Review

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Fabrication of microfluidic systems in poly(dimethylsiloxane)

Microfluidic devices are finding increasing application as analytical systems, biomedical devices, tools for chemistry and biochemistry, and systems for fundamental research. Conventional methods of fabricating microfluidic devices have centered on etching in glass and silicon. Fabrication of microfluidic devices in poly(dimethylsiloxane) (PDMS) by soft lithography provides faster, less expensive routes than these conventional methods to devices that handle aqueous solutions. These soft-lithographic methods are based on rapid prototyping and replica molding and are more accessible to chemists and biologists working under benchtop conditions than are the microelectronics-derived methods because, in soft lithography, devices do not need to be fabricated in a cleanroom. This paper describes devices fabricated in PDMS for separations, patterning of biological and nonbiological material, and components for integrated systems.

Keywords: Microfluidics / Rapid prototyping / Poly(dimethylsiloxane) / Review EL 3710

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Abbreviations: CAD, computer-aided design; **PDMS**, poly(dimethylsiloxane)

1 Introduction

Microfluidics, the manipulation of liquids and gases in channels having cross-sectional dimensions on the order of 10–100 μm, will be a central technology in a number of miniaturized systems that are being developed for chemical, biological, and medical applications. These applications can be categorized into four broad areas: miniaturized analytical systems, biomedical devices, tools for chemistry and biochemistry, and systems for fundamental research. In order for these systems to be successful, they must have the attributes that are required for the particular application – *e.g.*, optical properties and surface chemistry – and they must also be fabricated in materials that are inexpensive and rugged and use processes that are amenable to manufacturing. Here, we review the design, fabrication, and applications of microfluidic devices in one material – poly(dimethylsiloxane) (PDMS) – that shows particular promise in the fabrication of systems for biological and water-based applications. We begin by discussing the motivation underlying the development of microfluidic systems. We then describe the fabrication of microfluidic systems in PDMS by casting the polymer

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against models that are usually created by using photolithography. We emphasize systems fabricated by using a technique we call rapid prototyping [1, 2], which combines high-resolution commercial printing, photolithography, and soft lithography, and allows microfluidic systems to be designed and fabricated rapidly and inexpensively. We then discuss applications of microfluidic systems in PDMS that have been developed in separations, patterning of biological and nonbiological materials, and components for integrated systems.

1.1 Motivation behind the development of microfluidic devices

Microfluidic systems have the potential for wide application (Table 1). Miniaturization of devices for use in these areas leads to many benefits, including decreased cost in manufacture, use, and disposal; decreased time of analysis; reduced consumption of reagents and analytes; reduced production of potentially harmful by-products; increased separation efficiency; and increased portability. In addition, some studies are difficult or impossible in larger-scale devices. For example, microfluidic channels can approximate the size and flow conditions (~ 10 μm, 0.1 cm/s) found *in vivo* in capillaries [3]; use of research and diagnostic devices of the same sizes and with similar elasticity as found in biology could lead to more accurate information and greater understanding of physiology. In addition, smaller channels increase resolution while decreasing the overall size of the device, but small channels also make detection more demanding, are susceptible to blockages from particles, and are more sensitive to adsorption of species on the surface [4].

1.2 Historical background of the development of microfluidic systems

The first microfluidic device was a miniaturized GC developed at Standord University in the 1970s [5]. Although this miniaturized GC system was not developed further, the growth of molecular biology, especially genomics, has stimulated the development of technology for the analysis of complex mixtures of macromolecules, especially DNA and proteins, in aqueous solutions by CE and LC. Microfluidic systems that analyzed aqueous solutions developed originally in four laboratories: those of Manz [4, 6–9], Harrison [10–15], Ramsey [16–21], and Mathies [22–25]. Most of these early systems were fabricated by technology derived from microelectronics – photolithography and etching in silicon and glass – because these technologies were available and highly developed. Silicon is, however, a relatively expensive material; it has the further

Table 1. Potential applications of microfluidic devices

Area	Application
Miniaturized analytical systems	
Genomics and proteomics	Rapid, high density sequencing [22, 23, 25], DNA fingerprinting, combinatorial analysis, forensics, gene expression assays, integration of fluidics with DNA arrays
Chemical/biological warfare defense	Early detection and identification of pathogens and toxins; early diagnosis; triage
Clinical analysis	Rapid analysis of blood and bodily fluids [72, 73], point of care diagnostics based on immunological [14, 28, 74] or enzymatic assays [21], electrochemical detection, and cell counting [57]
High throughput screening	Combinatorial synthesis and assaying for drugs. Toxicological assays [8, 75]
Environmental testing	*In situ* analysis of environmental contamination [76]
Biomedical devices	
Implantable devices	Devices for *in vivo* drug delivery [36], *in vivo* monitoring for disease and conditions
Tools for chemistry and biochemistry	
Small-scale organic synthesis	Combinatorial synthesis [77]
Sample preparation	Purification of biological samples for further analysis [15]
Amplification of nucleic acids/sequences	PCR [9, 17, 31, 78, 79], RT-PCR
Systems for fundamental research	
Systems with which to study the flow of fluids	Studies on EOF and laminar flow in small channels [80], studies of diffusion
Studies of chemical reactions	Enzyme-substrate
Biomimetic systems	Development of machines that mimic biological functions
Systems to study small amounts of sample	Detection of single molecules [53, 58, 81]

disadvantage that it is opaque in the visible/UV region of the spectrum, thus making it unsuitable for systems that use optical detection. Glass is transparent, but because it is amorphous, vertical side walls are more difficult to etch than in Si. Although batch processing of both silicon and glass is possible, the commonly used processes for sealing these materials require that each device be made in a cleanroom environment. These sealing processes also typically require high voltages or temperatures. Glass and oxidized silicon, however, have desirable surface characteristics: they possess a negative charge and support electroosmotic flow (EOF), and the channels are fabricated by etching, which cleans the surfaces as it produces the channels. Glass systems have proved especially successful when applied to separating and sequencing DNA [22–26], but when used with proteins, adsorption can be a problem.

1.3 New materials for the fabrication of devices

Since the early work in the field, there has been a rapid expansion [27–37] into new types of materials, especially polymers [38–45]. Polymers, in contrast to silicon and glass, are inexpensive; channels can be formed by molding or embossing rather than etching; and devices can be sealed thermally or by using adhesives. The disadvantages of polymers are that more care must be taken to control their surface chemistry than with glass or silicon; they are often incompatible with organic solvents and low molecular weight organic solutes; and they are generally incompatible with high temperatures.

