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Capillary Electrophoresis Separation in the Presence of an Immiscible Boundary for Droplet Analysis

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Abstract

This paper demonstrates the ability to use capillary-electrophoresis (CE) separation coupled with laser-induced fluorescence for analyzing the contents of single femtoliter-volume aqueous droplets. A single droplet was formed using a T-channel (3 μm wide by 3 μm tall) connected to microinjectors, then the droplet was fluidically moved to an immiscible boundary that isolates the CE channel (50 μm wide by 50 μm tall) from the droplet generation region. Fusion of the aqueous droplet with the immiscible boundary effectively injects the droplet content into the separation channel. In addition to injecting the contents of droplets, we found aqueous samples can be introduced directly into the separation channel by reversibly penetrating and re-sealing the immiscible partition. Because droplet generation in channels requires hydrophobic surfaces, we have also investigated the advantages to using all hydrophobic channels versus channel systems with patterned hydrophobic and hydrophilic regions. To fabricate devices with patterned surface chemistry, we have developed a simple strategy based on differential wetting to deposit selectively a hydrophilic polymer (polystyrene sulfonate) onto desired regions of the microfluidic chip. Finally, we applied our device to the separation of a simple mixture of fluorescein-labeled amino acids contained within a ~ 10 fL droplet.

INTRODUCTION

Droplet based microfluidic systems have seen increased attention as an attractive platform for manipulating and analyzing discrete biological samples, owing to the small size and confined volume of droplets and the possibility to manipulate them in a combinatorial fashion.^{1–18} Recent applications of droplets include protein crystallization, protein expression, and single-cell enzymatic assays.^{3–5} Small aqueous droplets with femtoliter volumes in particular offer an attractive platform for single-cell and single-organelle analysis,^{2, 3} given the low copy number of proteins and molecules present in such biological samples.

Despite the numerous methods developed and employed for generating and manipulating droplets in microfluidic systems,^{1, 6–8, 12, 14, 17, 18} there is a lack of strategies for analyzing the contents of droplets. Most methods rely on microscopic imaging to visualize droplets or to read-out a fluorescence signal from the droplets. This paucity of read-out methods originates both from the limited samples present in small droplets and from the inability to “release” controllably the contents of the droplet back into aqueous solution for detailed analysis. To address this shortcoming, this paper describes the use of capillary electrophoresis (CE) to separate the contents of individual droplets after the contents have been emptied into a separation channel; the separated components are detected by laser-induced fluorescence (LIF).

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Our approach relies on the use of an immiscible-fluid partition to divide the portion of the fluidic system where droplet generation and manipulation occurs from the part where CE separation takes place. The contents of the droplet are emptied into the CE separation channel by fusing the droplet with the immiscible boundary. This approach restricts the use of techniques for droplet generation and manipulation to ones that do not fluidically perturb the immiscible partition. Practically, this requirement means that the formation and the transport of droplets to the immiscible boundary should produce low fluid flow or movement, and that the droplet generation and CE separation is fluidically decoupled. This fact may make it difficult to integrate continuous droplet-production schemes⁴⁻¹⁶ with our current approach of droplet analysis with CE-LIF.

Another integration issue is that the generation of aqueous droplets (especially small droplets with femtoliter volumes) requires hydrophobic walls, but CE separation benefits from hydrophilic channels. Most droplet generation work so far has used microchannels fabricated in poly(dimethylsiloxane) (PDMS),^{1-8, 10-18} which is hydrophobic in its native state. Here we have studied both the issues of performing CE separation in hydrophobic PDMS channels and have developed a new strategy based on differential wetting to pattern selectively the CE separation channel with charged polymers but not the droplet generation and manipulation regions of the system.

