Rapid Prototyping of Microfluidic Systems in Poly(dimethylsiloxane)

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This paper describes a procedure that makes it possible to design and fabricate (including sealing) microfluidic systems in an elastomeric material—poly(dimethylsiloxane) (PDMS)—in less than 24 h. A network of microfluidic channels (with width >20 μm) is designed in a CAD program. This design is converted into a transparency by a high-resolution printer; this transparency is used as a mask in photolithography to create a master in positive relief photoresist. PDMS cast against the master yields a polymeric replica containing a network of channels. The surface of this replica, and that of a flat slab of PDMS, are oxidized in an oxygen plasma. These oxidized surfaces seal tightly and irreversibly when brought into conformal contact. Oxidized PDMS also seals irreversibly to other materials used in microfluidic systems, such as glass, silicon, silicon oxide, and oxidized polystyrene; a number of substrates for devices are, therefore, practical options. Oxidation of the PDMS has the additional advantage that it yields channels whose walls are negatively charged when in contact with neutral and basic aqueous solutions; these channels support electroosmotic pumping and can be filled easily with liquids with high surface energies (especially water). The performance of microfluidic systems prepared using this rapid prototyping technique has been evaluated by fabricating a miniaturized capillary electrophoresis system. Amino acids, charge ladders of positively and negatively charged proteins, and DNA fragments were separated in aqueous solutions with this system with resolution comparable to that obtained using fused silica capillaries.

We report a method for creating microscopic channels in an elastomeric material, poly(dimethylsiloxane) (PDMS), that makes it possible to carry out a complete cycle of design, fabrication, and testing of microfluidic systems rapidly. We believe this system for design and fabrication should be useful in prototyping microfluidic systems, such as the microscale total analysis systems (μTAS),1–4 and specialized systems that are being developed for genetic analysis,1 clinical diagnostics,5–14 drug screening,15 and environmental monitoring.16

μTAS perform the functions of large analytical devices in small, often disposable, units.1–4 The potential benefits of μTAS, relative to systems of conventional size, include (i) reduced consumption of samples and reagents, (ii) shorter analysis times, (iii) greater sensitivity, (iv) portability that allows in situ and real-time analysis, and (v) disposability. As a consequence of these potential benefits, there has been considerable interest in the development of μTAS.8,9

μTAS must contain elements for the acquisition, pretreatment, separation, posttreatment, and detection of samples. These elements must be able to handle liquid or gas samples and be miniaturized and incorporated onto, or compatible with, chip-based substrates. Microfluidics is, therefore, central to the development of μTAS.17,18 It is important to have fabrication techniques that create the networks of microscopic (widths 1–100 μm) channels in substrates in which analytes are transported, mixed, and separated in μTAS.18 Although a number of microfluidic components—valves, pumps, mixers, filters, and interconnects18—are available, both new types of components and new systems that integrate these components effectively are needed.

Miniaturized capillary electrophoresis (CE) systems are benchmarks for \( \mu \)TAS and microfluidic systems. There are several reasons for the wide use of these systems. First, conventional CE in fused silica capillaries is a well-established separation technique that is amenable to miniaturization. Second, pumping of analytes is achieved simply by applying electric fields along the channels. Third, capillary channels can be defined on substrates using existing technologies.

Miniaturized CE was developed initially in four groups. The groups of Harrison, Manz, Widmer, and Effenhauser first reported miniaturized CE systems. Harrison’s group has since used these systems for immunoassays and on-chip chemical reactions in an organic solvent. Manz et al. demonstrated free-flow electrophoresis and synchronized cyclic CE devices.

Ramsey and Jacobsen have incorporated elements for pre- and posttreatments into miniaturized CE systems, extending them toward \( \mu \)TAS. They used these systems to digest DNA with a restriction enzyme and to analyze the products on-chip, and to follow the kinetics of the reaction between an enzyme and its substrate in microscopic channels. These workers have also transferred techniques used in conventional CE to miniaturized CE systems; for example, they developed a source of electrospay for a mass spectrometer and miniaturized versions of open-channel electrophoresis and micellar electrokinetic capillary chromatography (MECC).

M ost microfluidic systems, including miniaturized CE systems, have been fabricated in glass or oxidized silicon (Si/SiO\(_2\)). Microscopic channels are defined in these substrates using photolithography and micromachining. The channels are enclosed by sealing the substrates containing channels against flat substrates using anodic or fusion bonding. These materials and fabrication methods were adopted because they relied on technology already developed extensively in the microelectronics industry.

