Abstract

Electrophoresis devices have been microfabricated on polycarbonate substrates. A simple, 4 lithographic step, batch micromachining process was developed. Surface micromachined channels up to 2.7 cm in length have been fabricated and tested. The presence of access holes for the sacrificial release have been modeled using conformal transformations and predicted to contribute less than 10% of the total band variance. Experimental observations suggest this to be even lower. High-efficiency separations of dsDNA have been demonstrated in these devices. Separations on the order of 100,000 theoretical plates have been achieved. The use of polycarbonate reduces the substrate cost substantially and such devices have potential as disposable, single-use, electrophoresis chips for a variety of separation applications.

Introduction

Microfabrication of capillary electrophoresis devices has shown distinct promise in recent years. High efficiency and rapid separation have been shown in a variety of applications[1, 2, 3, 4, 5, 6]. The size miniaturization and potential for parallel processing make these devices attractive for high throughput applications.

While a variety of fabrication technologies have developed over recent years for these devices, most are fabricated on glass substrates. A few groups have constructed devices from polymer substrates. Aclara biosciences has shown a variety of separations and chemical assays on injection-molded poly(methyl methacrylate) (PMMA) substrates[7, 8]. Effenhauser et al.[9] has demonstrated dsDNA separations in casted silicone structures. A number of other groups are investigating plastic technologies for microfluidic devices on polycarbonate[10], PMMA[11], teflon[12], and poly(ethylene terephthalate) (PET)[13]. Plastic substrates are a low-cost alternative to glass devices that hold potential for disposable analysis tools.
Nearly all electrophoresis devices have been fabricated by bulk micromachining techniques. Bulk micromachining refers to the machining of the substrate material itself. In the case of electrophoresis devices, channels are etched into glass substrates. These techniques rely on processes such as assembling, drilling, wafer bonding, and using epoxies to fabricate a device that is ready to perform separations. Often these techniques cannot be performed in a batch fashion where many devices are processed at one time. Recently, a surface micromachining process using photoresist sacrificial layers has been developed that is capable of producing channels up to 1.5 cm in length[14, 15, 16]. By using surface micromachining techniques, electrophoresis devices can be fabricated in a batch fashion, eliminating potentially costly steps of assembly, and drilling. This paper presents the construction and testing of capillary electrophoresis devices on polycarbonate substrates using this method.

**Surface micromachined CE devices**

With surface micromachining methods, channel structures are built on top of sacrificial layers. The sacrificial layers are then removed by etching or dissolution processes leaving a free standing structure. Using photoresist as a sacrificial layer, channel lengths of 1.5 cm have been constructed. For longer channels, however, release times are too long for practical fabrication. For the case of silicon dioxide sacrificial layers, the release process has been studied in detail[17, 18]. For long channel lengths and fast etch rates, the problem is reduced to a one dimensional, single phase stefan problem where the release time is found to be proportional to the square of the distance:

$$t \propto \delta^2 D^{-1}$$

where $\delta$ is the channel length and $D$ is the diffusion coefficient. Therefore the release time for long channels such as 3 cm would be nearly 6 days. When long channel lengths are necessary for electrophoresis, access holes along the channel length must be made that allow the release agent to penetrate into the channel structure. Figure 1 shows a schematic of these types of holes. In the case of photoresist sacrificial layers, holes can be placed at a linear density of 1 per centimeter of length. This hole is open to the atmosphere during the separation. Therefore small convective flows are expected along with a local distortion of the electric field.

The electric field distribution in the access hole region can be found using the method of conformal transformations. Holzwarth used this method to evaluate the effect of step height changes on electrophoresis band shapes in slab gels[19]. A conformal transformation has been used that transforms the wall of the channel with the access hole to the real axis of another coordinate system. The electric field
is then transformed into a uniform electric field in the new coordinate system, and the problem is solved. Assuming the absence of electroosmotic flow, the migration time through the access hole region can be found by numerical integration along a constant stream line according to the equation:

\[ t = \frac{1}{\mu} \int_{V_i}^{V_f} \frac{1}{E_x^2 + E_y^2} dV \]  

(2)