EXPERIMENTAL SECTION

Fabrication of microfluidic chip

Silicon masters were fabricated using photolithography methods as described in detail elsewhere.^{19, 20} Briefly, SU-8 (Microchem, Newton, MA) was spin coated onto three-inch silicon wafers and exposed to UV radiation to generate the desired pattern before development. For masters that required two layers of features, two-step photolithography was used in which the master was spin-coated with a second thicker layer of SU-8 after exposure and development of the first thin layer. Features in the second layer were aligned to features on the first layer with a mask aligner. The T-channel employed in our device was 3 μm wide and 3 μm tall. Adjoining channels (sample, oil, and separation channels) were all 50 μm by 50 μm in cross section (Figure 1A). After the second layer was exposed and developed, the wafers were treated with tridecafluoro-1,1,2,2-tetrahydrooctyl-1-silane (Sigma-Aldrich, St. Louis, MO) overnight to facilitate the release of the mold from the master.

Microchannels were formed by casting poly(dimethylsiloxane) (PDMS) on silicon masters.^{19, 21, 22} After treatment with oxygen plasma, the microchannels were sealed to coverslips (Corning) that were spin-coated with PDMS. The sealed device was placed in a 120 °C oven to ensure the microchannel reverted to its native hydrophobic character. Spincoating the coverslip with PDMS was necessary since formation of discrete, small aqueous droplets required four hydrophobic walls to prevent the wetting of the aqueous phase onto the channel wall.³ Access ports for our microchannels were created using a blunt 16 gauge needle. Polyethylene tubing (PE100) was inserted into the access ports and the ends were fitted to a homemade pressure control system consisting of a syringe with an attached micrometer (Newport, Irvine, CA).

Patterning hydrophilic patches on native PDMS

Microfluidic chips were selectively patterned by applying negative pressure to one end (e.g. waste reservoir) of the separation channel to introduce polystyrene sulfonate (PSS) (Sigma-Aldrich, St. Louis, MO) into the channel from the opposite end (e.g. sample reservoir) of the channel. Alternatively, positive pressure was applied while observing filling of the channel under a microscope (TE 300, Nikon, Tokyo, Japan). The filled channels were allowed to sit

for a minimum of one hour at a PSS concentration of 1mg/mL before the PSS solution was removed. Selective patterning of the separation microchannel was observed by flowing Alexa488-tagged goat anti-mouse IgG at a concentration of 2 mg/mL (Invitrogen, A-11001, Carlsbad, CA) through the separation channel.

Measurement of electroosmotic flow (EOF)

EOF was characterized by the current monitoring method.^{23, 24} Straight channels of 4 cm long with a height of 50 μm and width of 50 μm were used to determine EOF values. EOF was generated with a high-voltage power supply (Stanford Research Systems, PS500, Sunnyvale, CA). Borate buffer of 10 mM and 20 mM concentrations were used as exchange buffers to determine EOF rates as has been detailed previously.²⁰ Linear flow velocities were calculated from the time required for complete buffer exchange.

Generation and separation of droplet encapsulated analytes

Fluorescently labeled amino acids (glycine, glutamic acid, aspartic acid) (Sigma-Aldrich, St. Louis, MO) were prepared by mixing 100 μL of 1 mM fluorescein isothiocyanate (FITC) in DMSO with 1 mL of 1 mM of each amino acid. Samples were then diluted to 20 μM of each amino acid in a 20 mM borate buffer solution. The immiscible phase used in the microchannel was AR 20 silicon oil (Fluka, Buchs, Switzerland). To ensure that oil did not wet the separation channel, filling of each channel was monitored under a microscope.

To fill the chip for each experiment, oil was injected into the microfluidic chip first, followed by running buffer consisting of 20 mM borate (pH $\sim$ 9). The amino acid sample to be separated was injected last. Generation of amino-acid containing droplets was achieved by actuating a micrometer attached to homemade microinjectors.¹ Transport of droplets to the separation channel was achieved by applying gentle positive pressure to a microinjector connected to the oil line, which caused a discrete droplet to move from the droplet-generation channels to the separation channel. When a droplet fused to the separation channel, voltage was applied to generate EOF and to initiate separation.

