Review

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Microfluidic devices fabricated in poly(dimethylsiloxane) for biological studies

This review describes microfluidic systems in poly(dimethylsiloxane) (PDMS) for biological studies. Properties of PDMS that make it a suitable platform for miniaturized biological studies, techniques for fabricating PDMS microstructures, and methods for controlling fluid flow in microchannels are discussed. Biological procedures that have been miniaturized into PDMS-based microdevices include immunoassays, separation of proteins and DNA, sorting and manipulation of cells, studies of cells in microchannels exposed to laminar flows of fluids, and large-scale, combinatorial screening. The review emphasizes the advantages of miniaturization for biological analysis, such as efficiency of the device and special insights into cell biology.

Contents

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1 Introduction

Microfluidic systems provide a powerful platform for biological assays [1–3]. In microfluidics, small volumes of solvent, sample, and reagents are moved through microchannels embedded in a chip. Examples of bioassays and biological procedures that have been miniaturized into a chip format include DNA sequencing, polymerase chain reaction (PCR), electrophoresis, DNA separation, enzymatic assays, immunoassays, cell counting, cell sorting, and cell culture [4–6]. Miniaturized versions of bioassays offer many advantages, including small requirements for solvents, reagents, and cells (critical for valuable samples and for high-throughput screening), short reaction times, portability, low cost, low consumption of power, versatility in design, and potential for parallel operation and for integration with other miniaturized devices.

In this review, we briefly describe the properties of poly (dimethylsiloxane) (PDMS) and techniques for the fabrication of PDMS microstructures, with an emphasis on the

advantages of using PDMS for miniaturized bioassays. We then discuss the panel of PDMS-based components available for microfluidics, paying attention to the challenges posed by the special physics of fluid flows in small channels, and the technologies developed to address and exploit these flows. Finally, we describe PDMS-based miniaturized bioassays that integrate components into functional devices. Whenever possible, we highlight the advantages of miniaturization, such as the efficiency of the device and special insights into biology that it provides.

2 PDMS: properties and fabrication

The use of PDMS elastomer for miniaturized bioassays has numerous advantages over silicon and glass. PDMS as a material is inexpensive, flexible, and optically transparent down to 230 nm (and therefore compatible with many optical methods for detection). It is compatible with biological studies because it is impermeable to water, nontoxic to cells, and permeable to gases. A final, major advantage of PDMS over glass and silicon is the ease with which it can be fabricated and bonded to other surfaces. For the development of bioassays, where many designs may need to be tested, the ease of rapid prototyping in PDMS is a critical advantage.

Procedures for the fabrication of PDMS structures for microfluidics have been described in detail elsewhere [7–9]. Briefly, the design of the microstructures is made in a computer-aided design (CAD) program. Using commercial services, the CAD-generated patterns are printed on transparencies (these services have overnight turnaround times). Lateral resolutions of 25 μm can be routinely achieved with image setters operating at 5080 dots per inch, and can be extended to 8 μm using photoplotters operating at 20 000 dots per inch [10]. (For features beyond 8 μm, chrome masks can be used, but they take longer to fabricate commercially, and are more expensive than transparencies.) The transparency is then used as a photomask in UV-photolithography to generate a master. In this procedure, a thin layer of photoresist (for example, the photocurable epoxy SU-8) is spin-coated onto a silicon wafer. Using different types of SU-8 of various viscosities, thicknesses of 1–300 μm can be reliably spin-coated. The photoresist is exposed to UV light through the photomask, and a developing reagent is used to dissolve the unexposed regions. The resulting bas-relief structure serves as a master for fabricating PDMS molds.

To create the PDMS mold, the surface of the silicon/photoresist master is treated with fluorinated silanes (which prevents irreversible bonding to PDMS), and a

liquid PDMS prepolymer (in a mixture of 1:10 base polymer:curing agent) is poured onto it. The PDMS is cured at 70°C for 1 h or more and peeled off the master, producing the final replica bearing the designed microstructures. Small holes are drilled into the PDMS using a borer to produce inlets and outlets. Finally, PDMS can seal to itself and other flat surfaces reversibly by conformal contact (*via* van der Waals forces), or irreversibly if both surfaces are Si-based materials and have been oxidized by plasma before contact (a process that forms a covalent O-Si-O bond). Seals are watertight and can be formed under ambient conditions (unlike silicon and glass, for which bonding requires high temperatures or adhesives). If desired, many PDMS replicas can be made from a single master. This procedure of producing the PDMS structure from the silicon master, called replica molding, can be carried out under normal laboratory conditions without an expensive clean room, and can replicate certain types of features with dimensions down to 10 nm. Replica molding, along with procedures such as microcontact printing, casting, injection molding and embossing, comprise a set of techniques for manipulating elastomeric structures called soft lithography [11]. Although we focus on PDMS in this review, soft lithography has been demonstrated for other elastomers (such as polyurethane and epoxy).

