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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 interconnects[18]—are available, both new types of components and new systems that integrate these components effectively are needed.

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Miniaturized capillary electrophoresis (CE) systems[4,15,19−55] are a benchmark for μTAS 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[56] 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[20−30] and Manz, Widmer, and Effenhauser[31−35] first reported miniaturized CE systems. Harrison's group has since used these systems for immunoassays[30] and on-chip chemical reactions in an organic solvent;[27] Manz et al. demonstrated free-flow electrophoresis[33] and synchronized cyclic CE devices.[34] Ramsey and Jacobsen[36−46] have incorporated elements for pre- and posttreatments[45] into miniaturized CE systems, extending them toward μTAS. They used these systems to digest DNA with a restriction enzyme and to analyze the products on-chip,[36] and to follow the kinetics of the reaction between an enzyme and its substrate in microscopic channels.[38] These workers have also transferred techniques used in conventional CE to miniaturized CE systems; for example, they developed a source of electrospray for a mass spectrometer[39] and miniaturized versions of open-channel electrochromatography[41] and micellar electrokinetic capillary chromatography (MECC).[43] Mathies et al.[47−51] have emphasized the potential application of these systems in DNA sequencing by performing restriction assays for genotyping using multichannel analyses and multicolor detection. For example, they performed 12[49] and 96[51] analyses of a DNA restriction digest on single substrates and carried out four-color sequencing of a single-stranded DNA template.[47] Work in μTAS is now ongoing in a large number of laboratories.[1−3,5−11,14,16,18,57−59]

Most microfluidic systems, including miniaturized CE systems, have been fabricated in glass or oxidized silicon (Si/SiO_2).[60−64] Microscopic channels are defined in these substrates using photolithography and micromachining.[1,18,65] The channels are enclosed by sealing the substrates containing channels against flat substrates using anodic or fusion bonding.[1,18] 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 μTAS—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)[52−54] and are faster and less expensive than those used on glass and Si/SiO_2.

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There are several reports of microfluidic systems based on PDMS:[52,66−69] the one of Effenhauser et al.[52] is particularly relevant to this report. We describe a CE system based on molding PDMS against a master, as did Effenhauser et al.;[52] 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.[70,71] This technique has been described previously and has been used to generate a variety of microstructures with dimensions >20 μm.[70,71] 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[68] 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.[56] 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.

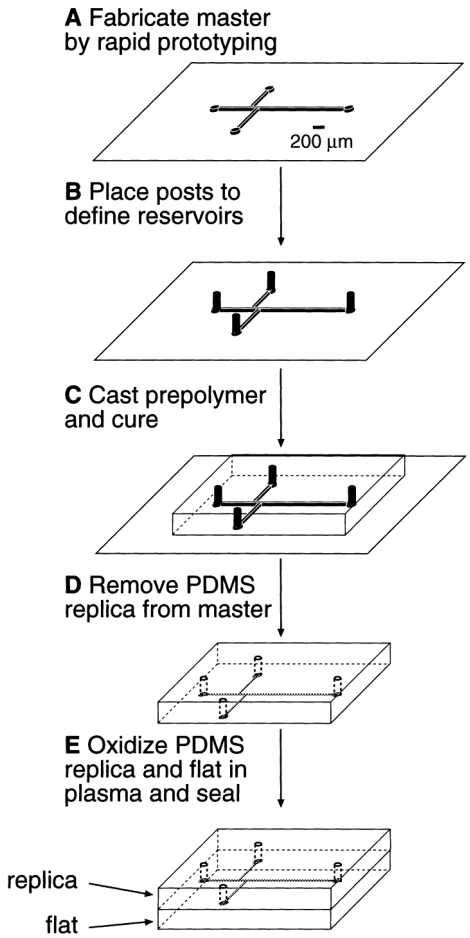

Figure 1. Scheme describing the fabrication of enclosed microscopic channels in oxidized PDMS. (A) A high-resolution transparency containing the design of the channels, created in a CAD program, was used as the mask in photolithography to produce a positive relief of photoresist on a silicon wafer. The 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 polymer replica of the master containing a negative relief of channels was peeled away from the silicon wafer, and the glass posts were removed. (E) 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.