The migration times of different streamlines across the channel width can be used to approximately determine the distortion introduced by the holes. Figure 2 shows the equipotentials and streamlines for half of the channel. Particles near the walls will follow the streamlines into the access hole region changing their velocity and increasing their pathlength. Figure 3 compares the magnitude of the electric field distribution along the channel walls with that of the centerline of the channel. Molecules in the center of the channel are unaffected by the presence of the access holes. However, along the walls molecules travel extremely slow due to the low electric field, this creates a band “tail” near the walls of the channel. The band shapes are shown in figure 4 for a 200 \( \mu \text{m} \) wide channel and varying width of the access holes. The variances of the bands are calculated with respect to the distance along the channel and summarized in table 1.

Access holes less than 25 \( \mu \text{m} \) wide should have little effect on separation performance. The injection plug variance contribution alone is 1875 \( \mu \text{m}^2 \) for a 150 \( \mu \text{m} \) wide injection channel. A 4 cm long electrophoresis channel requires 3 access holes. The total variance for the access holes is 294 \( \mu \text{m}^2 \). Diffusion contributes approximately 1000 \( \mu \text{m}^2 \) to the variance under typical dsDNA separation conditions. Therefore, the presence of the access holes contributes less than 10% of the total variance.

**Experimental**

**Materials** Plastic substrates were cut from 4’ \( \times \) 8’ sheets of clear polycarbonate purchased from AIN plastics (Mount Vernon, NY). AZ9260 photoresist and 400K developer were purchased from Clariant (Summerville, NJ). Siloxane copolymer was purchased from Gelest, Inc. (Tullytown, PA). The photoinitiator (2,2’- dimethoxy-phenyl-acetophenone) was purchased from Aldrich Chemical Co. (Milwaukee, WI).

**Solution preparation** The siloxane solution is prepared by melting the photoinitiator at 70°C and pouring it into the siloxane copolymer to a 1% solution by weight. The solution is then degassed for 10 minutes using a simple sink aspirator. The solution is allowed to set overnight to assure complete mixing.

**Device Fabrication** Capillary electrophoresis devices were fabricated using a 4 mask lithographic process according to figure 5. Since the polycarbonate substrates dissolve and swell in acetone, they must
first be coated with a thick, 12 \( \mu \)m layer of parylene-C (poly-p-xylylene) by vacuum deposition. The parylene-C layer is solvent and acid resistant. Prior to deposition, the substrate is cleaned in isopropyl alcohol (IPA) for 10 minutes, spin dried, and baked for 10 minutes at 100\(^\circ\)C. Adhesion of the parylene-C layer to the substrate is critical and can be achieved by treating the substrate in an oxygen plasma at 100 W, at a pressure of 250 mTorr for 2 minutes.

Electrophoresis electrodes are then deposited and patterned on the substrate. A 0.12 \( \mu \)m gold layer is evaporated on the substrate, patterned and etched with a wet etchant using standard lithographic techniques. Since gold adheres well to parylene-C there is no need for an additional adhesion metal. This is critical since typical adhesion metals such as chromium and titanium corrode very rapidly in TBE solutions. After the electrodes are patterned, a thin layer of chromium 0.02 \( \mu \)m thick is evaporated on the front of the wafer. On top of the chromium layer, a 20 \( \mu \)m thick layer of AZ9260 is patterned to define the channel regions. The direct patterning of thick sacrificial layers on top of plastic and glass substrates can be problematic. The chromium layer prevents these reflections from below the wafer from interfering with the patterning of the sacrificial photoresist. The chromium is then etch away in the field regions using a wet etchant. Next a second layer of approximately 6.5 \( \mu \)m of parylene-C is conformally deposited to form the top and sides of the channel. The adhesion of this layer is also assisted by a short oxygen plasma (100 W, 250 mTorr, 1 minute). Using a thick photoresist mask, reservoir, and contact pad openings are etched using an oxygen plasma (250 W, 50 mTorr, 30 minutes). A photo-definable silicone rubber (polysiloxane) is next used to make the reservoir rings. The wafer is partially released in acetone (10-20 minutes) to remove the photoresist mask and then treated in a short oxygen plasma (100 W, 250 mTorr, 1 minute) to improve adhesion. Using a technique similar to [20, 21], the siloxane solution is spin casted to a thickness of approximately 200 \( \mu \)m. The wafer is then carefully covered with a clear polyester sheet so as not to entrap air bubbles between the polyester and the siloxane coating. The polyester sheet prevents oxygen in the atmosphere from inhibiting the polymerization reaction. The wafer is then aligned and exposed (1200 mJ/cm\(^2\)). After exposure, the polyester sheet is removed and development is carried out by immersion in xylenes for 2 minutes followed by isopropyl alcohol for 2 minutes. The wafer is then dried. The channel is released in acetone for up to 36 hours depending on the channel length, rinsed in IPA, and dried. Finally, the wafer is immersed in chrome etchant several times to etch the remaining chromium on the bottom of the channel. For channels with bends, the wafer cannot be dried before etching the chrome. In this case after the IPA rinse, the wafer is rinsed in DI water and immersed into chrome etchant overnight. The release time can be shortened by constant agitation or
by heating the acetone. Although silicone rubber is known to swell in aqueous solutions, no significant swelling was seen.