PDMS consists of repeating -OSi(CH$_3$)$_2$- units; the CH$_3$ groups make its surface hydrophobic. This hydrophobicity results in poor wettability with aqueous solvents, renders microchannels susceptible to the trapping of air bubbles, and makes the surface prone to nonspecific adsorption to proteins and cells. The surface can be made hydrophilic by exposure to an air plasma (in a plasma cleaner for 1 min); the plasma oxidizes the surface to silanol (Si-OH). The plasma-oxidized surface remains hydrophilic if it stays in contact with water. In air, rearrangements occur within 30 min, which bring hydrophobic groups to the surface to lower the surface free energy. The surface of oxidized PDMS can be modified further by treatment with functionalized silanes.

3 Fluids in microchannels: the toolbox

Fluid flow in microchannels exhibits a number of characteristic features, the most important of which is laminar flow. We describe components such as valves and mixers, which have been developed to handle fluid flow in microchannels, as well as devices such as gradient generators, which exploit the special physics of microfluidics. We focus on techniques that have been shown to work in PDMS-based devices.

3.1 Fluid flow

There are two main methods for driving the flow of fluids in microchannels: pressure-driven and electrokinetic. Both methods are used in PDMS devices. In pressure-driven flow (also called hydrodynamic flow) (Fig. 1A), the flow rate Q (m³/s) is given by $Q = \Delta P/R$, where ΔP is the pressure drop across the channel (Pa), and R is the channel resistance (Pa·s/m³). The pressure drop can be created either by opening the inlet to atmospheric pressure and applying a vacuum at the outlet, or by applying positive pressure at the inlet (*e.g.*, *via* a syringe pump) and open-

ing the outlet to atmospheric pressure. Both methods work well, although for syringe pump-driven flow, it is necessary to form an irreversible seal for PDMS devices (irreversibly sealed structures can withstand pressures of 30–50 psi, whereas conformally sealed structures can withstand pressures of 5 psi). For vacuum-driven flow, both irreversible and conformal seals can be used.

For pressure-driven flow, the other determinant of flow rate is the channel resistance R. For a circular channel, $R = 8\,\mu L/\pi r^4$, and for a rectangular channel with a high or low aspect ratio ($w \ll h$ or $h \ll w$), $R = 12\,\mu L/wh^3$, where
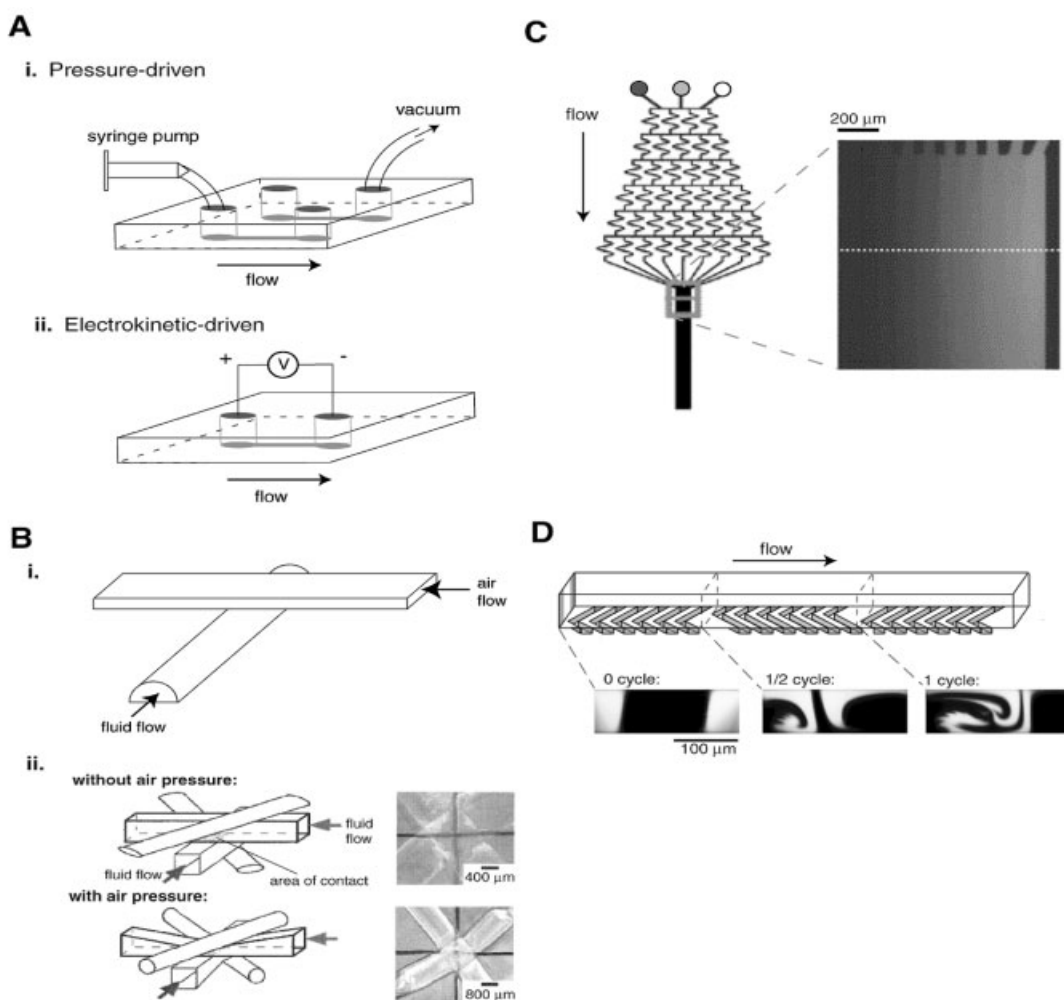

