence intensity along the length of the channel, clearly showing differences in the rate that each analyte crosses the membrane. The trend is consistent with the size dependence expected for diffusion based transport across the nanopores. Images were recorded near the center of the field of view to minimize heterogeneity in the illumination intensity. Sensitivity across this imaging area only varied by less than 1.9% (rsd).

Recovery and Size Discrimination

Recovery was assessed by comparing the fluorescence intensity of the analyte solution entering the device to that exiting on the opposite side of the membrane (see eq. 1). Solution was collected and measured offline in a fluorescence plate reader to eliminate the potential for calibration errors in the fluorescence images due to pixel position, differences in channel depth and background generated by the membrane. Recoveries of fluorescein, 10 nt ssDNA and 80 nt ssDNA were measured across a range of flow rates and plotted in Figure 5. As would be expected if diffusion dominates, recovery was higher at lower flow rates. The recovery for fluorescein increased from 18.5% to 90% while the recovery for 10 nt ssDNA increased from 9% to 70%. The increase in recovery observed for 80 nt ssDNA was a more modest 7% to 31%. The increase in recovery at lower flow rates is the result of the increased time for equilibration that the analyte is exposed to the membrane. For the 12.8 cm long channel used here, the equilibration time increases from 13 seconds to 4.3 minutes as the linear flow rate is decreased from 10 mm/s to 0.5 mm/s (1ml/hr to 0.05 ml/hr). Making

measurements at flow rates lower than 0.5 mm/s was challenging due to the corresponding increase in time required to collect a reasonable volume of sample.

The size dependence of recovery was maintained across all flow rates. For example, at 0.5mm/s, the recovery for fluorescein was 90% while the recovery for 10 nt and 80 nt DNA was 70% and 31%, respectively. The selectivity between 10 nt and 80 nt was 2.3. It should be noted both recovery and selectivity decreased with increasing flow rates (see Figure 5). For example, at 10mm/s, the recovery for 10 nt DNA was 9% and the recovery for 80 nt DNA was 7% resulting in a selectivity of only 1.3.

Considering the dimensions of the molecules under study is useful in assessing the mechanism that generates selectivity. The radius of gyration R_g of a chain of contour length $L_D = Mb$ can be estimated using the Kratky-Porod equation^{50, 51}:

$$R_g^2 = \frac{pL_D}{3} \times \left[1 - 3\left(\frac{p}{L_D}\right) + 6\left(\frac{p}{L_D}\right)^2 - 6\left(\frac{p}{L_D}\right)^3 \times \left(1 - e^{-\frac{L_D}{p}}\right) \right] \quad (2)$$

where p is the persistence length, M is the number of monomers and b is the contour length of a single monomer. Using $b=0.43\text{nm}$ ⁵² and $p=3\text{nm}$ ⁵³, common estimates for ssDNA, the radius of gyration for the 80 nt and 10 nt are approximately 5nm and 1nm, respectively. Both values are significantly smaller than the 50 nm pore size of the membrane suggesting that physical size alone is unlikely to give rise to the selectivity differences observed. Instead selectivity is likely to arise from a combination of steric factors, diffusion constants and interactions with both ions and solution and the surface of the pores.⁵⁴

Ionic Strength of the Buffer

Although the size of the pores is significantly larger than that of the molecules assessed here, the thickness of the double layer also impacts transport of DNA molecules²⁴. Sodium chloride was added to buffers to evaluate DNA and fluorescein transport properties as a function of ionic strength, which in turn impacts the double layer in the pores. As demonstrated in Figure 6(A), recovery of each analyte initially increased with increasing ionic strength before reaching a plateau at approximately 30 mM NaCl. Figure 6(B) illustrates the effect of ionic strength on the relative recovery of each analyte. The increase in relative recovery showed clear size dependence with 80 nt and 10 nt ssDNA increasing 4.6-fold and 2.1-fold, respectively. Fluorescein was largely unaffected, with relative recovery only increasing 20% with increased ionic strength.

The effect of ionic strength on recovery suggests that the double layer within the pores plays an important role in the transport of the highly negative analytes studied here. The surface of polycarbonate membranes is coated with polyvinylpyrrolidone (PVP) which renders the pore surface hydrophilic. It has been reported that the surface of PVP coated pores typically bears a negative charge at neutral pH.^{24, 55-57} The Guoy-Chapman theory can be used to estimate the Debye length κ^{-1} for dilute aqueous solutions⁵⁸:

$$\kappa^{-1} = \left(3.29 z C^{\frac{1}{2}} \right)^{-1} \quad (3)$$

where κ^{-1} is given in nm and C is the bulk z:z electrolyte concentration in mol/L. The Debye length describes the distance over which ionic effects are observed in solution and can be used to characterize the thickness of the diffuse double layer at a charged surface. The Debye length in the buffer was estimated to be 4.67nm in 2mM NaH_2PO_4 . Addition of 30 mM NaCl reduces the Debye length to 1.64 nm. Addition of 200 mM NaCl further reduces the Debye length to 0.67 nm.