The large body of work on miniaturized CE systems in glass and Si/SiO\(_2\) demonstrates that this approach to fabrication is successful. There are limitations to it, however, especially for the rapid development and testing of new concepts in microfluidic systems. The fabrication processes are slow: use of clean-room facilities is required each time a device is made. The bonding processes used to seal the channels are complicated and time-consuming. The materials are fragile and too expensive for disposables. Furthermore, silicon is optically opaque and electrically semiconducting and is, therefore, unsuitable for certain types of separation and detection schemes. Materials and fabrication methods are needed that allow the rapid evaluation of microfluidic systems—especially for the exploratory stages of \( \mu \)TAS—while maintaining low cost and using mechanically robust materials.

Polymers offer an attractive alternative to glass and Si/SiO\(_2\) as materials for fabrication of microfluidic systems. Polymers are less expensive and less fragile than glass and silicon. The fabrication processes used to create plastic devices are based on replication (casting, embossing, or injection molding) and are faster and less expensive than those used on glass and Si/SiO\(_2\).
There are several reports of microfluidic systems based on PDMS, particularly relevant to this report. We describe a CE system based on molding PDMS against a master, as did Effenhauser et al., our methods differ significantly from theirs, however, so for comparison their approach warrants a brief description. These workers created channels in PDMS by casting the polymer against a commercially obtained master that was composed of a positive relief structure of silicon. The master was created using a chrome mask in the photolithographic step and silicon micromachining. The channels were enclosed by the hydrophobic, conformal contact between the PDMS replica of the master and a flat piece of PDMS. This contact confined liquids to the channels without leakage but was reversible; i.e., the devices were not sealed tightly. The PDMS replicas and flats were not treated after curing of the polymer, and the walls of the channels were hydrophobic. As the walls of the channels were uncharged, they did not support electroosmotic flow (EOF). These workers reported separations of DNA fragments and small molecules in solutions containing a sieving matrix; they did not report separations in the absence of a sieving agent, i.e., capillary zone electrophoresis. The main benefit of this CE system, apart from the creation of multiple devices by replicating a master, was reusability: the reversible contact between the two pieces of PDMS allowed them to be dismantled and the channels cleaned after use.

In this work, we have created masters using a combination of high-resolution printing and contact photolithography—a process we refer to as rapid prototyping of masters. This technique has been described previously and has been used to generate a variety of microstructures with dimensions >20 μm. In this technique, a CAD file is printed on a flexible transparency by using a commercial printer; this transparency is used as a mask in contact photolithography. After development, the pattern of photoresist is used as the master to cast replicas of PDMS.

An important aspect of this work is the demonstration of the value of surface oxidation of the PDMS, both to make sealing straightforward and to support EOF. Enclosed channels are formed simply by oxidizing the PDMS replica containing a network of channels and a second, flat piece of PDMS in a plasma discharge. Bringing the two oxidized PDMS surfaces into conformal contact forms a tight, irreversible seal. An important consequence of oxidizing the PDMS surfaces for microfluidic systems that use electroosmotic pumping is that the walls of the channels present a layer of silanol (SiOH) groups to an aqueous solution in contact with them. These surfaces are charged (SiO⁻) when the solution is neutral or basic, and so the channels support EOF toward the cathode.

In this paper, we first describe the rapid prototyping of microfluidic systems in PDMS. We then describe a miniaturized CE system that we used to evaluate the performance of a microfluidic system fabricated by using these methods and present separations of amino acids, protein charge ladders, and DNA fragments.

RESULTS AND DISCUSSION

Fabrication of Elastomeric Microfluidic Devices. Figure 1 outlines the rapid fabrication of microfluidic systems in PDMS. 

(a) Rapid Prototyping of Masters. Designs of networks of channels were generated by using a computer drawing package. Figure 2 shows representative designs we used to fabricate miniaturized CE systems in oxidized PDMS. Transparencies of the designs were created and used as masks in contact photolithography, which was achieved by using a chrome mask and a commercial printer.

Figure 1. Scheme describing the fabrication of enclosed microscopic channels in oxidized PDMS. (A) A high-resolution transparency containing the design of the channels, created in a CAD program, was used as the mask in photolithography to produce a positive relief of photoresist on a silicon wafer. The scale bar gives an indication of the thickness and width of photoresist. (B) Glass posts were placed on the wafer to define reservoirs for analytes and buffers. (C) A prepolymer of PDMS was then cast onto the silicon wafer and cured at 65 °C for 1 h. (D) The PDMS replica and a flat slab of PDMS were oxidized in a plasma discharge for 1 min. Plasma oxidation had two effects. First, when two oxidized PDMS surfaces were brought into conformal contact, an irreversible seal formed between them. This seal defined the channels as four walls of oxidized PDMS. Second, silanol (SiOH) groups introduced onto the surface of the polymer ionize in neutral or basic aqueous solutions and support EOF in the channels.

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thography to yield masters composed of a positive relief of photoresist on a silicon wafer. Using transparencies printed from a high-resolution printer, we could rapidly and reliably create features in photoresist with widths >20 \( \mu m \). By using different photoresists, features could be generated with thickness between 1 and 200 \( \mu m \). For our studies, we used a photoresist thickness of 55 \( \mu m \) to define the channel depth; we could reproduce this thickness within \( \pm 5\% \). The dimensions of features of photoresist that can be achieved by rapid prototyping are, therefore, well suited for fabricating masters for molding channels in polymers that can be used in microfluidic applications.