Figure 1. Toolbox for PDMS-based microfluidic bioassays. (A) Methods for driving fluid flow. (i) Pressure-driven flow using a vacuum at the outlet or a syringe pump at the inlet. (ii) Electrokinetic flow using a voltage applied across the microchannel. (B) Switches and valves for control of fluid movement. (i) Pneumatically actuated monolithic valve. When pressure is applied above, flow in the bottom rounded channel stops [18]. (ii) Channel crossing in which the fluid flow can be switched. When air pressure is applied above and below the crossing, the fluid turns 90° instead of flowing straight. Figure adapted from [20]. (C) Gradient generator using laminar flow. A solution or surface-bound molecular gradient is generated perpendicular to the direction of fluid flow in the microchannel. Figure adapted from [31]. (D) Chaotic mixer. Neighboring streams of fluids are passively mixed. Homogeneity is observed after 16 cycles of the staggered herringbone structure. Figure adapted from [32].

μ is the fluid viscosity (Pa·s), L is the length (m), r is the radius (m), h is the height (m), and w is the width of the channel (m). (See [5] for the formula for a rectangular microchannel with an intermediate aspect ratio.) A long narrow channel therefore exhibits high fluidic resistance, and a short wide channel exhibits low fluidic resistance. Pressure-driven flow has the key advantages that it is effective for solvents with a wide range of compositions (*e.g.*, including solvents that are not electrically conductive), and for channels made of a wide range of materials (*e.g.*, even if electrically conductive, such as silicon). Pressure-driven flow, however, requires an external pump or a vacuum source. The method also suffers in assays requiring high-resolution separation because the velocity profile of a cross-section is parabolic, and samples in the form of plugs undergo axial dispersion and peak broadening. Finally, because of the relations $R \propto 1/r^4$ or $1/wh^3$, high pressure drops are needed to drive fluid flow in small microchannels.

Electrokinetic flow is based on the movement of molecules in an electric field due to their charges (Fig. 1A). There are two components to electrokinetic flow: electrophoresis, which results from the accelerating force due to the charge of a molecule in an electric field balanced by the frictional force, and electroosmosis, which creates a uniform pluglike flow of fluid down the channel. In electroosmotic flow in glass capillaries, a layer of fluid enriched in solvated cations forms at the surface of negatively charged silanol groups of the channel wall; an electric field drives the layer of cations towards the negatively charged cathode, and by viscous drag, transfers the motion to the rest of the liquid (given a sufficiently small cross-section in the microchannel). PDMS-based channels (normally uncharged at the surface) can be made to support electroosmotic flow effectively by plasma oxidation immediately before the addition of buffer; this oxidation generates silanol groups at the channel surface.

For electrokinetic flow, small channels have the advantage of a high surface-to-volume ratio, and thus they dissipate heat more efficiently than large channels. Also, electroosmotic flow results in flat velocity profiles, and gives rise to sharp peaks and high resolution separations in capillary electrophoresis. Another advantage of electrokinetic flow is that fluid flows in a microfluidic network can be controlled easily by switching voltages on and off; this control circumvents the need for valves. Nevertheless, electrokinetic flow has important drawbacks for bioassays, including buffer incompatibility (only buffers of appropriate pH and ionic strength are compatible), the need for an off-chip power supply, frequent changes of voltage settings (due to ion depletion, and to compensate for pressure and resistive imbalances in the channels),

electrolytic bubble formation, and evaporation of solvent due to heating. Also, electrophoretic demixing – the separation of components in a heterogeneous mixture due to different electrophoretic mobilities – is unfavorable in bioassays requiring a uniform flow for all species.

Fluid flow in microchannels using other principles has been described. Delamarche *et al.* [12] used capillary action in plasma-oxidized PDMS to deposit immunoglobulins onto a surface. Centrifugal force was used to drive fluid flow in PDMS channels on a plastic disk, on which enzymatic assays were performed [13]. In non-PDMS-based systems, fluid flow was directed using gradients in surface pressure due to redox-active surfactants [14], gradients in temperature [15], patterning of self-assembled monolayers with different surface free energies [16], and capillary action [17].

3.2 Fluid switching: valves

In electrokinetic flow, fluid flow can be controlled by applying voltages to electrodes integrated in microchannels. A more general strategy for manipulating fluid flow is the use of valves to open and close microchannels. The elastomeric property of PDMS can be exploited to make a mechanical valve. Quake *et al.* [18, 19] used a cross-channel architecture made of PDMS to fabricate a pneumatically actuated valve. In this design, pressure is applied to the upper channel, deflecting a thin PDMS membrane downward; this deflection closes the lower, rounded channel and stops fluid flow (Fig. 1B). We demonstrated an elastomeric switch in a PDMS system featuring two crossing channels, each in a different layer (Fig. 1B) [20]. Application of an external pressure above and below the crossing of the channel decreases the aspect ratio at the crossing, such that the fluid turns into the other channel due to lower fluidic resistance, instead of flowing straight through the crossing. Finally, pneumatically actuated PDMS valves can also be combined with glass microfluidic channels [21]. Advantages of pneumatically actuated valves include ease of fabrication (by multistep lithography), rapid response time, avoidance of air bubbles, and wide fluid compatibility. In the future, the integration of valves in microfluidics, although adding to the complexity of the system, will become more prevalent, especially for devices featuring large numbers of independent channels [22].