The formation of a double layer in the pores raises the potential for both coulombic interactions between the charged surface and charged molecules, and increased steric hindrance^{25, 59–62}. Increasing the ionic strength of the buffer is expected to reduce the size of the double layer, effectively decreasing the interaction between the charged pore walls and analytes, while increasing the pore size permitting larger molecules to pass through. For example reducing the double layer thickness from 4.67 nm to 0.67 nm increases the cross sectional area of a 50 nm pore by 43%. Beyond affecting the size of the physical barrier of the pore, reducing the Debye length reduces ionic interactions between the analyte and the surface or double layer. Similarly, the radius of gyration of ssDNA decreases as the ionic strength of the buffer increases due to the smaller persistence length of ssDNA^{50, 52, 53}. The diffusion coefficient of ssDNA also increases with ionic strength^{53, 63}. Through these combined effects increasing the ionic strength improved the recovery of 80 nt DNA 4.6 fold. As expected increasing the effective pore size and decreasing ionic interaction had less of an effect on the smaller and less charged 10 nt DNA, only improving recovery 2.2 fold. The effect on the recovery of fluorescein, already much smaller than the pore size, was negligible.

Discussion

The separation process was assumed to be diffusion driven, that was, molecules diffuse through the membrane due to concentration differences between two channels across the membrane. Due to differences in diffusion rates arising from different molecular size, separation between fluorescein, 10nt ssDNA and 80nt ssDNA was achieved. In order to characterize the recovery of each molecule, a mathematical model was built to simulate the separation process. In this model, several assumptions were made including a constant mass transfer coefficient, the boundary layer was ignored and convection through the membrane pores was considered minimal. The model domain is shown in Figure 7.

The sample molecules were transported by convection in the channel, whereas diffusion was considered the only transport mechanism to drive the molecules across the membrane. The following mass transport equations were formulated to describe the device.

$$\begin{cases} \frac{dC_1}{dt} = v \frac{dC_1}{dx} - \frac{K}{h} (C_1 - C_2) \\ \frac{dC_2}{dt} = v \frac{dC_2}{dx} - \frac{K}{h} (C_1 - C_2) \end{cases} \quad (4)$$

where C_1 was the sample concentration in the dialysis channel; C_2 was the sample concentration in the perfusion channel; K was the mass transfer coefficient; v was the average linear velocity in the channel; h was the height of the channel; L was the length of the channel; W was the width of the channel. The partition coefficient was assumed to be 1. R was recovery. When recovery was zero, no analyte was transported through the membrane. When recovery was one, all of the molecules were transported through the membrane. Recovery was obtained by solving the above equations.

$$R = \frac{C_2(x=L)}{C_1(x=L)} = \frac{KL}{vh + KL} = \frac{1}{\frac{Q}{KLW} + 1} \quad (5)$$

Thus the recovery is a function of value of how fast the molecules diffuse through the membrane (determined by KLW) versus how long the solution stays in the channel (determined by flow rate Q). The mass transfer coefficient K was obtained by linearly fitting the experiment data to the following equation.

$$\frac{1}{R} - 1 = \frac{1}{K} \cdot \frac{Q}{LW} \quad (6)$$

The data $\left(\frac{1}{2} - 1\right)$ can be plotted as a function of $\frac{Q}{LW}$ with the slope of the line being $\frac{1}{K}$. For example, the overall mass transfer coefficient (K) for 10 nt DNA was determined to be 6.9×10^{-4} mm/s using Figure 8 (2A). This value for K was used to calculate the theoretical recovery across a range of flow rates as shown in Figure 8(2B). Overall this strategy was successful in describing the trends observed in recovery, validating the assumptions made in developing this model.

Similarly, the mass transfer coefficients (K) were estimated to be 1.92×10^{-3} mm/s and 2.73×10^{-4} mm/s for fluorescein and 80nt ssDNA, respectively. The theoretical results are shown in Figures 8(1B) and 8(3B). For fluorescein, the model data fits the experiment data well for most flow rates. Although the model predicted the recovery trend for 80nt DNA, more deviation from the experimental data was observed.