Using rapid prototyping to create masters offers several advantages over the conventional photolithography and micromachining of silicon that has been used previously to create masters for molding polymeric microfluidic systems: (i) transparencies take less time to produce (hours compared to days or weeks) and are substantially less expensive than chrome masks (\$20 compared to \$500–\$1500); (ii) the development of photoresist to create a relief on silicon is easier and more flexible in the types of patterns that can be produced than the etching needed during micromachining of silicon. In particular, the orientation of features created by micromachining silicon is often limited by the crystallographic planes of silicon because of anisotropic etching; this property makes it difficult to create channels in microfluidic devices of arbitrary orientation or to create circular reservoirs. It is, however, straightforward to create features in photoresist that can be molded in polymer to yield channels of arbitrary orientation and circular reservoirs (Figure 2).

(b) Replica Molding of Masters. Glass posts were placed on the master to define reservoirs for liquids (Figure 1). PDMS was then cast against the masters to yield elastomeric replicas containing networks of channels. The angle of the walls of the photoresist to the silicon substrate was close to 90\( ^\circ \), so the channels in the PDMS replicas would be essentially rectangular (Figure 3). The SEM image in Figure 3 indicates that there is some roughness in the sides of the PDMS channels; this edge roughness arises from the limited resolution of the transparency used as a mask in photolithography to create the positive relief of photoresist.

(c) Oxidation and Sealing. To form enclosed channels, we sealed a PDMS replica containing a network of channels irreversibly to a flat slab of PDMS. Oxidizing both surfaces in a plasma discharge for 1 min and bringing them into conformal contact, without added pressure, achieved this seal in seconds (Figure 1). This procedure is detailed in the Experimental Section. The seal between the two pieces of PDMS was sufficiently strong that the
two substrates could not be peeled apart without failure in cohesion of the bulk PDMS.73

This strong seal enabled us to fill the microscopic channels easily by placing liquid in three of the reservoirs and applying vacuum on the fourth. We found it difficult to fill channels defined by the hydrophobic, conformal contact between an untreated PDMS replica and flat by vacuum because the two pieces of PDMS tended to be pulled apart by the force of the suction.

To understand the nature of the strong bond between two pieces of oxidized PDMS, we draw on the extensive surface characterization that has been performed previously by Morra et al.75 and Chaudhury et al.73,74 The advancing contact angle of water on PDMS oxidized for 1 min is 30°, compared to 108° for untreated PDMS. Oxidized PDMS has a hydrophilic surface.73–75 Static secondary ion mass spectrometry (SSIMS) indicated that the site of attack of the plasma was at the silicon atoms, and the fragments suggested that SiOH groups were at the surface.75 XPS revealed an increase in silicon and oxygen at the surface at the expense of carbon upon a plasma treatment. The ATR-IR spectrum of oxidized PDMS had a strong O–H stretching mode at 3400 cm⁻¹ and a Si–O–H stretching mode at 908 cm⁻¹.75 Oxidation also resulted in a weakening of the methyl modes.

All this experimental evidence indicates that oxidizing PDMS in a plasma discharge converts –OSi(CH₃)₂O– groups at the surface to –O₅Si(OH)₄⁻.75 We believe that the formation of bridging, covalent siloxane (Si–O–Si) bonds by a condensation reaction between the two PDMS substrates is the most likely explanation for the irreversible seal.

PDMS sealed irreversibly to a range of materials other than itself, including glass, silicon, silicon oxide, quartz, silicon nitride, polyethylene, polystyrene, and glassy carbon; in all cases, both surfaces were cleaned and exposed to an oxygen plasma for 1 min. This method of irreversible sealing to PDMS failed, however, with PMMA, polyimide, polycarbonate, and Saran Wrap. For the ceramic materials, the plasma probably removes organic contaminants on the surface; for the organic polymers, it introduces polar functional groups (COOH, OH, ketone) with which the SiOH groups can condense.76 Many of these materials are used commonly in μTAS, so this sealing method provides a rapid and simple way to enclose microfluidic devices. It is well established that plasma oxidation enhances the adhesion of polymers with low surface energies.77 The ability of oxidized PDMS to conform to surfaces over large area, and to bring both surfaces into atomic contact, further contributes to the success of sealing it to a wide range of substrates.

This method for sealing microfluidic systems made from PDMS is much simpler than others used commonly. Glass and Si/SiO₂ devices are sealed by either anodic or fusion bonding that require applying some combination of high pressures, temperatures, and voltages for an extended period (up to 18 h).18 Plastic devices are usually sealed by heating two substrates to their softening temperatures and bringing them into contact.52,54 This heating process can distort the geometry of the channels and requires careful attention to conditions.53 The method of sealing PDMS devices described here retains the integrity of the channels, is carried out at room temperature and pressures, and is complete in seconds to minutes.