Optical setup

Fluorescence images were obtained by epi-illumination of the separation and adjoining T-channels with an Ar^+ laser, and recorded with a CCD camera (Cohu 4910, San Diego, CA). Figure 1B illustrates the optical setup used for excitation and detection of separated amino acids. Briefly, an Ar^+ laser (wavelength at 488 nm, Spectra-Physics Lasers, Mountain View, CA) was used to excite fluorescent samples. An avalanche photodiode (APD) (Perkin Elmer, Wellesley, MA) was used for detection. The APD was attached to an MCS card (Ortec, Oak Ridge, TN), which recorded the number of photons emitted from the excited FITC labeled amino acids as a function of time.

RESULTS AND DISCUSSION

Sample injection

The first task in integrating droplet generation and CE separation is to be able to introduce the droplet content into the CE channel. To accomplish this, we have taken advantage of immiscible fluids, required for droplet generation, to decouple droplet generation from on-chip CE. Here we found two methods by which the sample can be injected into the CE channel, which we termed: (1) Droplet injection, and (2) Direct injection. Both droplet and direct injection utilized a T-channel to interface with the separation channel (Fig 1A). In our design, the oil filled T-junction acted as a boundary between the separation channel and the sample.

Droplet injection—Droplet injection is based on the fusion of the aqueous droplet with the immiscible boundary. In this case, we employed a T-junction to generate a single aqueous droplet, then fluidically moved the droplet to the immiscible boundary where it fused with the boundary. Although the droplet generation and transport we demonstrated here is simple, more complex droplet manipulation schemes can be incorporated, provided that they do not perturb the immiscible partition.

Figure 2 shows the generation of a discrete femtoliter-volume droplet at the T-junction (Fig 2A-2C), and the transport (Fig 2D, 2E) and subsequent fusion (Fig 2F) of the droplet with the immiscible boundary; the large channel on the other side of the boundary (partially seen at the top of the image) is the CE separation channel. This process required precise pressure manipulation, which was achieved with home-built microinjectors.¹ The droplet was generated by applying slight positive pressure to both the aqueous-phase and oil-phase channels. Additional pressure applied to the oil-phase channel moved the droplet to the immiscible partition and caused the fusion of the droplet with the oil boundary and the mixing of the droplet contents with the borate separation buffer contained in the CE channel.

Direct injection—Direct injection is based on the re-sealing capability of the immiscible boundary that separates the aqueous sample portion of the chip from the separation channel. Here no droplet is formed, but rather the aqueous sample is brought into direct contact with the buffer solution in the CE channel by breaking the thin oil layer that formed the partition between the two aqueous solutions. Injecting sample directly into the separation channel is similar in methodology to droplet generation. During direct injection, however, positive pressure applied to the oil phase was insufficient to overcome the Laplace pressure to generate a droplet, but was sufficient to divert the aqueous phase to the separation channel (Fig 3B). Because the T-channel is hydrophobic, the aqueous phase does not wet the walls of the channel; when slight negative pressure is applied to the sample reservoir, the aqueous phase retreats to its reservoir accompanied by a corresponding re-sealing of the immiscible boundary.

Typical sample injection schemes for chip-based CE require programming and the balancing of multiple applied voltages.²⁵⁻³² The direct injection technique is simple and allows for temporal control of sample introduction. Sample can be injected with defined volumes based on the flow rate of the aqueous sample phase and the duration of the applied pressure. We have taken advantage of this fact to illustrate the ease by which multiple injections at chosen intervals can be made (Fig 3). Figure 3C shows the aqueous sample being held in close proximity to the buffer solution in the separation channel as well as the oil layer that segregates the two solutions. The location of the sample/oil interface can be maintained and varied precisely, and the presence of the oil layer prevents any sample leakage into the separation channel. Figures 3D and 3F show two repeat injections that were spaced by 30 s. In between injections, the interface was positioned close to the separation channel (Fig 3E). In addition to the ease of performing multiple injections and the precise control this technique offers, the presence of the immiscible layer also prevents deleterious bleeding of sample into the CE channel during separation.