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 photoli-

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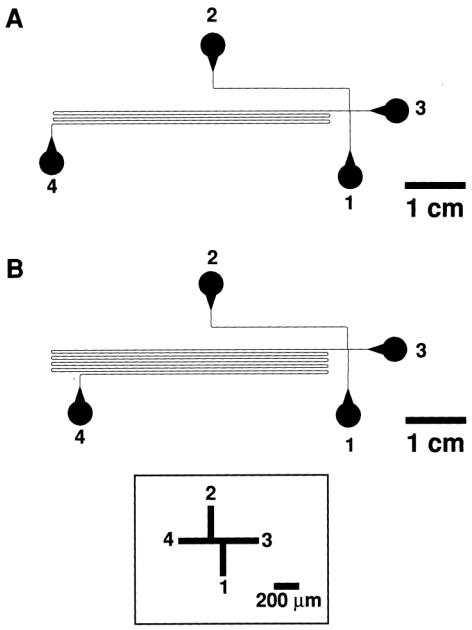

Figure 2. Designs of the network of channels used to create on-chip CE systems in oxidized PDMS. The designs are based on those used commonly in other miniaturized CE systems. A 50-μm-wide injection channel connected reservoirs for sample (labeled 1) and buffer (2). This channel crossed the 50-μm-wide separation channel that connected reservoirs 3 and 4 in a 150 μm-long double-T arrangement; this arrangement is used to inject sample into the separation channel, and a detail of it is shown in the inset. In A, the separation channel was 28 cm long; in B, the separation channel was 42 cm long.

thography to yield masters composed of a positive relief of photoresist on a silicon wafer.[70,71] Using transparencies printed from a high-resolution printer, we could rapidly and reliably create features in photoresist with widths >20 μm. By using different photoresists,[72] features could be generated with thickness between 1 and 200 μm. For our studies, we used a photoresist thickness of ~55 μm to define the channel depth; we could reproduce this thickness within ±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:[52−54] (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;[52] 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).

(72) SU8-5 and SU8-50 negative photoresists (Microlithography Chemical Corp., Newton, MA) could be used to span this range of thicknesses. The thickness of the photoresist was measured by profilometry.

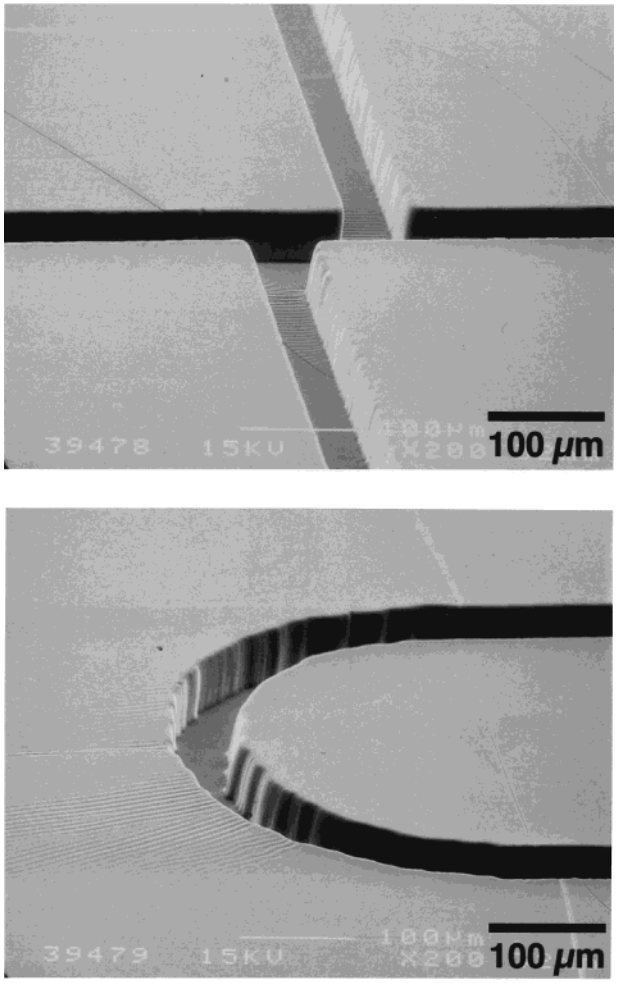

Figure 3. Scanning electron micrographs of channels in PDMS, created by molding the polymer against a positive relief of photoresist. The top image shows the double-T section of the network of channels (inset of Figure 2); the bottom image shows a turn in a channel. The roughness in the side walls of the PDMS channels arises from the limited resolution of the transparency used as a mask in photolithography to create the positive relief of photoresist.