(d) Nature of the Channels in Oxidized PDMS. As discussed above, silanol groups are present on the walls of oxidized PDMS channels. When in contact with neutral or basic aqueous solutions, the silanol groups deprotonate (SiO⁻). Charged PDMS/silicate walls provide two main benefits for microfluidic systems over hydrophobic walls:

(i) It is easy to fill oxidized PDMS channels with liquids. The high surface energy of oxidized PDMS means that it is wet by polar liquids more easily than native, hydrophobic PDMS.73–75 Oxidized channels are, therefore, easier to fill than hydrophobic channels with liquids with high surface tensions, such as water. As a consequence, we have been able to separate samples by capillary zone electrophoresis using a procedure that does not require the presence of a sieving matrix to reduce the surface tension of water and make it easier to fill hydrophobic channels. We found bubble formation to be a problem in channels with hydrophobic walls78 because aqueous solutions had a tendency to dewet from the surface of hydrophobic PDMS.

(ii) Oxidized PDMS channels support EOF toward the cathode. The direction of migration under an applied electric field of both a negatively charged molecule (fluorescein) and a positively charged molecule (4-(4-(diethylamino)styryl)-N-methylpyridinium iodide) in oxidized PDMS channels in 25 mM Tris–192 mM Gly buffer (pH 8.4) indicated that EOF was toward the cathode. This observation is consistent with the surface of oxidized PDMS being negatively charged.

We were concerned with the durability of the thin hydrophilic layer on PDMS produced by exposure to an oxygen plasma. Morra et al.75 found that the contact angle of water on oxidized PDMS changed from 30° to 79° in 15 min if the samples were left open to the atmosphere; the contact angle returned (93°) almost to the value of native PDMS after 45 min in air. These, and other, workers have reported, however, that oxidized PDMS was stable if placed under liquid immediately after oxidation; the advancing air-in-water contact angle of oxidized PDMS does not change appreciably over 80 min.75 An unstable charged layer would lead to unstable EOF in oxidized PDMS channels and could, therefore, limit the use of this material in μTAS. We tested the stability of the oxidized layer in our channels by measuring the mobility of fluorescein in a 5-cm-long oxidized PDMS channel over a period of 4 days. During this time, the channel underwent intermittent electrophoresis for a total of 8 h under an applied voltage of 200 V/cm in 25 mM Tris–192 mM Gly buffer (pH 8.4) at 20 °C. In the first 8 h of the experiment, during which an electric field was applied for a total of 3 h, the mobility of fluorescein fluctuated by ±5% around its mean value. Over the

(76) We found that irreversible sealing between oxidized PDMS surfaces failed if the pieces were not brought into contact within ~1 min of oxidation. The failure in sealing is presumably caused by adsorption of contaminants from the air or by intrinsic rearrangement of the surface.
(78) We created tightly sealed devices with channels with hydrophobic PDMS (contact angle > 90°) by heating the oxidized PDMS devices in air at 135 °C for 1 h. Morra et al. (ref 75) inferred from the temperature dependence of this change in wetting that it was due to diffusion of polar groups on the surface of oxidized PDMS into the bulk polymer and the condensation of silanol groups; this hypothesis was confirmed by SSIMS and XPS.
next 4 days, the mobility of fluorescein increased steadily to a value 26% greater than its initial value. The increase in mobility suggests an increase in the rate of EOF, possibly caused by the hydrolysis of siloxane bonds in the oxidized layer by the alkaline buffer. The useful lifetime of these devices for quantitative analyses requiring absolute values of mobilities is probably 3 h, but EOF pumping can be maintained for a longer time. Much longer operating lifetimes will be possible with an internal standard to compensate for drift (a common procedure in bioanalysis by CE), but we have not yet defined the operational limits of the system. Over a period of days, we did not observe loss in the polarity of the channels.

**Capillary Electrophoresis Performance of Oxidized PDMS.** We have developed a miniaturized CE system to evaluate the suitability of our fabrication methods and materials. As oxidized PDMS, fused silica, and glass all have silanol groups on their surfaces, it is useful to make comparisons of electrophoretic separations obtained in oxidized PDMS channels to those obtained from fused silica capillaries and channels defined in glass. CE experiments also allow us to determine the velocity of EOF supported by oxidized PDMS and the importance of adsorption of analytes to the polymer on electrophoretic separations.