Capillary electrophoresis separation in hydrophobic and hydrophilic channels

To integrate droplet generation and CE separation, we had to reconcile the need for hydrophobic channels in droplet formation and the preference for hydrophilic channels in CE separation. Because native hydrophobic PDMS does support electroosmotic flow (EOF) and has been used in CE,^{25, 26, 31, 33} we studied the pros and cons for using hydrophobic PDMS channels directly for use in CE separation.

Hydrophobic channels—We formed our channels by sealing two pieces of PDMS together after oxidizing the surface of each piece under an oxygen plasma. Because this oxidized PDMS

surface is hydrophilic, we baked our chips at 120 °C for 48 hours to revert the surface back to its native hydrophobic character.^{3, 34} We estimate the charge density of the hydrophobic PDMS surface after this treatment to be $\sim 7 \times 10^{-3} \text{ C/m}^2$, based on our EOF measurements. This value is about half of what has been reported previously for hydrophilic PDMS channels ($1.3 \times 10^{-2} \text{ C/m}^2$).³² Although the EOF rate is reduced in hydrophobic PDMS channels, it is sufficient for CE separation. Figure 4A shows the electropherogram obtained using a hydrophobic PDMS channel after direct injection of a mixture of three fluorescein-tagged amino acids. The sample retreated to its reservoir during separation; the inset in Fig 4A shows the position of the sample, oil, and separation buffer during separation.

An important advantage of using hydrophobic channel for CE separation is the ease by which the microfluidic system can be fabricated, which is especially critical when planning complex upstream and downstream processes, such as chemical and biological assays based on discrete generation and encapsulation of single cells and organelles. Integration of droplet fusion to perform chemical and biological assays in microfluidic chips can be challenging, especially in the small femto-liter volumes required for analyzing individual organelles considering their small volumes and low protein count.¹⁻³ The main disadvantage is the need to prevent any oil from entering the separation channel during the filling of the microfluidic system and the subsequent generation and manipulation of the droplets. The hydrophobic nature of the PDMS surface causes the preferential wetting of oil (as opposed to water) onto the walls of the channel. As a result, any oil that entered the CE channel and contacted the surface will remain wetted to the surface, in which case no measurable EOF is present (Table 1). In addition, we observed excess oil in the separation channel could cause plug formation, which resulted in shorting of our CE channel and dielectric breakdown of the PDMS. The practical consequence is that oil flow must be precisely controlled both during initial filling of the channels and the subsequent experiments. While our current method that uses microinjectors to control pressure and to position the immiscible interface is good, we nevertheless had issues with achieving good repeatability in our experiments with hydrophobic CE channels. If oil flow can be regulated more accurately, however, hydrophobic channels may be desirable for certain applications given the ease of fabrication.

Hydrophilic channels—The advantages to using hydrophilic channels for CE are the increase in EOF strength, the reduced wetting of oil on the channel walls, and the enhanced stability of the oil/aqueous interface at the entrance to the CE channel. To generate hydrophilic surfaces in the separation channel, we selectively patterned the CE channel with the polyelectrolyte polystyrene sulfonate (PSS). Polyelectrolytes have been employed to modify the hydrophilicity of surfaces of microchannels fabricated in PDMS and in other common microchip materials.^{35, 36} Figure 4B shows the electropherogram of the same mixture of fluorescein-tagged amino acids as in Fig 4A separated in a PSS coated channel after droplet injection. Because of the smaller sample volume of the injected droplet, the separation distance was reduced from 3.2 cm to 2 cm to allow for faster separations and thus minimize the amount of signal lost due to diffusion. The signal we were able to detect decreased as anticipated, due to the significantly reduced amount of sample contained in the small femto-liter droplet ($\sim 10 \text{ fL}$). This volume is much smaller than the pico-liter (or greater) volume introduced into the separation channel during direct injection.