In general our model was more successful in predicting recovery for smaller molecules such as fluorescein and 10 nt ssDNA than larger molecules such as 80nt ssDNA. There are two possible contributors to this phenomenon. The first relates to the boundary layer. As analytes cross the membrane a concentration gradient can be generated across the depth of the channel. Our model assumes constant concentration across the depth of the channels and does not account for this gradient. Molecules with higher diffusion constants equilibrate more quickly across the channel depth, decreasing the magnitude of this effect. Convection within the pores is a second potential contributor to the observed deviations. Our model assumes that diffusion is the only mechanism that contributes to transport across the membrane. However, a consequence of the counter current flow used in our device is a pressure difference across the membrane, which would be expected to generate a small convective flow. For small molecules this convective flow is small in comparison to diffusion and little deviation from the model is observed. For larger molecules, convection plays a more significant role in transport through the nanopores, leading to larger deviations from the model. In the future, the boundary layer and convection will need to be considered to extend the model to predict recovery for larger molecules or faster flow rates. Our model demonstrated that diffusion was the major mechanism of transport for the molecules and flow rates studied here, confirming the utility of our simplified model under these conditions.

CONCLUSION

We have demonstrated the fabrication and operation of a microfluidic device capable of making size based discrimination of DNA on microliter scale samples. A polycarbonate track etched membrane with 50 nm pores was sandwiched between two PDMS channels and a counter current flow strategy was adopted to maximize recovery. Trends in recovery measured at various flow rates support the premise that diffusion dominated the molecular transport in this device. Recovery increased as the linear velocity decreased. Recovery was also affected by the ionic strength of the buffer suggesting that the thickness and charge of the double layer in the pores play an important role in limiting transport. A theoretical model was built to simulate the transportation process. This model identified diffusion as the major transport mechanism for molecules through the nano pores.

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References

1. West J, Becker M, Tombrink S, Manz A. *Analytical Chemistry*. 2008; 80:4403–4419. [PubMed: 18498178]
2. Kovarik ML, Jacobson SC. *Analytical Chemistry*. 2009; 81:7133–7140. [PubMed: 19663470]
3. Di Ventra, M.; Evoy, S.; Heflin, JR. *Introduction to nanoscale science and technology*. Kluwer Academic; Boston: 2004.
4. Schoch R, Han J, Renaud P. *Reviews of Modern Physics*. 2008; 80:839–883.
5. Craighead H. *Nature*. 2006; 442:387–393. [PubMed: 16871206]
6. Jong J, Lammertink R, Wessling M. *Lab on a Chip*. 2006; 6:1125–1139. [PubMed: 16929391]
7. Lee C. *Electrophoresis*. 2001; 22:3857–3867. [PubMed: 11700714]
8. Piruska A, Branagan S, Cropek D, Sweedler J, Bohn P. *Lab on a Chip*. 2008; 8:1625–1631. [PubMed: 18813383]
9. Kim B, Yang J, Gong M, Flachsart B, Shannon M, Bohn P, Sweedler J. *Analytical Chemistry*. 2009; 81:2715–2722. [PubMed: 19271741]
10. Gong M, Kim B, Flachsart B, Shannon M, Bohn P, Sweedler J. *IEEE Sensors Journal*. 2008; 8:601–607.
11. Gong M, Flachsart B, Shannon M, Bohn P, Sweedler J. *Electrophoresis*. 2008; 29:1237–1244. [PubMed: 18288777]
12. Noblitt S, Kraly J, VanBuren J, Hering S, Collett J Jr, Henry C. *Analytical Chemistry*. 2007; 79:6249–6254. [PubMed: 17636868]
13. Long Z, Liu D, Ye N, Qin J, Lin B. *Electrophoresis*. 2006; 27:4927–4934. [PubMed: 17117457]
14. Kim B, Swearingen C, Ja-an A, Romanova E, Bohn P, Sweedler J. *J. Am. Chem. Soc.* 2007; 129:7620–7626. [PubMed: 17530757]
15. Jiang Y, Wang P, Locascio L, Lee C. *Analytical Chemistry*. 2001; 73:2048–2053. [PubMed: 11354489]
16. Lamoree M, Van der Hoeven R, Tjaden U, Van der Greef J. *Journal of Mass Spectrometry*. 1998; 33:453–460.
17. Tokuyama T, Fujii S, Sato K, Abo M, Okubo A. *Analytical Chemistry*. 2005; 77:3309–3314. [PubMed: 15889923]
18. Kurita R, Yabumoto N, Niwa O. *Biosensors and Bioelectronics*. 2006; 21:1649–1653. [PubMed: 16129595]
19. Kim M, Kim Y, Kim B. *Electrophoresis*. 2003; 24:200–206. [PubMed: 12652592]
20. Cooper J, Chen J, Li Y, Lee C. *Analytical Chemistry*. 2003; 75:1067–1074. [PubMed: 12641224]
21. Jong J, Ankone B, Lammertink R, Wessling M. *Lab on a Chip*. 2005; 5:1240–1247. [PubMed: 16234947]
22. Moskvina L, Nikitina T. *Journal of Analytical Chemistry*. 2004; 59:2–16.
23. Tulock J, Shannon M, Bohn P, Sweedler J. *Analytical Chemistry*. 2004; 76:6419–6425. [PubMed: 15516136]
24. Kuo T, Sloan L, Sweedler J, Bohn P. *Langmuir*. 2001; 17:6298–6303.
25. Nishizawa M, Menon V, Martin C. *Science*. 1995; 268:700–702. [PubMed: 17832383]
26. Zhou K, Kovarik M, Jacobson S. *J. Am. Chem. Soc.* 2008; 130:8614–8616. [PubMed: 18549214]
27. Hou Z, Abbott N, Stroeve P. *Langmuir*. 2000; 16:2401–2404.
28. Kovarik M, Jacobson S. *Analytical Chemistry*. 2008; 80:657–664. [PubMed: 18179245]
29. Kuo T, Cannon D, Shannon M, Bohn P, Sweedler J. *Sensors & Actuators: A. Physical*. 2003; 102:223–233.