**Separation conditions** Separations were performed in these devices using a sieving matrix consisting of 0.5\%(w/v) hydroxyethylcellulose (HEC) (Polysciences, Inc., Warrington, PA; MW 90000-105000) and 0.1x Tris/Boric Acid/EDTA buffer (Sigma Chemical Co., St. Louis, MO). Approximately 3 \( \mu \)l of the buffer solution is loaded one reservoir and allow to fill the entire channel by capillary action. The remaining reservoirs are filled with approximately 3 \( \mu \)l of buffer solution. A 0.2 \( \mu \)g/\( \mu \)l sample of DNA was labeled with SYBR Green I (Molecular Probes, Eugene, OR) intercalating dye at an intercalation ratio of 1:5, dye:DNA bp. Approximately 2 \( \mu \)l of the DNA sample was loaded into the injection reservoir. The samples were cross injected using a pinch injection scheme similar to [22]. The electric fields used during separation were 110 V/cm. For the case of the 1.5 cm devices the channels were pre-electrophoresis at 300 V/cm for 10 minutes. This was found to concentrate the HEC in the separation column to more than 0.5% resulting in a higher resolving power.

**Instrumentation** A stereo microscope (Olympus SZX12) equipped with a fluorescence illuminator (100W Mercury lamp illuminator) was used to observe the separations. The filter cube consisted of 470nm band pass excitation filter, 495nm long pass dichroic mirror, and a 500nm long pass emission filter. Separations were recorded to SVHS video tape using a Dage 68 SIT camera. Electropherograms were obtained by analyzing the video tape with an in-house program written for NIH-Image (Scion image).