(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°, so the channels in the PDMS replica were 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 printing on the transparency.

(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);[68] 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−OH 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 $-OSi(CH_3)_2O-$ groups at the surface to $-O_nSi(OH)_{4-n}$.[73,74] 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/SiO_2 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.[53,54] 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 walls[78] 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,[67] 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 electroosmosis 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

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(74) Chaudhury, M. K.; Whitesides, G. M. *Science* **1991**, *255*, 1230.
(75) Morra, M.; Occhiello, E.; Marola, R.; Garbassi, F.; Humphrey, P.; Johnson, D. *J. Colloid Interface Sci.* **1990**, *137*, 11.
(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.
(77) Chan, C.-M.; Ko, T.-M.; Hiraoka, H. *Surf. Sci. Rep.* **1996**, *24*, 1.
(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 EO 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.[20−51] A sample was injected into the separation channel using a "double-T" arrangement[31] 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).[20−51] 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[52] or a microscope with a long working distance.[23] 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.[20,31]

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, μ_{os}, defined as v_{os}/E, where v_{os} is the velocity of electroosmosis and E is the strength of the electric field.[56] The measured mobilities (μ_{meas}) of the amino acids (defined as 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

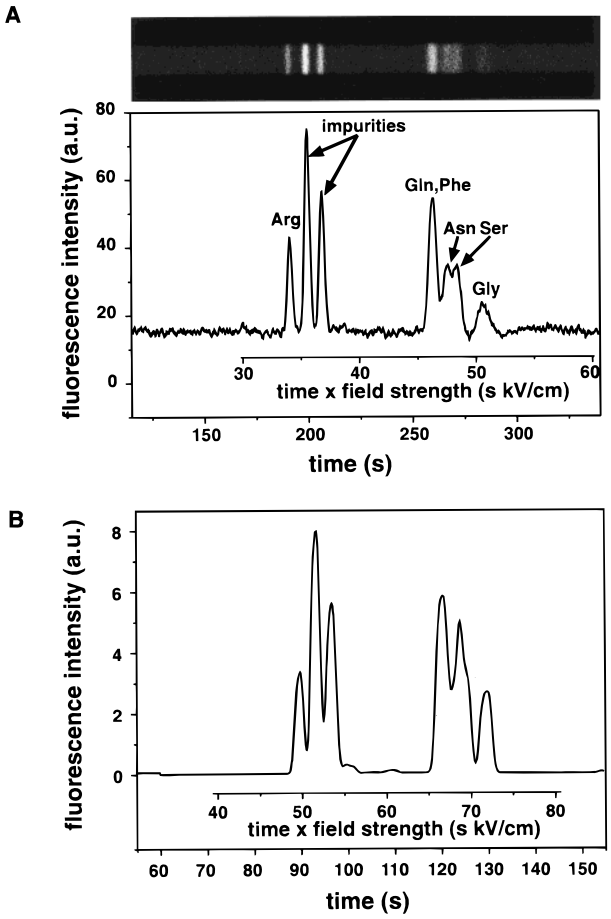

Figure 4. Electropherograms of fluorescence intensity against time of a mixture of six amino acids labeled with FITC, obtained in (A) an oxidized PDMS channel and (B) a fused silica capillary. A second *x*-axis is shown, i.e., time multiplied by the electric field strength. This correction takes into account differences in the length of the capillaries and separation voltages in A and B. In A, the network of channels in Figure 2A was used, i.e., the separation channel was 28 cm long. The cross section of the oxidized PDMS channels was 50 μm × 50 μm. The plug of injected sample was 150 μm long (~400 pL). The concentration of each amino acid in the injection sample was 10 μM in 100 mM Tris−20 mM boric acid (pH 9), and the ratio of FITC to amino acid was 1:100. Tris−boric acid was also used as the running buffer. The separation voltage was 5 kV. A laser beam (488 nm) was focused on the separation channel 21 cm from the injection point during acquisition of the electropherogram, and fluorescence was detected by a photomultiplier tube after passing through a band-pass filter at 520 nm. For each datum, the fluorescence signal from a 2-μm length of the channel was integrated for 70 ms; the image above the electropherogram in A shows the fluorescence from the oxidized PDMS channel as a function of time. In B, the same sample was separated in the same buffer, in a fused silica capillary with a 50-μm internal circular diameter. The separation voltage was 15 kV. The capillary was 27 cm long; the detector was 20 cm from the input end of the capillary. The assignment of the peaks was determined from CE experiments on the individual amino acids on fused silica capillary.