(a) **CE Experiment.** We used the designs of the networks of channels shown in Figure 2. The channels were 50 µm wide and 50 µm deep. The CE experiment was similar to those described extensively in the literature. A sample was injected into the separation channel using a "double-T" arrangement connecting the separation and injection channels (inset of Figure 2). The length and volume of the injected plug of sample were 150 µm and ~400 pL. Analytes were detected by laser-induced fluorescence (LIF). We employed relatively low electric field strengths (ranging from 35 to 185 V/cm) to separate the samples compared to those commonly used in miniaturized CE systems (typically >1000 V/cm). We found that, because we used a fluorescence microscope with a short working distance to detect analytes, shorting of the electric field through the optical elements tended to occur for field strengths greater than 200 V/cm. Higher electric field strengths can be achieved by using an inverted microscope or a microscope with a long working distance. With one of these experimental setups, our times of analysis could be shorter.

(b) **Separation of Amino Acids.** Figure 4 shows the electropherograms of a mixture of six amino acids labeled with fluorescein isothiocyanate (FITC) separated in an oxidized PDMS channel and in a fused silica capillary. The resolution of electrophoretic separations in the oxidized PDMS channels is slightly better than that in fused silica capillary. The resolution obtained is similar to that reported from miniaturized CE systems fabricated in glass.

Comparison of the electropherograms obtained from oxidized PDMS and fused silica capillary allowed us to quantify the strength of electroosmosis in oxidized PDMS channels. The strength of electroosmosis can be quantified by the electroosmotic mobility, \( \mu_{\text{os}} \), defined as \( \frac{v_{\text{os}}}{E} \), where \( v_{\text{os}} \) is the velocity of electroosmosis and \( E \) is the strength of the electric field. The measured mobilities \( \mu_{\text{meas}} \) of the amino acids (defined as \( \frac{v}{E} \), where \( v \) is the overall velocity of the analyte) in oxidized PDMS channels and fused silica capillaries are given by vector sums of the electroosmotic and electrophoretic mobilities:

\[
\mu_{\text{meas}}(\text{PDMS}) = \mu_{\text{os}}(\text{PDMS}) - \mu \tag{1}
\]

\[
\mu_{\text{meas}}(\text{silica}) = \mu_{\text{os}}(\text{silica}) - \mu \tag{2}
\]

where \( \mu \) is the electrophoretic mobility of the amino acid. 

![Electropherograms of fluorescence intensity against time](image-url)
determined by the mass, charge, and shape of the analyte molecules\textsuperscript{56} and is, therefore, the same in both oxidized PDMS channels and fused silica capillaries. From the total mobility of the amino acids in both oxidized PDMS and fused silica, and an independent measurement of $\mu_{\text{os}}$(silica), eqs 1 and 2 yield $\mu_{\text{os}}$(PDMS). The electropherogram of a neutral molecule ($\mu = 0$), p-methylbenzyl alcohol, detected by UV absorption, in the same fused silica capillary and buffer showed that $\mu_{\text{os}}$(silica) = 6.2 × 10$^{-4}$ cm$^2$/V·s$^{-1}$. The total mobilities of the amino acids on oxidized PDMS and fused silica were determined from the electropherograms in Figure 4. Substituting values of $\mu_{\text{os}}$(silica), $\mu_{\text{os}}$(PDMS), and $\mu_{\text{os}}$(PDMS) into eqs 1 and 2 yields $\mu_{\text{os}}$(PDMS) = 8.2 × 10$^{-4}$ cm$^2$/V·s$^{-1}$ at pH 9. As $\mu_{\text{os}}$ is proportional to the charge density of the walls of the capillary,\textsuperscript{56} and the charge density of fused silica is about 10$^{-2}$ C/m$^2$ at this pH,\textsuperscript{56} we conclude that the charge density of oxidized PDMS is $\sim$1.3 × 10$^{-2}$ C/m$^2$.

(c) Separation of a Charge Ladder of Carbonic Anhydrase.

Our group has used charge ladders\textsuperscript{79–81} of carbonic anhydrase (EC 4.2.1.1) as a model system to study the charge of a protein on the free energy of the binding of inhibitors,\textsuperscript{82} to evaluate coatings of capillaries,\textsuperscript{83} to determine the mass and charge of carbonic anhydrase,\textsuperscript{84} and to determine the pK$_a$ of the N-terminal $\alpha$-amino group of carbonic anhydrase.\textsuperscript{85}

A charge ladder is a set of derivatives of a protein that differ incrementally in the number of their charged groups that have been modified. Differences in the charges of these sets of derivatives often allow them to be resolved by CE into a group of distinct peaks, or “rungs”.\textsuperscript{79–81} Charge ladders are most conveniently formed by the partial acetylation of the $\epsilon$-amino groups of the lysine residues of proteins.