In another approach for constructing valves, Beebe *et al.* [5, 23] used pH-sensitive hydrogels. Although stimuli-responsive hydrogels have a slow response time, they are intriguing because they are autonomous, responsive only to the environment in the microchannel, and require no external control. Other strategies for the fabrication of

valves include electrochemically generated microbubbles [24], and thermally induced expandable microspheres [25].

3.3 Multiple fluid streams: laminar flow

Parallel streams of liquids can exhibit either laminar flow, where the streams flow parallel along each other and mixing occurs only by diffusion, or turbulent flow, where turbulence mixes the streams. The parameter that indicates whether flow is laminar or turbulent is the Reynolds number (dimensionless): $Re = vl\rho/\mu$, where v is the velocity of the fluid (m/s), l is the cross-sectional dimension (m), ρ is the density of the fluid (for water, 1000 kg/m^3), and μ is the viscosity of the fluid (for water, 10^{-3} kg/(m·s)). For aqueous solutions, ρ and μ are fixed parameters (characteristics of the fluid), and the rate of fluid flow v and channel dimension l are changeable. Under typical microfluidic conditions of small channels ($<$ 100 μm) and a low rate of fluid flow (1 cm/s), Re is almost always low ($Re < 1$), a value that correlates with laminar flow behavior (with Re above $\sim$2000, fluid usually exhibits turbulent flow).

The prevalence of laminar flow in microfluidics enables new technologies. For laminar flow, parallel streams of fluid mix only by diffusion at their boundary. Yager *et al.* [26, 27] used diffusion at the boundary as the basis for an immunoassay. We have demonstrated membraneless electrochemistry using the slowly diffusing boundary as a barrier [28], and microfabrication at the boundary using multiphase laminar flow patterning [29]. In another technique, we use controlled diffusive mixing of laminar flow fluids to generate stable molecular gradients perpendicular to the direction of flow (Fig. 1C) [30, 31]. The method is based on repeated splitting, mixing and recombination of neighboring fluid streams. The gradients can be generated in solution and on surfaces, and they are spatially and temporally stable. Moreover, we can generate gradients of complex shapes by using multiple microfluidic networks [31]. The use of solution and surface gradients for studying cell biology is described later in this review.

3.4 Multiple fluid streams: mixers

Diffusive mixing is a slow process. For example, the time for diffusion in one dimension is given by $t = d^2/2D$, where d is the distance a particle moves (in cm) in a time t (in s), and D is the diffusion coefficient (for most proteins, between 10^{-6} and 10^{-7} cm^{2}s^{-1}). Thus, a globular protein of 70 kDa needs only 1 s to diffuse 10 μm, but more than 10 days to diffuse 1 cm; the distance along the channel required for the mixing of the contents in two neighboring streams can be prohibitively long ($\gg$ 1 cm; estimated by vl^2/D) [32].

We designed a mixer that uses asymmetric grooves on the floor of the channel to introduce a transverse component to the flow (Fig. 1D) [32]. Using this structure, fluid elements are twisted and folded into one another; this folding increases the contact area between the two streams, and thus the rate of diffusive mixing. Neighboring streams of fluids mixed efficiently in a microchannel containing staggered grooves of different geometries (for two streams of protein-containing solutions, a microchannel of 1 cm length could produce nearly complete mixing). We believe that this design, which is easily fabricated by two-step lithography and compatible with steady pressure-driven flow, will find many applications is bioassays that require the mixing of fluids.

Other mixers have been demonstrated in PDMS-based systems. Quake *et al.* [33] fabricated a rotary, pneumatically actuated pump that actively mixes fluids from different inlets. Crooks *et al.* [34] built a device that achieved efficient mixing ($>$ 90%) by flowing fluid streams into the small spaces between the microbeads; this bed increased the interfacial area of the fluid elements and the rate of diffusive mixing. Ismagilov *et al.* [35] developed a mixer that initially flowed the reactants as laminar streams in a microchannel; injection of a water-immiscible phase (perfluorodecaline) generated uniform plugs, inside which the reactants mixed by chaotic advection. Other mixers have also been described in non-PDMS-based systems, using a serpentine channel [36], a T-channel [37], and intersecting channels [38].

4 PDMS-based microfluidic devices for biological studies

To build a functional microfluidic bioassay or a "lab-on-a-chip", one must effectively integrate components such as pumps, valves, and reservoirs. This section describes examples of functional microfluidic devices for applications in biology. We focus on PDMS-based systems, for which substantial progress has been made on the integration of components, because they both allow rapid prototyping and serve as final functional devices. Microfluidic components have been integrated using other materials to build impressive devices for bioassays [39].