electroosmotic and electrophoretic mobilities:[56]

$$\mu_{meas}(\text{PDMS}) = \mu_{os}(\text{PDMS}) - \mu \quad (1)$$

$$\mu_{meas}(\text{silica}) = \mu_{os}(\text{silica}) - \mu \quad (2)$$

where μ is the electrophoretic mobility of the amino acid. μ is

determined by the mass, charge, and shape of the analyte molecules[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 μ_{os}(silica), eqs 1 and 2 yield μ_{os}(PDMS). The electropherogram of a neutral molecule (μ = 0), *p*-methylbenzyl alcohol, detected by UV absorption, in the same fused silica capillary and buffer showed that μ_{os}(silica) = 6.2×10^{-4} cm^2/(V·s). The total mobilities of the amino acids on oxidized PDMS and fused silica were determined from the electropherograms in Figure 4. Substituting values of μ_{os}(silica), μ_{meas}(silica), and μ_{meas}(PDMS) into eqs 1 and 2 yields μ_{os}(PDMS) = 8.2×10^{-4} cm^2/(V·s) at pH 9. As μ_{os} is proportional to the charge density of the walls of the capillary,[56] and the charge density of fused silica is about 10^{-2} C/m^2 at this pH,[56] we conclude that the charge density of oxidized PDMS is $\sim 1.3 \times 10^{-2}$ C/m^2.

(c) Separation of a Charge Ladder of Carbonic Anhydrase. Our group has used charge ladders[79−81] of carbonic anhydrase (EC 4.2.1.1) as a model system to study the effect of the charge of a protein on the free energy of the binding of inhibitors,[82] to evaluate coatings of capillaries,[83] to determine the mass and charge of carbonic anhydrase,[84] and to determine the pK_a of the N-terminal α-amino group of carbonic anhydrase.[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".[79−81] Charge ladders are most conveniently formed by the partial acetylation of the ϵ-amino groups of the lysine residues of proteins.

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 ϵ-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