Our own work in microfluidics has focused on polymer systems made of PDMS. PDMS is an excellent material for the fabrication of microchannel systems for use with biological samples in aqueous solutions for a number of reasons: (i) features on the micron scale can be reproduced with high fidelity in PDMS by replica molding; (ii) it is optically transparent down to 280 nm so it can be used for a number of detection schemes (*e.g.*, UV/Vis absorbance and fluorescence); (iii) it cures at low temperatures; (iv) it is nontoxic; mammalian cells can be cultured directly on it; and devices made from it can be implanted *in vivo*; (v) it can be deformed reversibly; (vi) it can seal reversibly to itself and a range of other materials by making molecular (van der Waals) contact with the surface, or it can seal irreversibly after exposure to an air plasma by formation of covalent bonds (see Section 2.4); (vii) its surface chemistry can be controlled by reasonably well-developed techniques; and (viii) because it is elastomeric, it will conform to smooth, nonplanar surfaces, and it releases from delicate features of a mold without damaging them or itself.

2 Fabrication of microfluidic systems in PDMS

New methods and materials for fabricating microfluidic systems are needed because etching in Si and glass is too expensive and time-consuming. Some important issues to consider in a method of fabrication are the speed at which designs can be reduced to working devices and evaluated, the design parameters such as channel size and geometry, and the availability of required components, *e.g.*, for injection, separation, or detection. In this section we discuss methods for fabrication of devices in PDMS, how to address certain aspects of fabrication – sealing and surface chemistry – when using PDMS, and the design of devices in PDMS.

2.1 Soft lithography

Our approach to the fabrication of microfluidic devices is based primarily on the techniques of soft lithography [2, 46, 47], specifically rapid prototyping and replica molding. Soft lithography is a suite of nonphotolithographic methods for replicating a pattern. An elastomeric structure with the patterns embedded as a bas-relief on the surface acts as the pattern transfer agent. These methods have the characteristic that routine access to a cleanroom is not necessary when producing most structures relevant to microfluidics (20–100 μm). They also enable pattern transfer to curved materials. We use these techniques in the fabrication of channels in bulk polymer.

2.2 Rapid prototyping

Rapid prototyping begins with creating a design for a device in a computer-aided design (CAD) program. A high-resolution commercial image setter then prints this design on a transparency. This transparency serves as the photomask in contact photolithography to produce a positive relief of photoresist on silicon wafer (Fig. 1). We refer to this positive relief as a “master”; it is used for the casting of PDMS devices. A master in SU-8 photoresist, a photocurable epoxy, [48] on a silicon wafer is durable and can be used indefinitely; failure usually occurs from the user breaking the fragile silicon wafer or from the photoresist releasing from the wafer. Replication of the master as one piece in a hard polymer, *e.g.*, structural polyurethane or epoxy, can further extend its lifetime.

The hallmark of rapid prototyping is the reduction in time and cost for a cycle of design, fabrication, and testing of new ideas compared to methods that use a chrome mask in the photolithographic step. The chrome mask that the transparency replaces can be 20–100 times more expensive and can take weeks compared to hours to obtain.

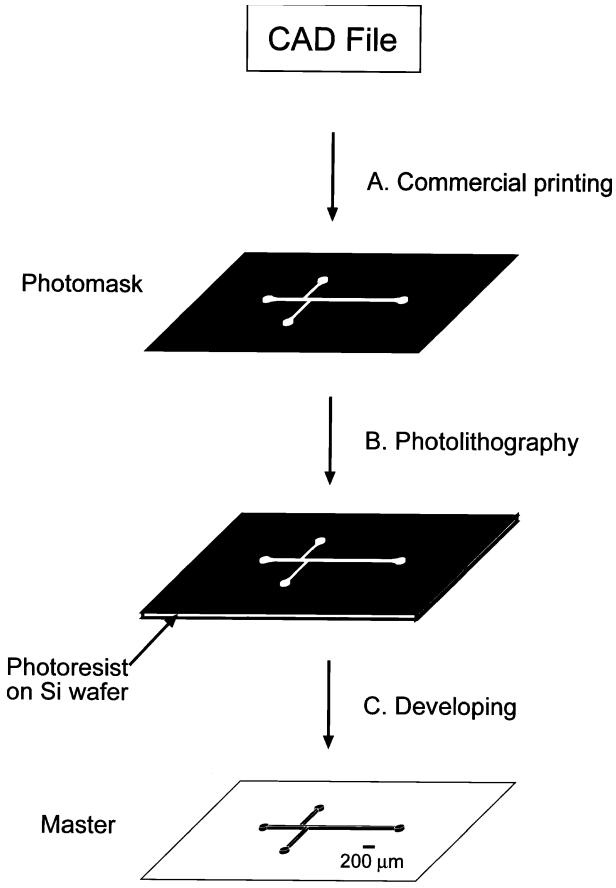

Figure 1. Scheme for rapid prototyping and replica molding of microfluidic devices in PDMS. A design for channels is created in a CAD program. (A) This file is printed on a high-resolution transparency. (B) The transparency then serves as the photomask in contact photolithography. (C) Dissolving away the unpolymerized photoresist leaves a positive relief that serves as a master.

The drawback is that the resolution of the transparency is lower (> 20 μm) than that of a chrome mask (~ 500 nm). Access to image setters with higher resolution (> 3386 dpi) would decrease the size of features obtainable with rapid prototyping. The channel or capillary diameter for most applications, however, ranges from 50–100 μm, which is within the capacity of rapid prototyping. For devices that require features smaller than 20 μm, a chrome mask needs to be used. The resolution of the transparency also leads to two walls with rough edges (Fig. 2). Experiments show that this edge roughness does not compromise resolution for 50 × 50 μm channels compared to separations in fused silica capillaries with circular cross sections [1].

2.3 Replica molding

Once a master is fabricated, we form channels in PDMS by replica molding. Replica molding is simply the casting