Table 1 summarizes the EOF properties of the five types of channel surfaces – hydrophilic PDMS, hydrophobic PDMS, hydrophobic PDMS that has been exposed to oil, PSS treated PDMS, and PSS treated PDMS exposed to oil – we encountered in our experiments; the results illustrate the advantage of using hydrophilic channels for CE separation. Hydrophilic PDMS channels, obtained by oxidation in an oxygen plasma, exhibited the fastest EOF ($6.54 \times 10^{-4} \text{ cm}^2/\text{Vs}$). The percentage values listed in Table 1 for the other four types of surfaces were normalized against this EOF rate of $6.54 \times 10^{-4} \text{ cm}^2/\text{Vs}$. For example, hydrophobic and PSS

treated channels had EOF rates that were 63 % and 95 % of the EOF observed in plasma-treated hydrophilic channels. To determine experimentally if exposure of the channel surface to silicon oil affected the rate of EOF, we flushed the channel with silicon oil followed by thorough perfusion of the channel with aqueous buffer. After this treatment, EOF in PSS coated channels decreased from 95 % to 76 %. In contrast, we were unable to detect any EOF in hydrophobic channels after this treatment, which explains the detrimental effect of inadvertent introduction of oil into the hydrophobic separation channel. PSS coated channels, therefore, have the advantages of both preventing oil from entering the CE separation channel and retaining EOF after transient exposure of the channel surface to oil.

Selective patterning of native PDMS

The main disadvantage to using hydrophilic CE channels is the need to pattern selectively hydrophobic and hydrophilic regions of the chip, which potentially can increase greatly the complexity of chip fabrication and preparation. To address this issue, we have incorporated a simple selective patterning procedure based on the difference in Laplace pressure between channels of different cross sectional dimensions.³⁷ Here the entire device initially had hydrophobic surfaces.

Laplace pressure (P_L) in a square-like channel can be estimated to be:

$$P_L \approx 2\gamma \cos \theta / r_e \quad (1)$$

where γ is the surface tension of the aqueous phase, θ is the contact angle of the aqueous phase on hydrophobic PDMS, and $r_e = aa / (a + a) = a / 2$ is the equivalent radius calculated from the side length (a) of the cross sectional area of the channel.³⁸ For two channels with different side lengths:

$$\frac{P_{L1}}{P_{L2}} \approx \frac{r_{e2}}{r_{e1}} = \frac{a_2}{a_1} \quad (2)$$

The side length of our small T-channel (a_1) is 3 μm and the side length of our separation channel (a_2) is 50 μm . Based on Equation (2), $P_{L1} / P_{L2} \approx 17$, that is, the Laplace pressure of the small T-channel is about 17 times that of the separation channel. The much larger Laplace pressure in the small T-channel thus provides resistance and prevents aqueous solution from entering. To ensure that the aqueous phase stayed inside the separation channel, a slight positive or negative pressure was applied to the separation channel to induce the aqueous phase to flow at a slow rate.

Figure 5A schematically depicts this process. Figure 5D is an image of the channel in its initial condition before the introduction of any fluid into the system. Figure 5E shows the wetting of the large separation channel by an aqueous solution that contained Alexa488-tagged fluorescent IgG antibodies; the arrow points to the air/water interface and clearly shows that the small hydrophobic T-channel was not wetted by the aqueous solution. After removal of the aqueous solution, we imaged our chip with epi-fluorescence; figure 5F shows that the dye-tagged antibody coated the large separation channel but did not enter the smaller T-channel.