30. Cannon D Jr, Kuo T, Bohn P, Sweedler J. *Analytical Chemistry*. 2003; 75:2224–2230. [PubMed: 12918959]
31. Kuo T, Cannon D Jr, Chen Y, Tulock J, Shannon M, Sweedler J, Bohn P. *Analytical Chemistry*. 2003; 75:1861–1867. [PubMed: 12713044]
32. Hulteen J, Jirage K, Martin C. J. *Am. Chem. Soc.* 1998; 120:6603–6604.
33. Gatimu E, Sweedler J, Bohn P. *The Analyst*. 2006; 131:705–709. [PubMed: 16732357]
34. Jirage K, Hulteen J, Martin C. *Science*. 1997; 278:655–658.
35. Schmuhl R, van den Berg A, Blank D, ten Elshof J. *Angewandte Chemie International Edition*. 2006; 45:3341–3345.
36. Osborn JL, Lutz B, Fu E, Kauffman P, Stevens DY, Yager P. *Lab on a Chip*. 2010; 10:2659–2665. [PubMed: 20680208]
37. Weigl BH, Yager P. *Science*. 1999; 283:346–347.
38. Helton KL, Yager P. *Lab on a Chip*. 2007; 7:1581–1588. [PubMed: 17960289]
39. Brody JP, Yager P. *Sensors and Actuators A: Physical*. 1997; 58:13–18.
40. Brody JP, Yager P, Goldstein RE, Austin RH. *Biophysical journal*. 1996; 71:3430–3441. [PubMed: 8968612]
41. Liu, D. *Handbook of nucleic acid purification*. CRC Press; Boca Raton: 2009. Editon edn.
42. Heller, M.; Guttman, A. *Integrated microfabricated biodevices*. Ramanujam, R.; Sacks, W.; Kang, J., editors. Marcel Dekker, Inc.; New York: 2002. p. 71-84. Editon edn.
43. McDonald J, Duffy D, Anderson J, Chiu D, Wu H, Schueller O, Whitesides G. *Electrophoresis*. 2000; 21:27–40. [PubMed: 10634468]
44. Jo B, Van Lerberghe L, Motsegood K, Beebe D. *Journal of microelectromechanical systems*. 2000; 9:76–81.
45. Truskey, G.; Yuan, F.; Katz, D. *Transport Phenomena in Biological Systems*. Pearson Prentice Hall; Upper Saddle River, N.J.: 2004. p. 263-265. Editon edn.
46. Truskey, G.; Yuan, F.; Katz, D. *Transport Phenomena in Biological Systems*. Pearson Prentice Hall; Upper Saddle River, N.J.: 2004. p. 375-384. Editon edn.
47. Skogestad, S. *Chemical and energy process engineering*. CRC Press; Boca Raton, FL: 2009. Editon edn.
48. Baehr, HD. *Heat and mass transfer*. Springer; Berlin, New York: 1998. Editon edn.
49. Frost N, Bowser M. *Lab Chip*. 2010
50. Kratky O, Porod G. *Rec Trav Chim Pays-Bas*. 1949; 68:1106–1122.
51. Teraoka, I. *Polymer solutions : an introduction to physical properties*. Wiley; New York: 2002.
52. Record M Jr, Anderson C, Lohman T. *Quarterly reviews of Biophysics*. 1978; 11:103–178. [PubMed: 353875]
53. Tinland B, Pluen A, Sturm J, Weill G. *Macromolecules*. 1997; 30:5763–5765.
54. Salieb-Beugelaar GB, Dorfman KD, Vvan den Berg A, Eijkel JCT. *Lab on a Chip*. 2009; 9:2508–2523. [PubMed: 19680576]
55. Lettmann C, Mockel D, Staude E. *Journal of Membrane Science*. 1999; 159:243–251.
56. Gasparac R, Mitchell DT, Martin CR. *Electrochimica Acta*. 2004; 49:847–850.
57. Huisman IH, Pradanos P, Calvo JI, Hernandez A. *Journal of Membrane Science*. 2000; 178:79–92.
58. Bard, AJ. *Electrochemical methods: fundamentals and applications*. Wiley; New York: 2001. Editon edn.
59. Kemery PJ, Steehler JK, Bohn PW. *Langmuir*. 1998; 14:2884–2889.
60. Martin CR, Nishizawa M, Jirage K, Kang M, Lee SB. *Advanced Materials*. 2001; 13:1351–1362.
61. Schmuhl R, Sekulic J, Roy Chowdhury S, van Rijn CJM, Keizer K, van den Berg A, ten Elshof JE, Blank DHA. *Advanced Materials*. 2004; 16:900–904.
62. Plecis A, Schoch RB, Renaud P. *Nano Letters*. 2005; 5:1147–1155. [PubMed: 15943459]
63. Rant U, Arinaga K, Fujiwara T, Fujita S, Tornow M, Yokoyama N, Abstreiter G. *Biophysical journal*. 2003; 85:3858–3864. [PubMed: 14645075]