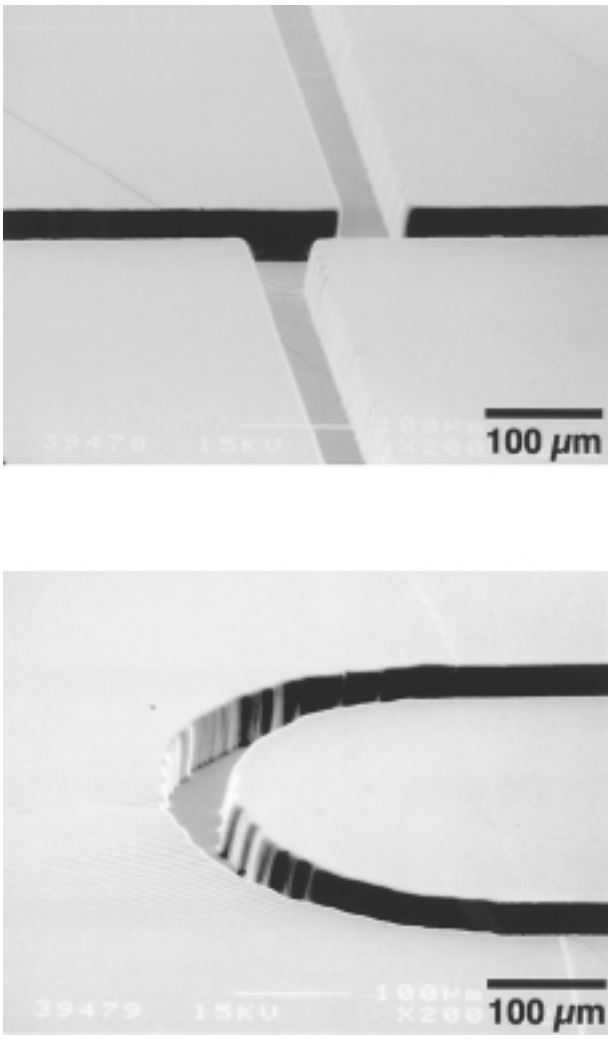

Figure 2. Scanning electron micrograph of 50 × 50 μm^2 channels of a miniaturized CE device that was created by molding PDMS against a photolithographic master. The top image shows straight sections of the channel. The bottom image shows a curve in the channel. The roughness of the vertical side walls arises from the resolution (3386 dpi) of the transparency used to create the channels. This edge roughness is more pronounced in the curved section. Reprinted from [1], with permission.

of prepolymer against a master and generating a negative replica of the master in PDMS, *i.e.*, ridges on the master appear as valleys in the replica (Fig. 3). The PDMS is cured in an oven at 60°C for 1 h, and the replica is then peeled from the master. Access holes for channels and reservoirs for buffer can be added in the replication step by appropriate placement of posts on the master or punched out of the cured layer by using a borer. Although we use masters produced in photoresist from rapid prototyping, masters can be fabricated by many techniques including etching in silicon, electroforming metal, or con-

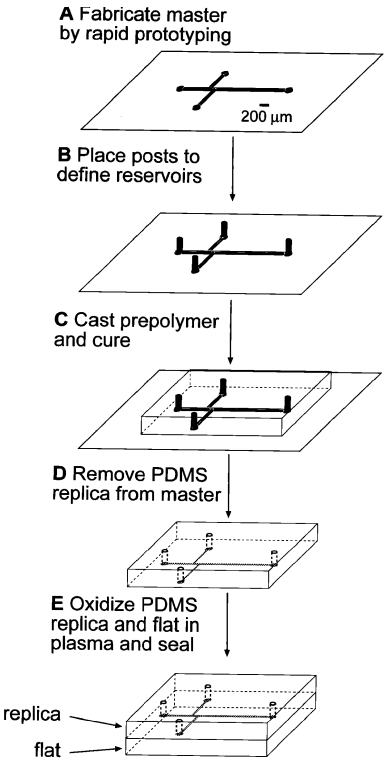

Figure 3. Scheme describing replica molding of microfluidic devices. (A) A master is fabricated by rapid prototyping. (B) Posts are placed on the master to define reservoirs. (C) The prepolymer is cast on the master and cured. (D) The PDMS replica is removed from the master. (E) Exposing the replica and an appropriate material to an air plasma and placing the two surfaces in conformal contact makes a tight, irreversible seal. Reprinted from [1], with permission.

ventional machining of other hard materials. The composition of the master used in production may depend on the production run. Masters made from metal or other hard materials may be used in manufacturing when the production run is large because of their durability; the expense of the master becomes negligible after many uses. For prototyping new devices and for limited run production, however, metal or silicon molds are time-consuming and expensive to make, especially in a program of research and development where several iterations are necessary for the development of a final design.

2.4 Sealing

Molding provides a PDMS replica that contains three of the four walls necessary for enclosed channels. Sealing the replica to a flat surface provides the fourth wall. This flat material can be PDMS, to give channels in which all four walls are made from the same material, or another material. Sealing occurs in two ways: (i) reversible, conformal sealing with a flat surface, and (ii) irreversible sealing to certain substrates upon exposure of both surfaces to an air plasma [49]. Reversible sealing occurs because PDMS is flexible and can conform to minor imperfections in a "flat" surface making van der Waals contact with this surface. This method of sealing is watertight and fast and occurs at room temperature. Simply peeling the PDMS off the flat surface breaks this reversible seal. It does not withstand high pressures (> 5 psi) in the capillaries. Removal of the PDMS leaves little or no residue on the other material, and resealing can occur numerous times without degradation in the PDMS.

PDMS comprises repeating units of $–O–Si(CH_3)_2–$. Exposing a PDMS replica to an air plasma introduces polar groups on the surface. We believe the plasma introduces silanol groups (Si-OH) at the expense of methyl groups ($Si\text{-}CH_3$) [49–51]. We believe these silanol groups then condense with appropriate groups (OH, COOH, ketone) on another surface when the two layers are brought into conformal contact. For PDMS and glass, this reaction yields Si-O-Si bonds after loss of a water. These covalent bonds form the basis of a tight, irreversible seal: attempting to break the seal results in failure in the bulk PDMS [1, 49]. The seal withstands pressures of 30–50 psi. It is possible to seal PDMS irreversibly to the surfaces of a number of materials: PDMS, glass, Si, SiO_2, quartz, silicon nitride, polyethylene, polystyrene, and glassy carbon [1]. This method, however, does not work with all polymers, *e.g.*, Saran, polyimide, poly(methylmethacrylate), and polycarbonate [1].

2.5 Surface chemistry

One of the most important issues in the selection of an appropriate material for a device is surface chemistry. Unmodified PDMS presents a hydrophobic surface. Channels in hydrophobic PDMS are difficult to wet with aqueous solutions, are prone to the adsorption of other hydrophobic species, and easily nucleate bubbles. Exposure to plasma oxidation, however, renders the surface hydrophilic because of the presence of silanol groups. Aqueous solutions then easily wet these oxidized channels. In addition, the presence of silanol groups on the walls of the channels provides ionizable groups ($SiOH \leftrightarrow SiO^- + H^+$) that, when in contact with neutral or basic solutions, support a strong EOF towards the cathode that can be used for capillary zone electrophoresis (CZE). These negatively charged channels have greater resistance to adsorption of hydrophobic and negatively charged analytes than unmodified PDMS, but some proteins still adsorb on the surface [1]. Adsorption of charged and neutral polymers [1] and covalent attachment of trichlorosilanes [49, 50] on oxidized PDMS can also modify the sur-