Formation of a stable immiscible boundary at the hydrophobic/hydrophilic interface—Because the surfaces in the separation channel were hydrophilic while the surfaces in the small T-channel were hydrophobic, the oil phase preferentially wet the walls of the T-junction where the two channels met and did not wet the walls of the separation channel. As a result, samples in the aqueous droplet could be injected into the separation channel with little or no oil accompanying it. The difference in interfacial tensions between PSS treated and untreated microfluidic chips can be approximated using Young's equation.³⁹ The force balance in the horizontal direction where oil enters the separation channel is (Figure 5B-5C):

$$\gamma_{SO} - \gamma_{SW} = \gamma_{OW} \cos \theta_{OW} \quad (3)$$

where γ_{SO} is the solid–oil interfacial tension, γ_{SW} is the solid–water interfacial tension, γ_{OW} is the oil–water interfacial tension, and θ_{OW} is the contact angle of the oil–water interface. This equation holds true for systems in a state of equilibrium. Although in our system it was difficult to achieve true equilibrium, the equation was used to obtain a qualitative explanation of the difference in interfacial energy between PSS treated and untreated microfluidic chips.

Figure 5B schematically illustrates the higher degree of curvature between oil and water that was observed in PSS treated microfluidic systems. PSS treatment led to high interfacial tension between the oil and aqueous phases and thus prevented oil from wetting the CE channel. In contrast, the interfacial tension between the oil and aqueous phases is low in untreated hydrophobic channels (Figure 5C). Even in microfluidic chips selectively patterned with PSS, however, we observed increased oil wetting at the entrance to the CE channel when the interface was repeatedly (> 3 times) moved in and out at the junction. This observation implies that PSS can be removed to some extent from the PDMS wall with repeated contact from the oil phase, which is consistent with the decrease in EOF in PSS coated channels that have been exposed to oil (Table 1).

Based on the values of the interfacial tensions between silicone oil and water reported in the literature,^{39, 40} we estimate the interfacial tension between silicone oil AR 20 and water (γ_{OW}) to be 40 mN/m. In the case of the PSS coated separation channel (Fig 5B), we measured the contact angle between the oil and water phase to be ~ 65 degrees. According to Equation (3), $\gamma_{SO} - \gamma_{SW} = 17$ mN/m; this force difference has a direction pointing toward the oil phase, and thus holds the oil together and prevents oil from spreading over the water-wetted PSS coated surface. Thus, the oil–water interface at the entrance to the separation channel had barrier-like properties to pressure exerted from the continuous oil phase.

When the PDMS separation channel was not coated with PSS (Fig 5C), the dynamic contact angle between the oil and water phases was ~ 150 degrees, which yields $\gamma_{SO} - \gamma_{SW} = -35$ mN/m. This force difference has a direction pointing away from the oil phase, and thus drives oil to wet and spread over the hydrophobic PDMS surface. The displacement of water by oil at the channel surface is thus energetically favorable, which made wetting of the separation channel by oil difficult to prevent even with good pressure control. This fact also explains the inability to recover EOF once the hydrophobic channel surfaces have been exposed to oil.

CONCLUSION

In summary, we have described a method for integrating droplet generation with CE separation. This method relies on the ability to perform CE in the presence of an immiscible boundary. The presence of the immiscible partition effectively divided our microfluidic system into a droplet generation and manipulation region and a CE-LIF based droplet analysis region. In developing this platform, we discovered the possibility of directly injecting samples into the CE channel by reversibly breaking and re-sealing the immiscible boundary. We found this method to be easy to control and implement, characteristics that may make it a useful technique in general outside the context of droplet analysis.

In the process of integrating droplet generation and CE separation, we have investigated the advantages and drawbacks of using hydrophobic and hydrophilic separation channels. The use of hydrophobic channels does not require patterning the surface chemistry of the microfluidic chip, but in exchange requires a very regulated and precise method to control the oil flow in the system. Hydrophilic channels, on the other hand, can be potentially more difficult to fabricate because it requires selective surface patterning of the channel system, but hydrophilic

CE channels both prevent the entrance of oil into the separation channel and retain EOF in the case when oil does inadvertently wet the hydrophilic surface. To overcome the difficulty of selectively patterning the channels of the microfluidic system, we reported a simple surface patterning technique that relies on differential wetting of channels having different cross sectional dimensions. The ability to use CE-LIF for droplet analysis greatly expands the current repertoire of techniques used to study the chemistries that occur within aqueous droplets.