We analyzed a fluorescent charge ladder of bovine carbonic anhydrase II (BCA, pI 5.9) as a model system to study the effect of the charge of a protein on the free energy of the binding of inhibitors,\textsuperscript{82} to evaluate coatings of capillaries,\textsuperscript{83} to determine the mass and charge of carbonic anhydrase,\textsuperscript{84} and to determine the pK$_a$ of the N-terminal $\alpha$-amino group of carbonic anhydrase.\textsuperscript{85}

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We analyzed a fluorescent charge ladder of bovine carbonic anhydrase II (BCA, pI 5.9); this protein is negatively charged at pH 8.4. We created the fluorescent charge ladder by modifying the $\epsilon$-amino groups of the Lys residues first with a fluorescent molecule, 5-carboxyfluorescein succinimidyl ester (F-NHS), and then with acetic anhydride (see Experimental Section). Figure 5 shows electropherograms of a charge ladder of BCA separated in an oxidized PDMS channel and a fused silica capillary. Each peak in the electropherograms corresponds to a set of regioisomers of the protein that have the same charge. Adjacent rungs differ by 1 in the number of Lys groups modified. BCA has 18 Lys residues; acetylation can, in principle, yield 18 sets of derivatives and hence a maximum of 18 rungs in the ladder. The conditions of the modification reaction we used, however, produced only eight (or possibly nine) rungs that were detectable by LIF.

The appearance of the electropherograms obtained from oxidized PDMS and fused silica are again similar. As with the amino acids, the resolution from the oxidized PDMS CE system is slightly better than that from a fused silica capillary. It appears that neither the turns in the microfluidic channel that might, in principle, lead to broadening of the peaks nor adsorption of protein to the polymer negatively affects separations of proteins in these devices.

(d) Separation of a Charge Ladder of Insulin.

We analyzed a charge ladder of another protein, insulin (pI = 5.3), that is also negatively charged at pH 8.4. To reduce adsorption of insulin to the walls of the PDMS channels, we added a zwitterionic surfactant, 3-quinoiulidinopropanesulfonate (QPS),\textsuperscript{86} to the running buffer. The addition of QPS greatly increased the strength of EOF. This increase in the strength of EOF required that lower field strengths be used in order to obtain sufficient resolution of the rungs. Figure 6 shows the electropherograms of a charge ladder of insulin obtained in an oxidized PDMS channel and a fused silica capillary.

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Insulin has three amino groups that can be modified on the Phe (R-amino, $pK_a = 7.1$), Gly (R-amino, $pK_a = 8.4$), and Lys (ε-amino, $pK_a = 11.1$) residues. Seven possible regioisomers can be formed upon modification of these amino groups: three derivatives with one amino group modified, three with two amino groups modified, and one with all three amino groups modified. Because of differences in $pK_a$ of these amino groups, the charge ladder can be separated into a set of seven peaks (Figure 6); the assignment of the rungs (labeled in Figure 6A) was determined from the $pK_a$ values. Only six rungs were clearly resolved in the fused silica capillary.

(e) Separation of a Charge Ladder of Lysozyme. It is difficult to analyze positively charged proteins by CE in negatively charged capillaries because these proteins tend to adsorb to the walls. We have separated a charge ladder of a protein, lysozyme (pI = 10.9), that is positively charged in 25 mM Tris–192 mM Gly buffer (pH 8.4), in oxidized PDMS channels by coating the negatively charged walls with a cationic polymer, Polybrene. Because of differences in $pK_a$ of the amino groups, the charge ladder can be separated into a set of seven peaks (Figure 6); the assignment of the rungs (labeled in Figure 6A) was determined from the $pK_a$ values. Only six rungs were clearly resolved in the fused silica capillary.

Figure 6. Electropherograms of a charge ladder of insulin obtained in (A) an oxidized PDMS channel and (B) a fused silica capillary. The fluorescent charge ladder was created by modifying insulin in phosphate buffer with 8 equiv of F-NHS. This sample was diluted for injection to 0.5 mg/mL protein in the running buffer, 25 mM Tris–192 mM Gly buffer containing 100 mg/mL QPS. In A, the network of channels and laser position were as described in Figure 4; the separation voltage was 1 kV. In B, the capillary was the same as described in Figure 4; the separation voltage was 1.8 kV. The first seven peaks arise from the seven fluorescent members of the charge ladder formed from the modification of the two α-amino groups (Phe and Gly) and the Lys ε-amino group. The last two, strong peaks arise from fluorescent impurities.

Figure 7. Electropherograms of a charge ladder of a positively charged protein, lysozyme, obtained in (A) an oxidized PDMS channel coated with Polybrene and (B) a fused silica capillary coated with Polybrene. The fluorescent charge ladder was created by modifying lysozyme with 0.5 equiv of F-NHS and then with 20 equiv of acetic anhydride. A 4-fold dilution of this sample in the running buffer, 25 mM Tris–192 mM Gly–100 mg/mL QPS, gave a final concentration of protein of 0.375 mg/mL. In A, the network of channels and laser position were as described in Figure 4; the separation voltage was 1 kV. In B, the capillary was the same as in Figure 4; the separation voltage was 1.8 kV. In both A and B, the capillaries were coated with a cationic polymer, Polybrene, that made the walls of the channels positively charged; the direction of EOF was reversed. The six peaks arise from sets of fluorescent derivatives of protein produced by the modification of the ε-amino groups of the six Lys residues of lysozyme.