Figure 1.

(A) Schematic of the counter current dialysis device. Two PDMS channels are separated by a track etched polycarbonate membrane (50 nm pore size). Transport occurs in areas where the channels overlap. A counter current geometry was employed to maximize recovery. (B) Side view illustrating the counter current geometry and transport across the membrane. Not to scale.

Figure 2.
A) Schematic of the fabrication procedure of the microfluidic counter current dialysis device. B) Image of the fully assembled device.

Figure 3.

Fluorescence image of the membrane region of the counter current dialysis device when perfused with (A) fluorescein, (B) 10 nt DNA and (C) 80 nt DNA. Analyte enters in the lower left channel and exits in the upper right. Buffer enters in the lower right channel and exits in the upper left.

Figure 4. Plot of fluorescence intensity vs. distance along the channel for fluorescein (circles), 10 nt ssDNA (squares) and 80 nt ssDNA (diamonds) at 0.5 mm/s in a buffer containing 2 mM NaH_2PO_4 , 50 mM NaCl and 300 μM Triton X-100.

Figure 5.

A) Plot of recovery vs. buffer flow rate for fluorescein (circles), 10nt ssDNA (squares) and 80nt ssDNA (diamonds). Error bars are the standard deviation of three replicate measurements. B) Effect of buffer velocity on the selectivity between 10nt and 80nt ssDNA. The buffer contained 2 mM NaH_2PO_4 , 300 μM Triton X-100, and 50 mM NaCl.

Figure 6.

(A) Plot of recovery vs. NaCl concentration for fluorescein (circles), 10 nt DNA (squares) and 80 nt DNA (diamonds). (B) Plot of recovery normalized to 0 mM NaCl vs. NaCl concentration for fluorescein, 10 nt DNA and 80 nt DNA. Data was recorded in triplicate. Error bars are smaller than the symbols used in the plots. The buffer contained 2mM NaH_2PO_4 , 300 μM Triton X-100, and 0 – 200 mM NaCl.

Figure 7. Schematic of the model. L is the length of the channel, h is the height of the channel. C_1 is the sample concentration in the dialysis channel. C_2 is the sample concentration in the perfusion channel. At $x=0$, $C_2=0$. At $x=L$, $C_1=1\mu\text{M}$.

Figure 8.

Top: Plot of $(1/R-1)$ as a function of $\frac{Q}{LW}$ for fluorescein(1A), 10nt DNA (2A) and 80nt DNA(3A). The slope of each curve is $\frac{1}{K}$. Bottom: Plot of recovery vs. flow rate for fluorescein(1B), 10 nt DNA(2B) and 80nt DNA(3B) comparing experimental data (squares) vs. values predicted by the model(diamonds).