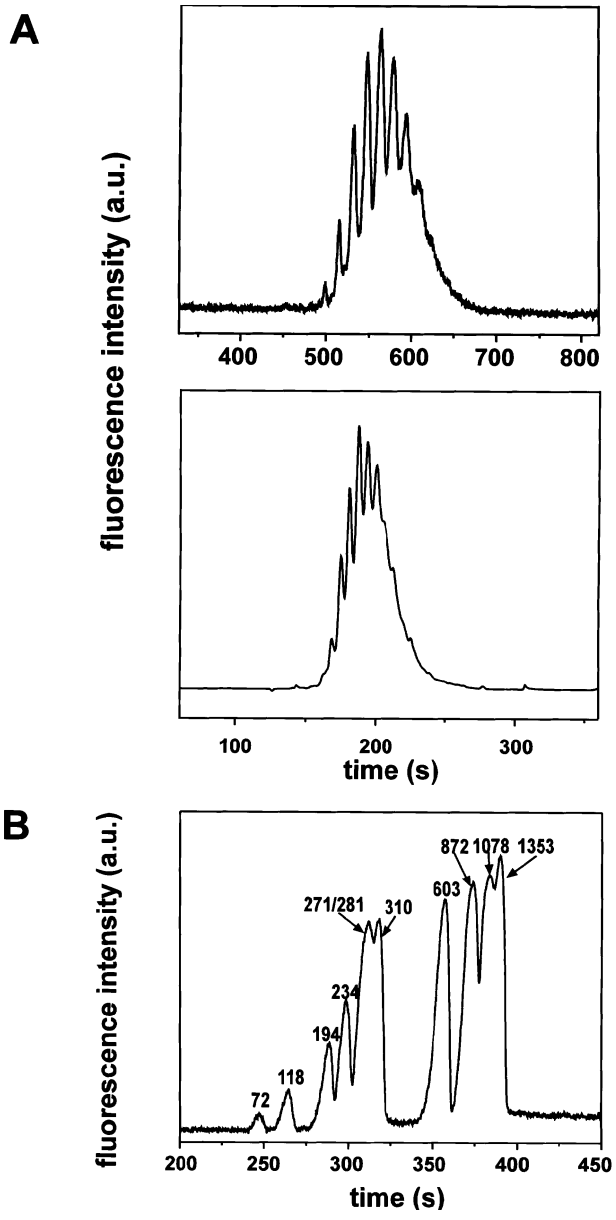

Figure 4. (A) Electropherograms of a fluorescently labeled charge ladder of bovine carbonic anhydrase. The charge ladder was formed by modifying the ε-amino groups of lysine residues of the protein first with 0.5 eq. of 5-carboxyfluorescein, succinimidyl ester and then with 20 eq. of acetic anhydride. The sample injected was 3 mg/mL of modified protein. The running buffer was 25 mM Tris-192 mM glycine, pH 8.4. In the miniaturized CE device (top), the oxidized PDMS channel was 42 cm long with a cross-sectional area of 50 × 50 μm, the sample was detected 35 cm from the point of injection, and the separation voltage was 5 kV. In the commercial CE (Beckman P/ACE 5000; bottom), the 50 μm ID fused silica capillary was 37 cm long, the detector was 30 cm from the point of injection, and the separation voltage was 15 kV. The resolution obtained with the PDMS device compared favorably to the resolution obtained with the commercial instrument. (B) Electropherogram of the ϕX-174/*Hae*III DNA restriction fragments intercalated with YOYO-1 fluorescent dye separated in an oxidized PDMS channel that had a cross-sectional area of 50 × 50 μm and was 28 cm long. The sample was detected 21 cm from 6 the injection point, and the separation voltage was 5 kV. The separation buffer contained 40 mM Tris, 40 mM acetic acid, 1 mM Na_2EDTA, 10 μM 9-aminoacridine, and 0.75% w/v hydroxypropyl cellulose (pH 8). The DNA concentration was 100 ng/μL; the base pair to YOYO-1 ratio was 1:10. This high concentration of DNA caused the relatively poor resolution in the separation [82], but when lower concentrations were used, the smaller fragments were no longer detectable with our instrument. The number of base pairs in each fragment is indicated. Adapted from [1].

face properties of PDMS. The oxidized surface of PDMS is unstable in air and reverts to being hydrophobic in ~30 min. Keeping the oxidized PDMS in contact with a polar liquid, however, protects the surface although the long-term stability of the oxidized layer is unknown [1, 49, 51].

2.6 Design of microfluidic systems

Each application requires different components in a device, *e.g.*, those for injection, separation, detection, heating, mixing, and post-treatment. The design of a device must therefore take into account how to fabricate these components and how to move samples from one component to the next. In general, components with few moving parts are desirable since these parts complicate fabrication and can break or become clogged. Usually EOF or pressure is used to move fluids, although some workers have used ultrasonic, bubble, and rotary pumps [52]. PDMS is an excellent material for EOF and pressure pumping since its surface can be charged and, when sealed irreversibly, can withstand high pressure. In addition, since PDMS is elastomeric, membrane pumps and check valves are easy to incorporate into a system.

3 Microfluidic systems in PDMS

Several microfluidic systems in PDMS have been developed. Most applications have been in separations, patterning of biological and nonbiological materials on substrates by using channels in PDMS, and components for integrated analytical systems. We consider each of these areas in turn.

3.1 Separations

3.1.1 Miniaturized CE systems

The first miniaturized CE system in PDMS was developed by Effenhauser *et al.* [53]. This system used a commercially obtained positive relief of silicon as the master in replica molding. The device sealed reversibly against a flat piece of PDMS. The walls of the channel were not modified and therefore were hydrophobic and uncharged; the channels thus supported at most a very weak EOF, and negatively charged molecules migrated towards the anode. These workers used a polymer sieving matrix to separate restriction fragments of DNA and fluorescently labeled peptides. They also explored single DNA molecule (many fluorophores) detection limits with λ DNA achieving 50% detection. The greatest advantage of this device was that it could be disassembled, easily cleaned, and reused.

We developed a PDMS device for CE whose properties differ significantly from that of Effenhauser *et al.* [1]. The device was fabricated by using rapid prototyping and was irreversibly sealed by using plasma oxidation. We have successfully separated fluorescently labeled amino acids, and protein charge ladders using CZE, and DNA restriction fragments using a sieving matrix. Figure 4 shows an electropherogram of a charge ladder of carbonic anhydrase obtained in these devices. A charge ladder is a set of modified proteins obtained by successive acylation of lysine amino groups [54, 55]. These separations utilized several surface modifications of PDMS. For the separation of amino acids and negatively charged proteins, no surface modifications other than plasma oxidation were necessary, although for a separation of an insulin charge ladder, a zwitterion had to be added to the running buffer to reduce adsorption of the protein and its derivatives onto the walls [56]. For the separation of a charge ladder of lysozyme – a positively charged protein – the channels were coated with Polybrene®, a polymer containing quaternary amines, to make them positively charged. This modification reduced the adsorption of the positively charged protein to the walls of the channels and reversed the direction of EOF to be toward the anode. The separation, however, also required the addition of a zwitterionic component to the buffer [56]. The sieving matrix used in the separation of restriction fragments of DNA eliminated EOF. The device could be used to separate different samples by flushing the channels between uses. Blockages of the channels, however, were difficult to remove since the device was irreversibly sealed.