4.1 Detection using microfluidic immunoassays

4.1.1 Immunoassays

Immunoassay is widely used to detect analytes using antibodies. Most immunoassays are heterogeneous: the antigen-antibody complex is bound to a solid substrate, and free antibodies are removed by washing. In homogeneous immunoassays, the free and bound antibodies do not need to be separated *via* a solid substrate. These types of procedures minimize washing steps and fluid handling, but they require that the free and antigen-bound antibodies exhibit different electrophoretic mobilities. Miniaturization of homogeneous immunoassays offers advantages [26, 40], but more work has been done on the miniaturization of heterogeneous immunoassays than of homogeneous immunoassays.

A significant disadvantage of heterogeneous immunoassays (such as enzyme-linked immunosorbent assay, or ELISA) in microtiter wells is that they require a long time to perform. Incubation times of hours are required

to allow diffusion of the analyte from the solution to the surface. Microfluidics can shorten the incubation times needed for surface events by minimizing the diffusion distance in microchannels, and by replenishing the diffusion layer with a fixed concentration of molecules. In one study, an immunoassay detecting immunoglobulin G (IgG) was performed in a PDMS microchannel, requiring incubation times of only 1–6 min [41]. Also, ELISA was performed on a microchip of polyethylene microchannels featuring 5 min incubation times; this assay was able to detect about 1 nM D-dimer, a protein used as a negative indicator for deep vein thrombosis [42].

In a typical microwell ELISA assay for detecting serum antibodies, serial dilutions of the sample are accomplished manually, and the assay repeated for each antigen to be tested. Thus, the analysis of a single sample typically requires many microwells. We developed a microfluidic immunoassay that automatically serially diluted the sample and presented multiple antigens on the surface for analysis (Fig. 2A) [43]. The device em-
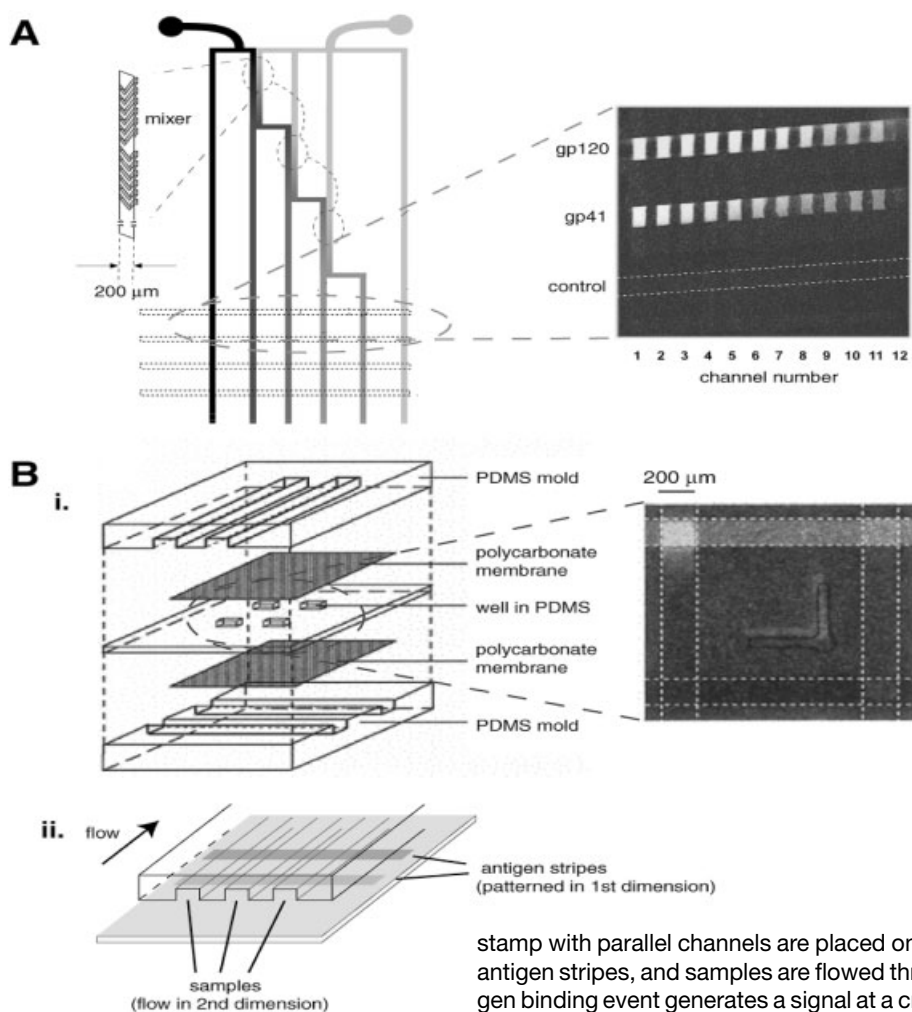

Figure 2. Detection of biomolecules using microfluidics. (A) Immunoassay employing a microdilutor network. The microdilutor network uses chaotic mixers to mix neighboring streams of fluids, serially diluting the sample with buffers. Anti-HIV antibodies from a patient are serially diluted and detected using two antigens (gp120 and gp41) in parallel. Figure adapted from [43]. (B) Two-dimensional microfluidic arrays. (i) Microwell system where the channel crossings are separated by two porous membranes and a thin PDMS membrane with embedded microwells. Reactions take place in the microwells, which produce a fluorescent signal. In this example, the colored chamber corresponds to a reaction between the fluorescent dye, fluo-3, and Ca^{2+}. Figure adapted from [44]. (ii) Two-dimensional immunoassay [45]. In the first dimension, parallel antigen stripes are patterrned onto a substrate using microfluidic delivery. In the second dimension, a PDMS stamp with parallel channels are placed onto the substrate at right angles to the antigen stripes, and samples are flowed through the channels. An antibody-antigen binding event generates a signal at a crossing.