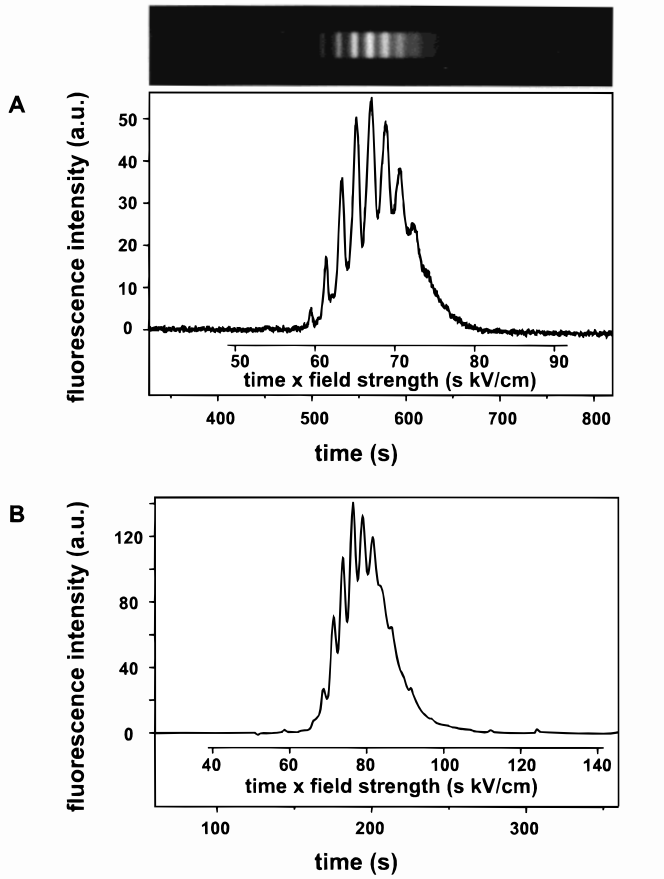

Figure 5. Electropherograms of a charge ladder of BCA obtained in (A) an oxidized PDMS channel and (B) a fused silica capillary. The fluorescent charge ladder was created by modifying the ϵ-amino groups of the lysine groups of BCA first with 0.5 equiv of F-NHS and then with 20 equiv of acetic anhydride. The sample used for injection contained 3 mg/mL of modified BCA. In A, the 42-cm-long separation channel (Figure 2B) was filled with 25 mM Tris−192 mM Gly buffer (pH 8.4); the separation voltage was 5 kV. The image above the electropherogram in A shows the fluorescence from the oxidized PDMS channel 35 cm from the injection point as a function of time. In B, the same sample was separated in the same buffer; the separation voltage was 15 kV. The 50-μm-i.d. fused silica capillary was 37 cm long; the detector was 30 cm from the input end of the capillary.

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-quinuclidinopropanesulfonate (QPS),[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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(86) Mammen, M.; Gomez, F.; Whitesides, G. M. *Anal. Chem.* **1995**, *67*, 3526.

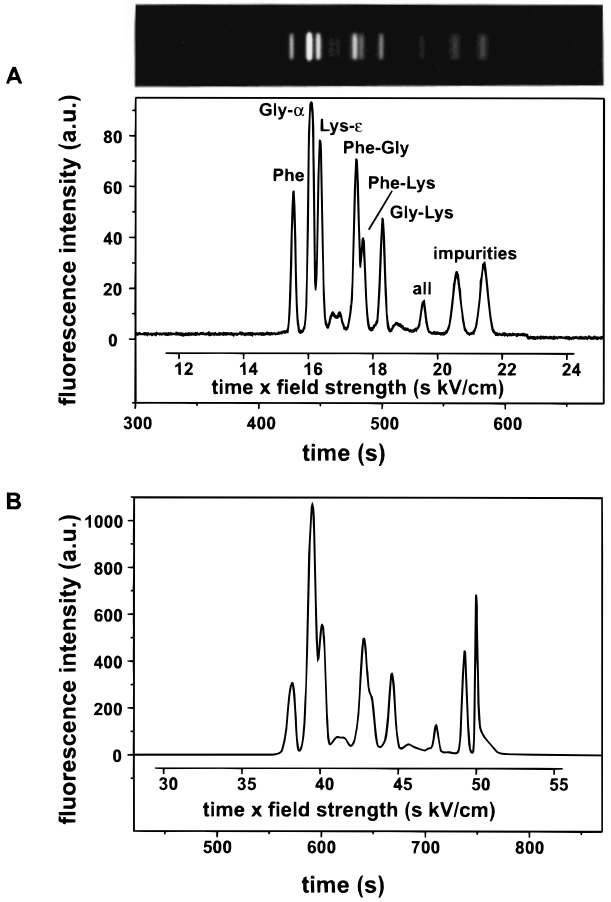

Figure 6. Electropherograms of a charge ladder of insulin obtained in (A) an oxidized PDMS channel and (B) a fused silica capillary. The fluorescent charge ladder was created by modifying insulin in phosphate buffer with 8 equiv of F-NHS. This sample was diluted for injection to 0.5 mg/mL protein in 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.