3.1.2 Sorting of cells

Bakajin *et al.* [57] used a PDMS device to sort white blood cells. They fabricated a lattice of small channels of varying lengths that mimic the size restrictions imposed on cells by capillaries *in vivo*. They used PDMS because devices fabricated in glass and silicon dioxide were too adhesive for the cells studied. Using this device, they separated two classes of white blood cells, T-lymphocytes and granulocytes, and showed that the cells' passage through the lattice depended strongly on size and nuclear morphology.

3.1.3 Sizing and sorting of DNA

Chou *et al.* [58] developed a microfluidic device for the sizing and sorting of restriction fragments of DNA based on single DNA molecule detection. The device was molded against a silicon master and had features ranging from 5 to 100 μm. The channels were only 3 μm deep, and posts were necessary in the 100 μm sections to prevent the PDMS from sagging and blocking the channels. They rendered the channels hydrophilic by soaking the channels in dilute HCl, which hydrolyzes some of the Si-O-Si bonds, and sealed the device reversibly to a glass cover slip. The reversible seal allowed all data to be taken with the same device. The device sized DNA on the basis of fluorescence intensity from an intercalated dye; individual DNA molecules that were detected had between 500 and 5000 dye molecules intercalated. DNA was pumped through the channels by a mixture of capillary action and EOF. Individual DNA molecules were detected as they passed through the narrow portion (5 μm) of the channel. The size of each molecule was determined by the intensity of the fluorescence. Counting the number of times that each fluorescence intensity (corresponding to a specific number of base pairs) was detected gave the concentration of each restriction fragment. Detection took place at a T-junction; each fragment could therefore go one of two directions after being detected. The fragments could be sorted by steering them down one of the possible paths using electric fields.

3.2 Patterning of biological and nonbiological materials on substrates

Controlled deposition or removal of material from substrates finds application in many areas. Patterned deposition of materials in small (< 100 μm) features is important

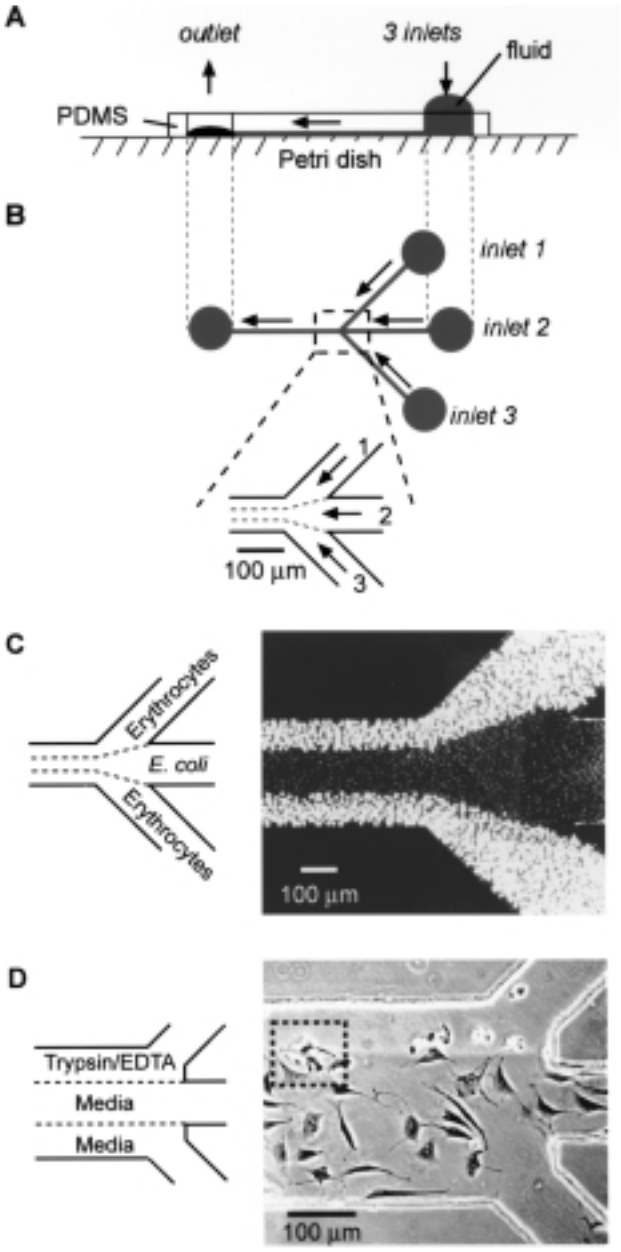

Figure 5. Laminar flow patterning of biological materials. (A) Side view of a PDMS membrane containing microscopic channels and reversibly sealed to a polystyrene Petri dish. Fluids were pumped by gentle aspiration at the outlet or by pressure from the inlets. (B) Top view of the channels. The closeup shows the paths that the flows from the three inlets take in the main channel. (C) Two different types of cells, chicken erythrocytes and *E. coli*, can be patterned next to each other. A suspension of chicken erythrocytes was placed in inlets 1 and 3, and PBS in inlet 2, and allowed to flow by gravitational force for 5 min followed by a 3 min wash with PBS; these flows patterned the outer lanes. Next, a suspension of *E. coli* in inlet 2 and PBS in inlets 1 and 3 were allowed to flow for 10 min followed by a 3 min wash with PBS. This flow patterned the middle lane. Cells adhered by nonspecific adsorption and were visualized by fluorescence microscopy. (D) Patterned detachment of bovine capillary endothelial cells with trypsin/EDTA. Cells adhered and spread in a fibronectin-coated region. Trypsin/EDTA and media were then allowed to flow from the designated inlets. Trypsin/EDTA caused the detachment of cells where it flowed. The patterning is well defined, and it was possible to detach only portions of a cell (dashed box). The cells were visualized by phase contrast microscopy. Adapted from [63].

for applications of miniaturized systems in biochemistry and cell biology. Many assays are based on attaching ligands to a surface and determining the locations of binding of species of interest. Patterned attachment of cells is also important in cell-based sensors where the reaction of cells to stimuli in specific areas of a device is necessary for detection of species of interest. There are several options for patterning materials from solutions, including ink-jet printing, stamping, patterning from fluids through stencils, and patterning in capillaries. We focus on patterning in capillaries by using microfluidic systems. This method relies on the reversible sealing of a network of channels in PDMS directly on the surface to be patterned. Fluids containing the patterning species flow through these channels and pattern the surface through covalent attachment, adsorption, or dissolution of material already present.