**Results and Discussion**

Figure 6 shows a 4” polycarbonate wafer containing approximately 30 electrophoresis devices. All fabrication steps are process on the whole wafer reducing the cost of individual devices. A perspective view of a straight channel electrophoresis device is shown in figure 7. Longer channel devices incorporated 2 turns and access holes for the sacrificial etch according to figure 8. The separation lengths for the straight channels are 1.5 centimeters and for the meandering device, 2.7 cm. Figure 9 shows an optical micrograph of the reservoir structure of the devices. The hydrophobic silicone rubber ring surrounding the channel opening is capable of holding 3 \( \mu \)l of water. Gold electrodes in the back of the reservoir apply the potentials for the electrophoresis. Figure 10 shows a scanning electron micrograph of the cross section of a parylene channel on top of a silicon wafer. The channel height is 20 \( \mu \)m and the channel width is 200\( \mu \)m. Since the channel cross section is defined by photoresist, nearly vertical sidewalls are obtained. Figure 11 shows the separation of fragments present in the HaeIII digest of \( \Phi X174 \) RF DNA.
All fragments are resolved with the exception of the 271- and 281-base pair doublet. Figure 12 shows the separation in the long channel device. Although the doublet is still not resolved in this case, better resolution is obtained among the longer fragments. The plate number for this separation is indeed higher than in the case of the shorter channel, but not as high as expected for an equivalently long, straight channel. This is consistent with the results of Culbertson[23] for channels with two 180° bends. While the presence of the second turn removes some of the variance introduced by the first, transverse diffusion between turns will contribute to the final band variance. The band variance of the 603 bp fragment was found to be approximately $9000 \mu m^2$. The contribution to the variance of the access holes alone was predicted to be $8220 \mu m^2$. Considering the other factors such as injection width, diffusion and the turns, this suggest that the variance due to the access holes is in fact much less than predicted. In fact during separations no significant band tailing was observed. This may be due to two factors. The band tails produced by the access holes have a significantly lower concentration than the center of the band. Therefore, the band tail can be truncated by the detection limits of the system reducing the band variance. Considering this factor the total variance due to the access holes could be as low as $2100 \mu m^2$. The second factor that reduces the variance is the presence of a small convective flow. Since the access holes are open to atmosphere, electrophoresis buffer will evaporate at the interface. Therefore a small convective flow of buffer from the reservoirs will flow into the access hole region to counteract the evaporation. The convective flow is too small to effect separation performance, however it is large enough to trap DNA in the access hole. The model predicts that DNA flowing into the access hole will eventually move out given enough time. Convective flow, however, traps a small amount of DNA in the hole region and therefore truncates the band tail reducing the variance.

**Conclusions**

In this paper we demonstrate high-efficiency separations in plastic electrophoresis devices on polycarbonate substrates. A surface micromachined process was developed for the fabrication of these devices that is completely batch fabricated. Bonding, drilling, and epoxy steps frequently used in microfabricated electrophoresis devices have been replaced by surface micromachining of polymer films. The inherent limitations of surface micromachined methods have been addressed and attempts to overcome this limitation implemented. Access holes required for the release of long sacrificial channels were modeled and demonstrated in these devices. Modeling and experimental results suggest that the presence of access holes at a linear density of one per centimeter of separation length have little effect on overall separation performance provided the holes are less than $50 \mu m$ wide. The inherent autofluorescence of
plastic substrates can be problematic for electrophoresis devices. Polycarbonate has been shown to have a comparably low autofluorescence and detecting at longer wavelengths will reduce the autofluorescence even further.

Acknowledgments

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References


Figure 1: Electrophoresis channel with access holes for sacrificial etching.

Figure 2: Schematic of one half of a channel showing the electric field equipotentials and streamlines near the access hole region. The equipotentials at $V=-1$, $V=0$ and $V=1$ are shown as dotted lines.

<table>
<thead>
<tr>
<th>Access hole width</th>
<th>$\sigma^2$ ($\mu m^2$)</th>
<th>plate height per cm ($\mu m$)</th>
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<tr>
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<tr>
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Table 1: Spatial variances due to the presence of access holes in electrophoresis channels
Figure 3: Magnitude of electric field along the centerline and near the channel walls. $V=0$ corresponds to the center of the access hole region. The width of the access hole is 50 $\mu$m.
Figure 4: Band shape in a 200 \( \mu \text{m} \) channel after passing through the access hole region. Curves correspond to different width of the access hole. The time \( T_0 \) is the time for the sample in the center of the channel to pass through the region.
Figure 5: Simplified process flow for the plastic electrophoresis device.
Figure 6: 4” polycarbonate wafer containing approximately 30 CE devices.

Figure 7: Schematic of a plastic capillary electrophoresis device.
Figure 8: Channel structure for 2.7 cm long separation devices.

Figure 9: Photograph of 20 μm high, 200 μm wide plastic capillary with silicone rubber reservoir.
Figure 10: SEM cross section of 20 μm-high, 200 μm-wide plastic capillary on a silicon substrate.
Figure 11: Electropherogram of HaeIII digest of ΦX174 RF DNA in a plastic device. Separation performed in a straight 1.5 cm long channel.
Figure 12: Electropherogram of HaeIII digest of \(\Phi X174\) RF DNA in a plastic device. Separation performed in a curved, 2.7 cm long channel with 50 \(\mu\)m wide access holes.