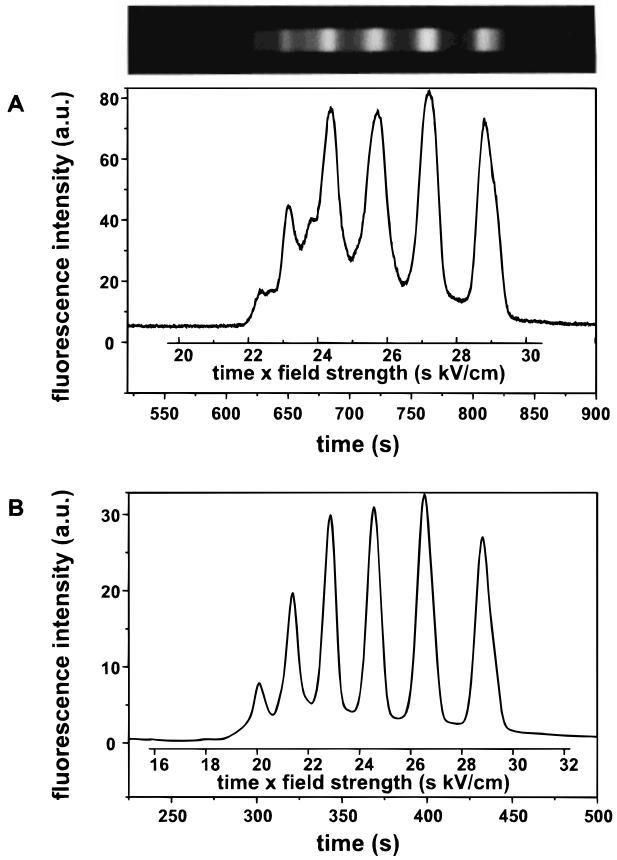

Figure 7. Electropherograms of a charge ladder of a positively charged protein, lysozyme, obtained in (A) an oxidized PDMS channel coated with Polybrene and (B) a fused silica capillary coated with Polybrene. The fluorescent charge ladder was created by modifying lysozyme with 0.5 equiv of F-NHS and 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.

Insulin has three amino groups that can be modified on the Phe (α-amino, pK_a = 7.1), Gly (α-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.[85] 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.[85] 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.[56,83] We have separated a charge ladder of a protein, lysozyme (pI = 10.9), that is positively charged in 25 mM Tris−192 mM Gly (pH 8.4), in oxidized PDMS channels by coating the negatively charged walls with a cationic polymer, Polybrene.[83] 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 the 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. The resolution obtained from the PDMS device (Figure 7A) is slightly poorer, however, than that from a fused silica capillary (Figure 7B).

(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.[50,52] The sample was separated in a buffer containing a sieving matrix, hydrox-

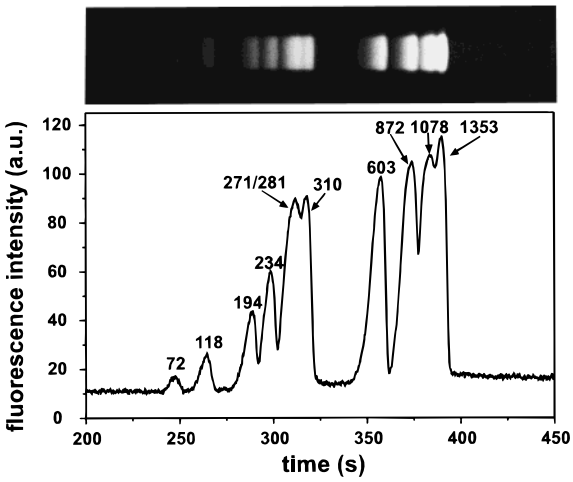

Figure 8. Electropherogram of the ϕX-174/*Hae*III DNA restriction fragments intercalated with YOYO-1 fluorescent dye separated in an oxidized PDMS channel. The network of channels, laser position, and separation voltage were as described in Figure 4. The separation buffer contained 40 mM Tris, 40 mM acetic acid, 1 mM Na_2EDTA, 10 μM 9-aminoacridine, and 0.75% (w/v) hydroxypropylcellulose (pH 8). The DNA concentration was 100 ng/μL; the base pair-to-YOYO-1 ratio was 1:10. The number of base pairs in each fragment is labeled.

ypropylcellulose, to resolve the DNA fragments on the basis of size. The migration of positive and negative fluorescent molecules indicated that EOF in the oxidized PDMS channels was greatly reduced, or even eliminated, by the presence of the polymer in the buffer.

We resolved 10 of the 11 fragments of the digest of DNA using the oxidized PDMS chip. The resolution is slightly inferior to those in untreated PDMS channels; Effenhauser et al.[52] resolved all 11 peaks. We believe broader peaks were observed in our experiment in part because of the high concentrations (100 ng/μL) of DNA used; Mathies also observed a poorly resolved electropherogram at this same concentration.[87] We used 100 ng/μL DNA to generate sufficient signal to observe the weaker fragments of the digest of DNA, rather than the 1 ng/μL recommended by Mathies[87] and Effenhauser[52] to optimize resolution. When we carried out separations at lower DNA concentrations, the three strongest peaks were better resolved, but the weaker peaks were not observed.