3.2.1 Patterning of cells and proteins

Delamarche *et al.* [59, 60] first reported the use of channels in a PDMS replica that was reversibly sealed to an underlying substrate to pattern proteins on the substrate. These workers used channels with cross sections of 1.5 μm × 3 μm to pattern mm^2 areas; the channels allowed for simultaneous patterning of different proteins

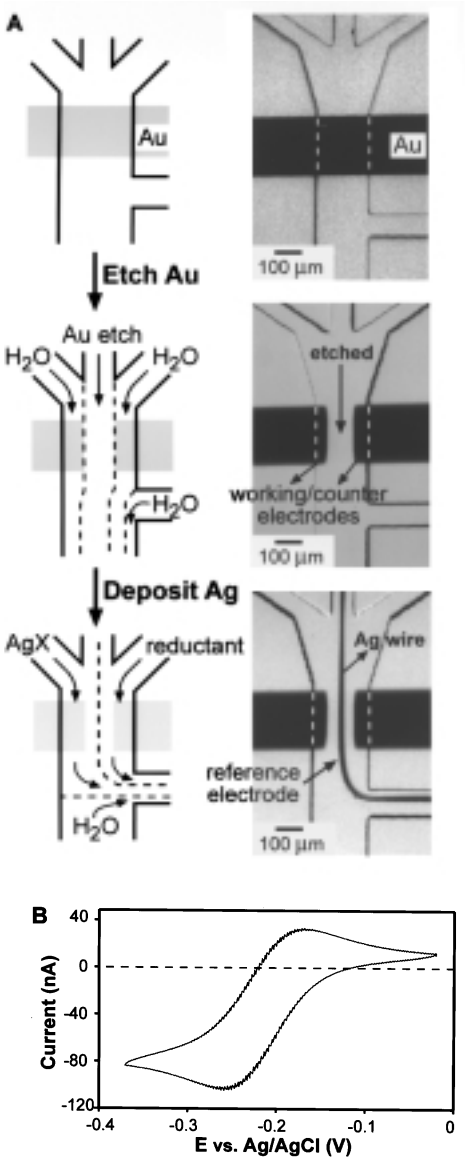

Figure 6. (A) Scheme for the fabrication of a three-electrode detector by using laminar flows. Two gold electrodes are fabricated by selectively etching an evaporated stripe of gold. The silver reference electrode was fabricated at the interface of flows of silver ion and reductant. (B) Cyclic voltammogram of 5 nL of 2 mM $Ru(NH_3)_6Cl_3$ in 0.1 M NaCl as recorded by the three-electrode system (scan rate = 100 mV/s). Adapted from [64].

in well-defined areas adjacent to each other. They also demonstrated the use of these patterns in bioassays. One important aspect of this work was the wettability of the PDMS surface and the propensity for the surface to adsorb proteins. In nonflowing systems, the substrate was patterned only about 100 μm from the inlet. In that distance, all of the protein had adsorbed onto the walls of the channel (three walls of PDMS and one wall of substrate) leaving no reagent to couple to the surface. The workers overcame the problem by creating flowing systems that replenished the supply of protein available to couple to the surface. They also used bovine serum albumin as a blocking agent to prevent protein adsorption on the PDMS walls; the albumin also has the benefit of rendering hydrophobic PDMS slightly hydrophilic [60].

Folch and Toner [61] and Folch *et al.* [62] used PDMS microchannels to pattern proteins and cells. They used deeper channels (20–30 μm) than Delamarche *et al.* [59, 60] in order to slow transport of material. In one experiment, they nonspecifically patterned collagen or fibronectin on a variety of substrates and then seeded cells on these patterns. A second culture with a different cell type was possible simply by seeding the areas not already patterned with cells that need no specific protein to promote adhesion [61]. They also used the microchannels to pattern cells directly on a substrate [62]. Using this method, they could pattern two different types of cells simultaneously [62].

Takayama *et al.* [63] have also used microfluidic channels to pattern proteins and cells. Their method differed from previous approaches, however, in that they used a single channel to pattern multiple species simultaneously. This approach relies on the fact that flow in these channels is laminar. The nature of a flow of fluid is characterized by the Reynolds number, $R_e = \rho v l/\eta$, where ρ is the density of the fluid, v is the velocity of the flow, l characterizes the shape and dimensions of the channel, and η is the viscosity of the fluid. Flows that have $R_e < 2000$ are laminar. When two or more streams with low R_e are combined into a single stream, the combined streams flow side by side without turbulent mixing; mixing occurs only by diffusion. By combining two or more streams containing different patterning materials, stripes of these materials can be placed adjacent to one another on a substrate (Fig. 5). These workers exploited laminar flow to pattern proteins and cells side by side in well-defined areas (Fig. 5). This method allowed for the selective patterning of a substrate and/or for the selective patterning of cells and even portions of cells. They used these methods to pattern *Escherichia coli* and chick erythrocytes side by side. In addition, they performed an enzymatic assay that used trypsin/EDTA to cleave fibronectin and thus detach cells or portions of cells in the affected area.

3.2.2 Patterning of nonbiological materials

Kenis *et al.* [64] have used multiple parallel flows to pattern nonbiological materials within channels. They used this technique to fabricate a three-electrode system that could potentially be used as a detector or a sensor

(Fig. 6). First they evaporated a thin stripe of gold on a glass slide. They then placed a PDMS replica on the slide with the channels oriented perpendicular to the stripe of gold. A portion of the gold was then etched by flowing three streams, two water flows flanking a central flow of gold etchant, over the gold. The removal of the gold from the center of the channel made two gold electrodes, a working and a counter electrode, separated by a distance controllable by the relative rates of flow of the three streams. A third, reference electrode was made by flowing two streams – one of silver ion and one of reductant – in the channel. Diffusion mixed the two flows at the interface, and silver metal plated onto the channel; the electrode was then treated with HCl to make an Ag/AgCl reference electrode. A cyclic voltammogram of $Ru(NH_3)_6Cl_3$ in water was obtained with these electrodes. This laminar flow-based method of fabrication both fabricates devices inside preformed channels and eliminates the need for an additional alignment step.

3.3 Components

While several systems have been developed for use in patterning and separations, the addition of certain components – especially in detection and fluid handling – can help to extend the range of applications for these systems. Most systems currently use fluorescence detection, but to reduce the complexity of the fabrication of a system, other methods of detection that are simple and compact, and reduce the need for labeling, may be needed. In addition, fine control of fluid motion by using pumps and valves, for example, will be necessary in order to effectively inject, pretreat, separate, and post-treat samples under analysis. Here we outline a sensor, two detectors, and a switch that have been developed in PDMS; these components illustrate the range of functional structures that can be fabricated easily in PDMS by using rapid prototyping.