From the reversal of the direction of EOF in these channels, we conclude that Polybrene caused the walls of the channels to become positively charged. Although coating with Polybrene reduced greatly the adsorption of lysozyme compared to uncoated oxidized PDMS, we also had to add surfactant, QPS, to the running buffer to get a well-resolved charge ladder. Figure 7 shows the electropherograms of a charge ladder of lysozyme separated in an oxidized PDMS channel and a fused silica capillary that had been coated with Polybrene. Lysozyme has six Lys residues, whose modification gives rise to six rungs.

(f) Separation of DNA Fragments. Figure 8 shows an electropherogram of a digest of DNA that has been used previously as a model sample for CE systems. The sample was separated in a buffer containing a sieving matrix, hydrox-
CONCLUSIONS

We have demonstrated the fabrication of a miniaturized CE system—the type of device most commonly used to benchmark microfluidic systems—using these methods and materials. We successfully separated both small molecules (amino acids) and large biological molecules (proteins and DNA) using this system. The resolution of electrophoretic separations of negatively charged amino acids and protein charge ladders obtained in oxidized PDMS channels is comparable to (or slightly better than) those in the same length of fused silica capillaries used in conventional CE. The separations are comparable to those obtained from other miniaturized CE systems.

There are a number of benefits to our approach for creating microfluidic systems. First, networks of microfluidic channels are designed directly by the user in a CAD program. Second, these systems are inexpensive: low-cost transparencies are used as masks in photolithography to create masters, and channels are created by molding a low-cost polymer against these masters. Third, many devices (>30 from each master) can be created by replica molding. In comparison, each separate device fabricated in glass or Si/SiO$_2$ requires photolithography and micromachining. Fourth, both channels of arbitrary orientation and circular reservoirs can be created, as the masters are composed of a positive relief of photoresist. Fifth, sealing of channels in oxidized PDMS is simple and fast. Sixth, microfluidic devices fabricated from PDMS are physically robust; for example, they can be dropped or bent without damage.

There are two main advantages to using oxidized PDMS in $\mu$TAS rather than other polymers. First, oxidized PDMS is charged under neutral and basic aqueous solutions, and, therefore, these channels support EOF, unlike the channels in some of the other polymers used previously to create microfluidic systems. We have also used EOF to actuate microfluidic devices, such as fluidic switches, and to characterize the flow in devices, such as fluidic rectifiers. Second, there are several strategies to control the surface chemistry of oxidized PDMS. We reversed the surface charge of oxidized PDMS by coating it with Polybrene and eliminated the charge on the surface using hydroxypropylcellulose. It should also be possible, in principle, to use self-assembled monolayers formed by functionalized trichlorosilanes at the surface of oxidized PDMS to modify its chemistry. Materials used to fabricate $\mu$TAS must be able to resist the adsorption of analytes that can cause blockages of microscopic channels: the ability to change the surface chemistry of oxidized PDMS may enable it to be tailored to minimize blockages.

Our system also has potential or real disadvantages. First, two of the walls of the molded polymer are rough compared to those molded against masters created by micromachining silicon (Figure 3). We did not observe any significantly adverse effects on the performance of these devices from this roughness (which arises from the resolution of the transparency), but in some circumstances it might be important. Second, because the devices were irreversibly sealed, they could not be dismantled and cleaned, as is possible in a CE system fabricated in unoxidized PDMS. We have, however, been able to separate different samples in different buffers on the same CE device by flushing the channels thoroughly in NaOH, water, and buffer between uses. Furthermore, the low cost and short time needed to create devices means that they can be treated as single-use disposables.

In summary, the ease of designing networks of microfluidic channels in a CAD program, the straightforward molding and

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sealing of devices, and the speed and low cost of the whole process should make the rapid prototyping of PDMS microfluidic systems useful in the exploratory stages of the development of μ TAS. Rapid prototyping should, therefore, facilitate the transfer of designs for microfluidic systems to materials and fabrication methods suited to high-volume production.

**EXPERIMENTAL SECTION**

**Fabrication. (a) Rapid Prototyping.** Designs of networks of microfluidic channels (widths > 20 μm) were created in a CAD program (Freehand 7.0, M acromedia, San Francisco, CA). High-resolution (3386 dpi) transparencies were produced by a commercial printer from the CAD files with the design clear and the background ink. Transparencies were used as masks in photolithography on negative photoresist (SU-8 50, Micro lithography Chemical Corp., Newton, MA) spin-coated onto silicon wafers to create masters. We spin-coated at 5000 rpm for 20 s to create features of photoresist, and hence channels, 50 μm deep. After development in propylene glycol methyl ether acetate (Aldrich, Milwaukee, WI), the masters were placed in a desiccator under an atmosphere of nitrogen (99.95%, Matheson, Woodstock, NJ). The running buffer was 25 mM Tris–192 mM Gly (pH 8.4).