ployed a microdilutor network that mixed the sample with buffer using a chaotic mixer. Each mixing achieved a dilution factor of 2; ten mixing steps resulted in a dilution factor of $2^{10} \approx 10^3$. The serially diluted samples then flowed over a polycarbonate membrane, onto which stripes of antigens have been patterned. Using a fluorescently labelled secondary antibody, we demonstrated the detection of anti-human deficiency virus (anti-HIV) antibodies in HIV$^+$ serum with an automated serial dilution profile, using two different HIV antigens in parallel.

4.1.2 Multiplexing

Microfluidic systems have the potential to perform a large number of biochemical assays in parallel, and enable large-scale combinatorial processes. An intriguing approach is a two-dimensional array where two sets of microfluidic channels are crossed at right angles. In these approaches, the screening of a library of N samples against a library of M reagents requires only a single chip, instead of N chips for conventional arrays of the titer well format. We fabricated a three-dimensional system where two PDMS molds of crossing channels are placed orthogonally to each other, separated either by a porous polycarbonate membrane, or by two polycarbonate membranes and a microwell (Fig. 2B) [44]. The whole system is conformally sealed. The membranes allow for diffusion of the reactants and provide a high resistance to convective flow through the crossing, thereby minimizing cross-contamination between the crossing channels. We showed that a variety of biochemical reactions can be performed in this system, such as enzymatic reactions and detection of *Staphylococcus aureus* by bead agglutination. The system, however, is more difficult to fabricate than a microtiter plate, and requires pressure balancing to control the flow across the membrane that mixes the reactants.

Delamarche *et al.* [45] demonstrated a different implementation of a two-dimensional immunoassay (Fig. 2B). In this method, parallel stripes of antigens are first patterned onto the surface using PDMS microfluidic channels. The channel system is demounted, and a second system of parallel microfluidic channels is placed onto the patterned antigens at right angles. Samples containing the analytes are caused to flow onto the patterned antigens. The method was effective in detecting antibodies using either a sandwich ELISA or fluorescently labelled secondary antibodies. Compared to conventional ELISA assays, this method required only nanoliter volumes and took only minutes to complete.

4.2 Separation of proteins and DNA

4.2.1 PDMS open channels: capillary electrophoresis

Techniques for separating proteins and DNA – such as capillary electrophoresis and liquid chromatography – can be performed on a microfluidic chip. Advantages of miniaturization include reduced cost and analysis time, and potential for high-throughput analysis and for integration with other microfluidic components (for example, sample filtration and extraction). The ease with which fluid flow can be controlled electrokinetically has made capillary electrophoresis a popular technique for miniaturization onto a chip. In comparison, the difficulty in miniaturizing high-pressure systems for driving fluid flow in packed columns has limited the work on miniaturizing liquid chromatography (see [46] for a discussion of recent work).

PDMS can be easily molded to form channels for the separation of biological molecules. It has the added advantage that plasma oxidation of its surface generates silanol groups that are negatively charged at neutral or basic pH; this charged surface enables electroosmotic flow towards the negatively charged cathode [7]. In an initial demonstration of capillary electrophoresis in PDMS microchannels, Effenhauser *et al.* [47] achieved efficient separation of DNA fragments in native PDMS channels using electrokinetic flow in a sieving matrix. Joule heating was effectively dissipated by PDMS for field strengths less than 1 kV/cm. We demonstrated capillary zone electrophoresis in plasma-oxidized PDMS channels, which supported uniform electroosmotic flow (Fig. 3) [7]. The device efficiently separated amino acids and protein charge ladders, and, in the presence of a sieving matrix, DNA fragments. Harrison *et al.* [48] showed that native PDMS could also support a reproducible and stable electroosmotic flow (the origin of the surface charge may stem from silica fillers in the polymer). The ability of oxidized and native PDMS to support electroosmotic flow may depend on the ionic strength of the buffer [49].