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Figure 1. Schematic showing the design of our microfluidic chip and an overview of our experimental setup

(A) The separation channel was 50 μm by 50 μm in cross section; the small T-channel where the sample and oil met had a height of 3 μm and a width of 3 μm . The inset highlights the difference in dimension between these two channels. (B) Our experiments were performed on an inverted microscope using an Ar⁺ laser for exciting fluorescence; a CCD camera was used for wide-field imaging and an avalanche photodiode (APD) for point detection. A set of home-built microinjectors and a high-voltage power supply were connected to the microfluidic chip for droplet generation and capillary-electrophoresis separation.

Figure 2.

A sequence of images showing the generation and transport of a single aqueous droplet to the separation channel. Pressure was first applied to the sample-containing aqueous channel, which caused the aqueous interface to protrude into the channel that contained the continuous phase (A, B). Subsequent flow of oil then sheared off an aqueous droplet (C) and moved the droplet to the immiscible boundary (D, E), where it was injected into the separation channel (F) before a high-voltage was applied to separate the contents of the droplet.

Figure 3. Direct injection of aqueous sample into the separation channel

(A) In direct injection, the T-channel was first filled with oil. (B) Pressure applied to the aqueous sample caused displacement of the oil, and brought the sample into direct contact with the separation buffer. (C-F) shows two cycles of direction injection. Note any retraction of the sample solution led to re-sealing of the immiscible interface and re-establishment of the immiscible boundary (C, E). The sample solution contained fluorescein-labeled amino acids and was visualized under bright-field and epi illumination. Plumes of injected sample are seen in B, D, and F.

Figure 4. Electropherogram showing the separation of fluorescein isothiocyanate (FITC) and FITC-labeled glycine, glutamate, and aspartate
 (A) Separation was performed in a hydrophobic PDMS separation channel after direct injection; the applied field strength was 650 V/cm and the separation distance was 3.2 cm. During separation, the sample-oil interface was retreated back to the sample channel (inset).
 (B) Separation of the contents of a single 10 fL volume droplet, which was performed in a polystyrene sulfonate coated separation channel; the applied voltage was 500 V/cm and the separation distance was 2 cm. The sample-oil interface has retracted during the separation as in (A). The Y-axis in (A) and (B) have different scales.

Figure 5. Selective patterning of microchannels and the formation of a stable immiscible boundary (A) Schematic showing the selective wetting of the large separation channel by a polyelectrolyte-containing aqueous solution; all channel surfaces initially were hydrophobic. (B, C) Under positive pressure from the oil phase, the polystyrene sulfonate patterned channel exhibited a high degree of curvature (B), but in untreated all-hydrophobic channels, the oil wetted the walls of the separation channel that resulted in a low degree of curvature (C). (D-F) A sequence of images that show the selective patterning of the separation channel. The channels initially were all empty (in contact with air) and hydrophobic (D). A solution containing fluorescently-labeled IgG antibodies were then flowed through the separation channel; note the formation of the stable air-water interface (arrow) at the entrance to the T-channel (E). The fluorescent antibodies were laid down only on the surfaces in the separation channel, as visualized by epi-fluorescence after the solution has been removed (F).

Table 1

Experimentally determined EOF values for the five types of channel surfaces — hydrophilic PDMS (Hydrophilic), hydrophobic PDMS (Hydrophobic), hydrophobic PDMS that has been exposed to oil (Hydrophobic / Oil), polystyrene sulfonate (PSS) coated PDMS (PSS treated), and PSS coated PDMS exposed to oil (PSS treated / Oil).

PDMS Sample	cm^2/Vs	(EOF as a percentage of the value in hydrophilic channel)
Hydrophilic	6.5×10^{-4}	100%
Hydrophobic	4.1×10^{-4}	63%
Hydrophobic / Oil	-	-
PSS treated	6.2×10^{-4}	95%
PSS treated / Oil	5.0×10^{-4}	75%