**Charge Ladder of Insulin.** One milligram of insulin (Sigma) was suspended in 500 μL of 0.05 N NaOH, and then 500 μL of 50 mM Na₂HPO₄–50 mM NaH₂PO₄ was added. A 200-μL aliquot of this suspension was mixed with 56 μL of 5 mM F-NHS (8 equiv) in DMF. This sample was then purified on a NICK spin column. The injection sample was prepared by mixing equal amounts of the sample of charge ladder and 25 mM Tris–192 mM Gly buffer containing 100 mg/mL of 3-quinuclidinopropanesulfonate (QPS). Tris–Gly–QPS was also used as the running buffer.

**Charge Ladder of Lysozyme.** A 200-μL aliquot of 1.5 mg/mL lysozyme ( Worthington, Freehold, N J) in water was mixed with 1 μL of 0.1 N NaOH and 2 μL of 5 mM F-NHS (0.5 equiv) in DMF. After 30 min, 19 μL of 0.1 N NaOH, followed by 10 μL of 40 mM acetic anhydride (20 equiv) in dioxane, were added to the solution of protein. This sample was diluted with 3 parts 25 mM Tris–192 mM Gly–100 mg/mL QPS. Tris–Gly–QPS was used as the running buffer.

**Digest of DNA:** The labeling of the φX-174/HaeIII digest of DNA (Pharmacia) was taken from the literature. Briefly, 40 μL of stock solution of the DNA digest (500 μg/mL) was added to 157 μL of 40 mM Tris, 40 mM acetic acid, 1 mM Na₂EDTA buffer (pH 8). Then 3.1 μL of 1 mM YOYO-1 (Molecular Probes) in DM SO was added to the solution; the base pair-to-YOYO-1 ratio was 1:1. The sample was injected without further dilution. The running buffer contained Tris–acetic acid–EDTA with 10 μM 9-aminoacridine and 0.75% (w/v) hydroxypropylcellulose.

**Preparation of Oxidized PDMS Channels.** For the devices used to separate amino acids, BCA, and DNA, the channels were flushed sequentially with 0.1 N NaOH, deionized water, and buffer solution (10 min each) by applying vacuum to one of the reservoirs; flushing with base was omitted for separations of insulin. The devices used to separate lysozyme were flushed sequentially with deionized water, 7.5% (w/v) Polybrene ( Sigma) in buffer, and buffer with QPS for 10 min each.

**Injection and Separation.** The designs of the networks of channels used to create miniaturized CE systems in PDMS are shown in Figure 2. A solution of sample was placed in reservoir 1, and running buffer was placed in reservoirs 2–4. A potential was applied between the sample (1) and waste (2) reservoirs through Pt electrodes connected to a high-voltage power supply (CZE 1005, Spellman, Hauppauge, NY). The resulting EOF in the injection channel connecting reservoirs 1 and 2 pumped sample into the 150-μm-long double-T section of the separation channel (inset of Figure 2). This plug of sample was separated by applying a voltage between reservoirs 3 and 4. Small (300 V) voltages (VWR105 Power Supply, VWR Scientific, Boston, MA) with the same polarity as the separation voltage were applied to the sample and waste reservoirs to prevent leakage of sample into the separation channel. Warning: extreme care should be exercised when using high-voltage power supplies.

**Detection.** Fluorescent samples were detected by a scanning laser-induced fluorescence (LIF) microscope (TC54D confocal scanner on a DM RBE microscope, Leica, Heidelberg, GmbH). An Ar⁺ laser (488 nm) was used to generate fluorescence that was detected by a photomultiplier tube after passing through a band-pass filter at 520 nm. We used a 10× objective to give a...
field of view of 1000 \( \mu m \times 1000 \mu m \) to align and focus the laser beam. During acquisition of data, the fluorescence signal from a 2\( \mu m \)-long section of the 50\( \mu m \)-wide channel was recorded at 14 Hz; the electropherogram was, therefore, recorded as a fluorescence image of this narrow section of channel as a function of time. Images of fluorescence from channels were converted into plots of total fluorescence intensity against time using NIH Image 1.61 software.

**CE in Fused Silica Capillaries.** Electrophoretic separations in 50\( \mu m \)-i.d/365\( \mu m \)-o.d fused silica capillary (Polymicro Technology, Phoenix, AZ) were performed on a PACE 5010 CE instrument (Beckman Instruments, Fullerton, CA) fitted with an LIF source and detector. The capillaries were pretreated with NaOH, water, and buffer in the same way as the oxidized PDMS channels.

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