One-dimensional sodium dodecyl sulfate (SDS) capillary gel electrophoresis (CGE) has been performed in a microchannel [50]. The microchannel-based SDS/CGE separated a six-protein mixture with greater efficiency and speed than a conventional capillary-based SDS/CGE. To separate components in complex mixtures of proteins such as cell lysates, two-dimensional (2-D) gel electrophoresis is often used. In this method, the first dimension is isoelectric focusing (IEF) and the second dimension is SDS gel electrophoresis. Compared to a slab gel, a miniaturized format of 2-D gel electrophoresis would require
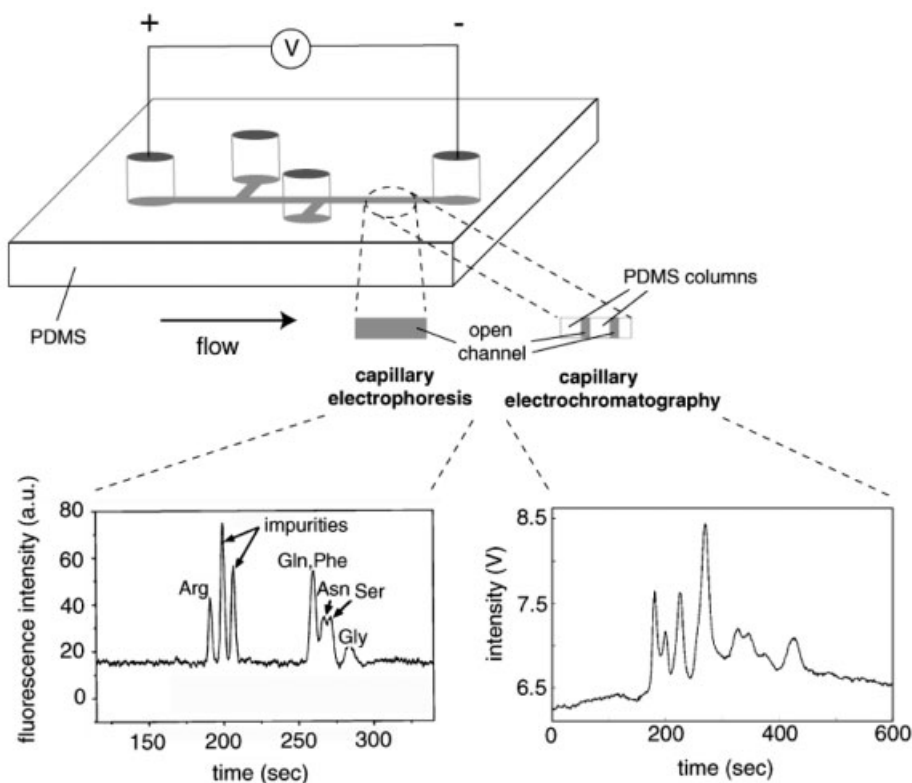
Figure 3. Separation of biomolecules using PDMS-based microfluidic devices. One-dimensional separation in a PDMS-based microchip. (Left) In an open channel, capillary zone electrophoresis was used to separate a mixture of FITC-labeled amino acids (figure adapted from [7]). The separation voltage was 5 kV, and laser-induced fluorescence was detected by a photomultiplier tube. (Right) In a channel with PDMS monolithic posts, capillary electrochromatography was used to separate a tryptic digest of FITC-BSA (reprinted from [55], with permission). The separation voltage was 1 kV, and laser-induced fluorescence was detected by a photomultiplier tube.

less sample and may exhibit less heat-induced peak broadening due to more efficient heat dissipation (from a high surface area-to-volume ratio).

Previous methods to miniaturize 2-D gel electrophoresis (and other 2-D separations) have focused on the injection of effluent from the first dimension into a second dimension. This process is slow and serial. In an initial demonstration, we have built a PDMS-based channel system to perform all separations in the second dimension in parallel, similar to conventional 2-D gel electrophoresis [51]. PDMS was a particularly appropriate material for this design because of convenient procedures for fabricating 3-D microfluidic channels, and the facility with which PDMS-based systems can be assembled and disassembled. After separation using IEF in the first dimension, we disassembled the channel system and connected the IEF gel (filled with the partially separated protein mixture) to a 3-D channel for SDS gel electrophoresis. As a proof of concept, we demonstrated the separation of three proteins in a mixture. This demonstration is at an early stage and does not represent a practical method of separation. Higher efficiency of separation may be achieved by optimizing the design of the channel system.

PDMS channels can also be used to separate DNA. Since DNA fragments of different sizes exhibit similar charge-to-mass ratios, they separate poorly in an open channel.

Doyle *et al.* [52] demonstrated the use of a stationary phase consisting of a self-assembled magnetic matrix for separating DNA in a PDMS channel. Large DNA fragments (10–50 kbp) were effectively separated in this device. In another approach, PDMS was used as an intermediate layer between a high-voltage source and the separation channel; the hybrid PDMS-glass microchip effectively separated DNA samples [53]. Finally, PDMS was used as a cover slip on nanochannels (as small as 150 by 180 nm) fabricated in silicon [54]. The electrophoretic behavior of individual λ-DNA molecules was studied in the nanochannels.

4.2.2 PDMS as stationary phase

Whereas PDMS open channels have been well studied, less focus has been placed on using PDMS as a stationary phase for separations of proteins and of DNA. There are numerous advantages to this approach, the most significant one being that PDMS microstructures can be precisely and inexpensively fabricated. In this way, different microfabricated patterns of stationary phase can be rapidly prototyped and precisely controlled. The high degree of control allows for high channel homogeneity and total control of channel dimensions and geometry, compared to conventional packed columns (which have inhomogeneous beds).

Regnier *et al.* [55] fabricated a microcolumn consisting of PDMS support structures of 10 μm dimensions; these structures covered over 60% of the surface area of the separation section of the device (Fig. 3). Operating in the capillary electrochromatography mode, the device separated peptides from a tryptic digest of bovine albumin. As hydrophobic stationary phases are most effective for peptide separations in high-performance liquid chromatography, PDMS support structures derivatized with hydrophobic moities (silanes containing phenyl groups or C8-C18 alkyl groups) gave rise to better separation than native PDMS, which is only moderately effective as a hydrophobic support (it is roughly the equivalent of a C1 phase). An important consideration in using this method is the nonspecific interaction of analytes with the PDMS walls.