3.3.1 Cell-based biosensor

DeBusschere *et al.* [65] developed a prototype cell-based biosensor using both PDMS and silicon. The PDMS portion comprises the fluid channels, interconnects, injection ports, and sensing chambers. The sensor relies on the reaction of cells exposed to irritants compared to a reference cell population. The cells are electrically active, and when the detecting population of cells is exposed to a toxin or irritant, they will react differently than the reference populaltion that has not been exposed to any contaminants. A difference in the electrical signals between the two populations of cells is used as the basis of detec-

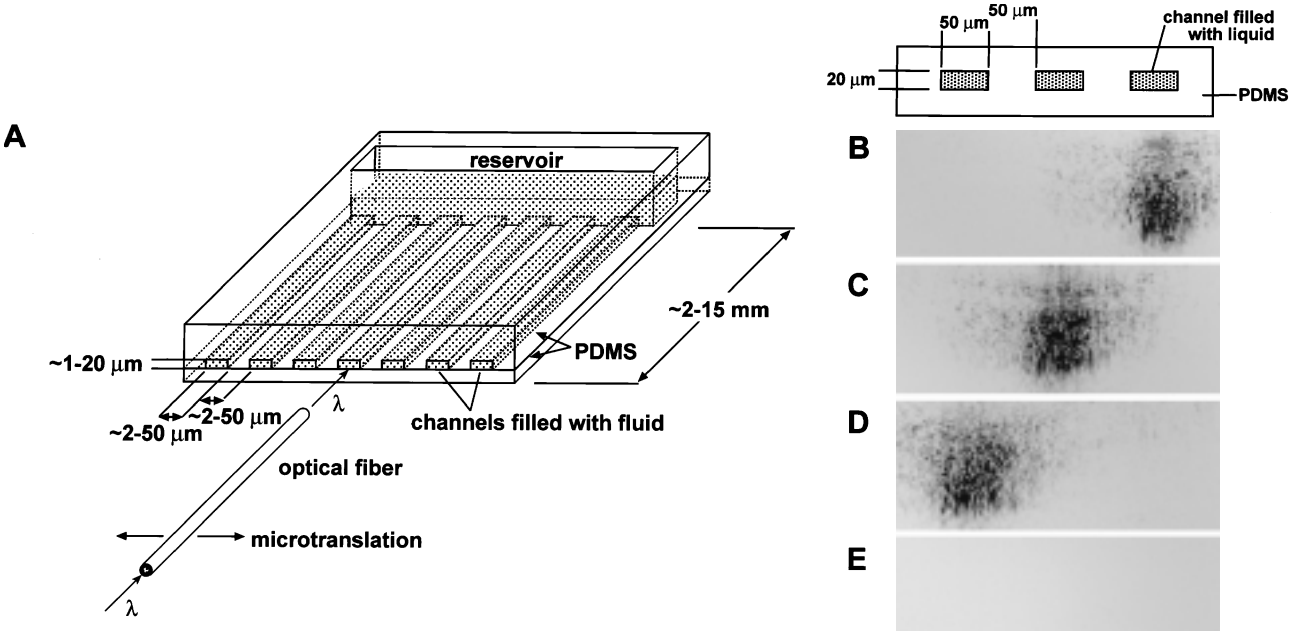

Figure 7. Schematic diagram of the optical coupling to the waveguides. (A) An optical fiber is used to couple light from a laser into the fluid-containing channels. High index fluids in the channels guide light by total internal reflection. A CCD camera images the output of the waveguides. (B)–(D) Photographs of the output of $20 \times 50\ \mu m^2$ channels as a function of the location of the coupling fiber. Selection of a particular waveguide is accomplished by changing the lateral position of the fiber. Light is coupled into and guided through the channels when the end of the fiber is directly facing the end of a channel. (E) No output is seen when the fiber is not aligned with one of the channels. Adapted from [68].

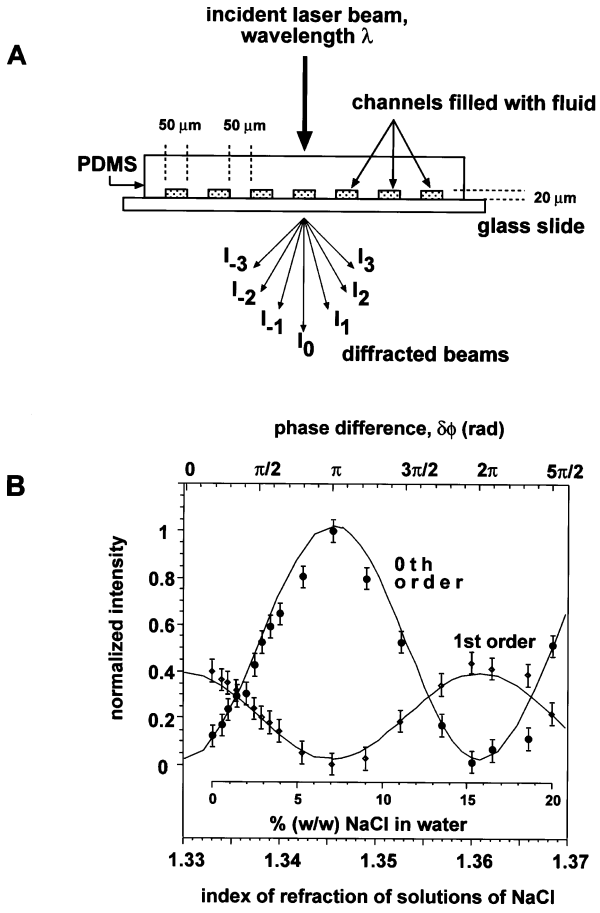

Figure 8. (A) An array of parallel channels embedded in PDMS is sealed against a glass slide. When filled with fluids that differ in index of refraction from PDMS, the array acts as a diffraction grating. If the fluid does not absorb the incident light, only the phase of the light is affected. If the fluid absorbs the incident light, both the phase and amplitude of the light change. Diffraction results from the interference of light passing through the PDMS and light passing through the channels. The changes in phase and amplitude modulate the relative intensities of the diffracted beams. (B) Variation in the intensities of the 0^{th} (filled circles) and 1^{st} (open diamonds) order beams of the diffraction pattern from a microfluidic grating as a function of the concentration of NaCl in solutions filling the microchannels. Adapted from [67].

tion. PDMS is especially suitable in this application because it is highly permeable to oxygen and carbon dioxide, so that the cells are able to respire normally even when not directly exposed to the atmosphere.

3.3.2 Detectors

One alternative to detecting analytes by fluorescence is to detect changes in index of refraction of the liquid in the channels caused by the presence of analytes. Detection of index of refraction has the following advantages: it is universal, *i.e.*, it does not require labeling of analytes; it is concentration dependent; and it is optically simple and less expensive than fluorescence [66]. Some disadvantages of the method are the dependence of refractive index on the temperature and poor detection limits achieved thus far [67]. Two components that use index of refraction to manipulate beams of light are liquid-core waveguides and diffraction gratings.

A component that exploits differences in index of refraction is a liquid-core waveguide (Fig. 7) [68]. These waveguides were fabricated by sealing channels molded in PDMS, either reversibly or irreversibly, against an appropriate material. When a liquid with a higher index of refraction than PDMS ($n = 1.41$) fills the channel, incident light is guided along the channel by total internal reflection. Changes in the index of refraction of the liquid core will change the output of the waveguide; this change provides the basis for a simple detector.