CONCLUSIONS

We have developed a system for fabricating networks of channels (>20 μm width) rapidly and at low cost, based on rapid prototyping of masters using high-resolution printing and contact lithography, molding of PDMS, and contact sealing of oxidized PDMS surfaces. The entire fabrication process, from concept to realization, takes less than 24 h; these methods significantly reduce the time to develop a working prototype of a microfluidic system.

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 μTAS 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,[88] and to characterize the flow in devices, such as fluidic rectifiers.[88] 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.[73,74] Materials used to fabricate μTAS 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.[52] 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 μTAS. 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, Macromedia, San Francisco, CA). High-resolution (3386 dpi) transparencies were produced by a commerical 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, Microlithography 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 vacuum for 2 h with a vial containing a few drops of tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (United Chemical Technologies, Bristol, PA); silanization of the master facilitates the removal of the PDMS replica after molding.

(b) Molding. A 10:1 mixture of PDMS prepolymer and curing agent (Sylgard 184, Dow Corning, Midland, MI) was stirred thoroughly and then degassed under vacuum. Glass posts were placed on the master to define reservoirs. The prepolymer mixture was poured onto the master and cured for 1 h at 65 °C. After curing, the PDMS replica was peeled from the master, and the glass posts were removed. Flat pieces of PDMS were formed by casting prepolymer against a silanized silicon wafer and curing.

(c) Sealing. A PDMS replica and a PDMS flat were rinsed in ethanol and dried under a stream of argon. The two pieces of PDMS were placed in a plasma cleaner (PDC-23G, Harrick, Ossining, NY) and oxidized for 1 min. Immediately after removal from the plasma cleaner, the substrates were brought into conformal contact, and an irreversible seal formed spontaneously. To seal PDMS to other polymers, the same cleaning and oxidizing procedures were performed. To seal PDMS against nonpolymeric materials, such as glass and silicon, the same procedure was adopted, but the nonpolymeric substrate was first cleaned in heptane before rinsing in ethanol.

Capillary Electrophoresis. (a) Preparation of Samples. *Amino Acids.* Arg, Asp, Gly, Glu, Phe, and Ser (Sigma. St. Louis, MO) were labeled with FITC (Molecular Probes, Eugene, OR) using a procedure described previously.[20,31] Briefly, 10 μL of 1 mM FITC in acetone was added to 1 mL of 1 mM amino acid in 100 mM Tris−20 mM boric acid (pH 9) buffer. An injection sample was prepared by adding 10 μL of each sample of FITC-labeled amino acids to 940 μL of buffer. Tris−boric acid was also used as the running buffer.

Charge Ladder of BCA. Two hundred microliters of 3 mg/mL BCA (Sigma) in 80 mM borate (pH 8.9) was mixed with 2 μL of 5 mM F-NHS (Molecular Probes) in DMF (0.5 equiv). After 30 min, 10 μL of 40 mM acetic anhydride in dioxane (20 equiv) was added to this mixture. This sample was purified on a NICK spin column containing G-50 Sephadex gel (Pharmacia Biotech, Piscataway, 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 Na_2HPO_4−50 mM NaH_2PO_4 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, NJ) 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/*Hae*III digest of DNA (Pharmacia) was taken from the literature.[50,52] 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 Na_2EDTA buffer (pH 8). Then 3.1 μL of 1 mM YOYO-1 (Molecular Probes) in DMSO was added to the solution; the base pair-to-YOYO-1 ratio was 10:1. 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.

(b) Pretreatment 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.

(c) 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 (CZE1000R, 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.*

(d) Detection. Fluorescent samples were detected by a scanning laser-induced fluorescence (LIF) microscope (TCS4D confocal scanner on a DMRBE 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 μm × 1000 μm to align and focus the laser beam. During acquisition of data, the fluorescence signal from a 2-μm-long section of the 50-μm-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-μm-i.d/365-μm-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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