Schueller *et al.* [67] report the fabrication of a microfluidic diffraction grating. The grating is based on a network of parallel channels in PDMS (Fig. 8). These channels were 50 μm wide and 20 μm deep and spaced by 50 μm. Filling the channels with liquids that differ in refractive index from PDMS produces a phase grating: light incident on the grating will be diffracted because of the phase differences caused by the different indexes of refraction. Use of a dye in the channels that absorbs the light of interest leads to an additional modulation of the amplitude. Filling the channels with different liquids easily reconfigures the grating. Since the diffraction and amplitude patterns are sensitive to the composition of the liquid filling the channels, this device could find use as a detector or as an actuator for wavefront engineering or beam steering.

3.3.3 Microfluidic switch

Duffy *et al.* [69] demonstrated a microfluidic deflection switch. This switch could be used to control the direction of flow in complex microfluidic devices that require splitting of flows into or out of a channel. They fabricated this switch using rapid prototyping and sealed the devices irreversibly using plasma oxidation. The fluidic deflection switch is a forked channel with control channels at the junction that steer the fluid down one of the paths of the fork (Fig. 9). The direction of EOF or hydrodynamic flow emanating from the control channels determines which path the flow from the main channel will take. This switch could be used in microfluidic systems where fractionation of species is desired. The switch contains no moving

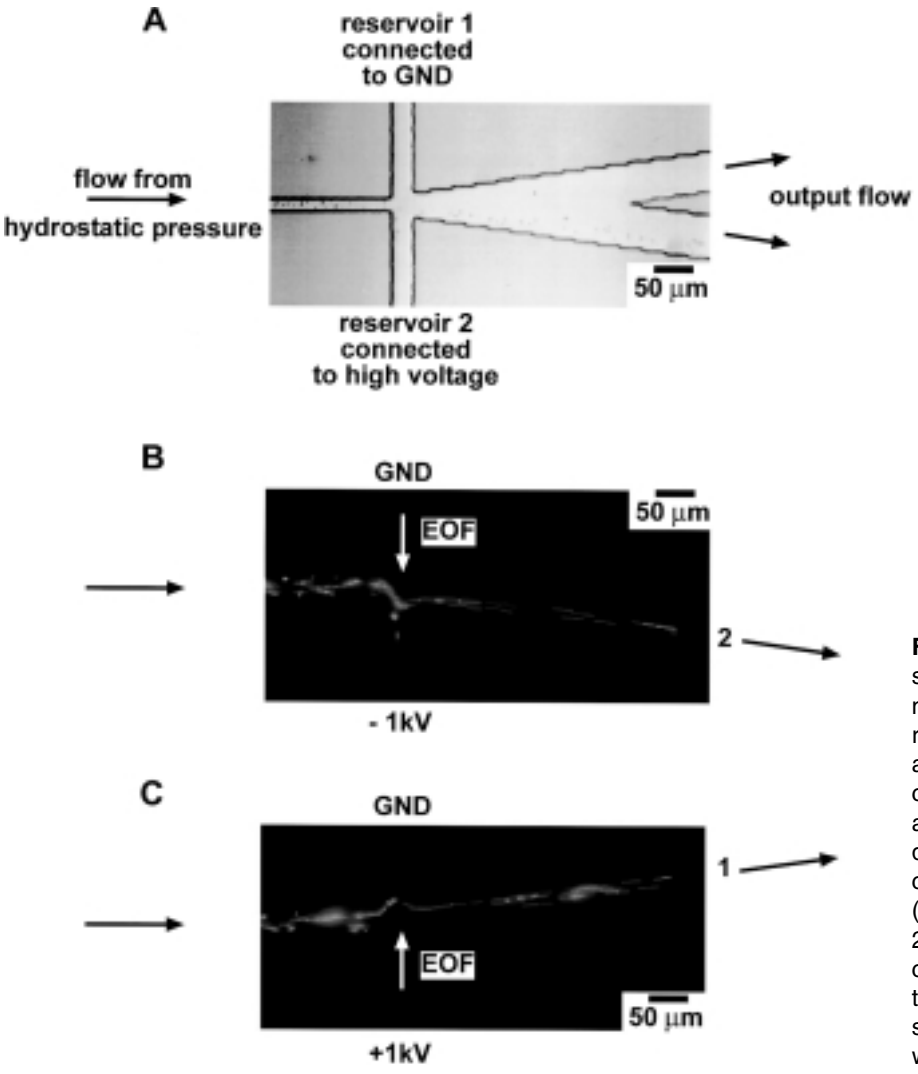

Figure 9. Microfluidic deflection switch actuated by EOF. (A) Optical micrograph of the switch. (B) Fluorescent micrograph of 1 μm diameter beads labeled with fluorescein. A negative potential (–1 kV) applied to control reservoir 2 deflected the beads into output channel 2. (C) A positive voltage (+ 1 kV) applied to control reservoir 2 deflected the beads into output channel 1. The beads flowed from the inlet to the outlet under hydrostatic pressure. Reprinted from [69], with permission.

parts that can fail or become clogged by small particles. Careful control of the magnitude of the flow in the control channel must be taken to prevent the flow from the main channel from being diluted by fluid from the control channels or being directed into the control channel.

4 Conclusions

While PDMS has many desired attributes as a material for fabrication of microfluidic systems, it also has some limitations. While it is compatible with aqueous media and some alcohols, most organic solvents are soluble in bulk PDMS and swell the polymer. In addition, small organic analytes with appreciable solubility in water may also dissolve in the bulk PDMS. While PDMS reproduces masters with high fidelity, some geometries tend to collapse because of the elasticity of the material [60, 62, 70]. Other properties of PDMS that could be advantageous or detrimental depending on the circumstances are its poor thermal conductivity (~ 0.2 Wm/K) [71] and high permeability to gases. A low thermal conductivity can lead to a rise in temperature due to resistive heating in electrophoresis; this rise in temperature causes broadening of peaks and lowers the resolution of the system. Conversely, a low thermal conductivity could find use in systems such as an incubator where an elevated temperature is desired. Permeability to gases like water vapor, oxygen, and ammonia could be disadvantageous, for example, when evaporation of water in channels can occur in devices in storage. The gas permeability could be useful in systems requiring gas, but not liquid, exchange.

Although it is still in the early stages of development in this field, we believe that PDMS is an extremely promising material for the fabrication of microfluidic systems that use aqueous media. Since PDMS can be molded at low temperatures and by using rapid prototyping, chemists and biologists working on a benchtop can make devices

quickly and easily. PDMS is robust, and devices made in it, unlike hard materials, can be dropped and bent without loss of performance. PDMS is ideal for the fabrication of devices that require more than one material in fabrication, since it can seal to a variety of materials. In addition, fabrication of 3-D devices will be straightforward since layers of PDMS with channels, reservoirs, valves, and through holes can easily be aligned and sealed.

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