4.2.3 Interfacing PDMS microchannels with mass spectrometry

Mass spectrometry is a powerful tool for postcolumn analysis of peptides, proteins, and small molecules. Several approaches have been taken to connect PDMS microchannels to electrospray ionization-mass spectrometry (ESI-MS). Aebersold *et al.* [56] connected a fused-silica capillary to the outlet of a prefabricated PDMS channel; the other end of the capillary was connected to the ESI-MS. Interfaces between PDMS and silica capillary can be formed with minimal dead volumes by taking advantage of the molding properties of PDMS. For example, PDMS was cast directly on a fused-silica capillary; after curing the PDMS, removal of a part of the embedded capillary generated a PDMS microchannel that formed a smooth interface with the remaining embedded capillary [57]. In another study, PDMS was cast on a metal wire inserted into a silica capillary; removal of the metal wire generated a PDMS microchannel that connected to the silica capillary with no dead volume [58].

Alternatively, the PDMS-capillary interfaces can be eliminated altogether by fabricating PDMS microchannels with tapered ends; these ends functioned as ESI emitters [59]. This device employed pressure-driven flow for sample infusion. In another study, ESI was obtained by direct spraying from PDMS microchannels using electrokinetic flow [60].

4.3 Sorting and manipulation of cells

The two most common methods for sorting and enriching cell populations are the fluorescence activated cell sorter and magnetic filtration. Both methods can be miniaturized to devices that are sensitive, cost-effective, and easy-to-operate. This section also describes PDMS-based systems for manipulating and culturing reproductive cells.

4.3.1 Cell sorting by flow cytometry

Using soft lithography, Quake *et al.* [61] microfabricated a fluorescence activated cell sorter (FACS) driven by electrokinetic flow. The sample was introduced into a T-shaped junction, and upon detection of fluorescence near the junction, voltages were switched to divert the sample to the collection channel. For a sample containing fluorescent and nonfluorescent *Escherichia coli*, the cell sorter enriched fluorescent *E. coli* by 30-fold, and 20% of the recovered cells were viable. In a subsequent study, the microfabricated cell sorter was modified with valves and pumps to use pressure-driven flow instead of electro-osmosis (Fig. 4A) [62]. The pressure-driven device exhib-
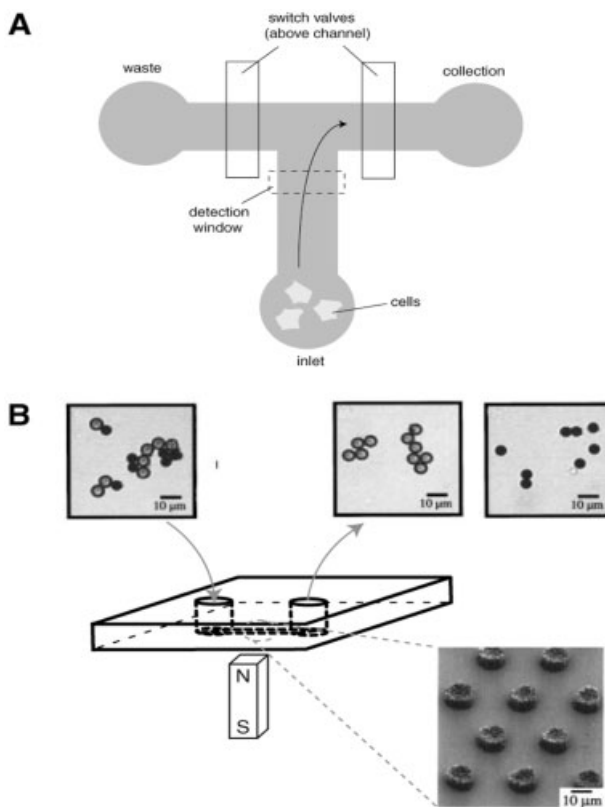
Figure 4. Miniaturized sorting devices. (A) Microfabricated fluorescence-activated cell sorter. Samples containing fluorescently labelled molecules flow from the input well to the detection window. Detection of fluorescence triggers pneumatically actuated switch valves that force the cells to flow to the collection well [62]. (B) Magnetic filtration device. Next to a permanent magnet, the nickel posts create a large local magnetic field gradient and capture 4.5 μm superparamagnetic beads (colored) from a mixture also containing nonmagnetic beads (not colored). Figure adapted from [64].

ited higher cell viability and sorting accuracy than the electrokinetically driven sorter. Takayama *et al.* [63] fabricated a PDMS-based flow cytometer using pressure-driven flow. In this device, the use of air as the sheath fluid (instead of liquid in conventional FACS) to focus the sample flow stream eliminated the need of large liquid reservoirs, and may allow for higher flow rates and higher throughput than systems that do not use sheath fluids. Microfabricated cell sorters exhibit a lower cost than benchtop systems, and they have the potential for single-cell studies and integration with other microfluidic components. Disadvantages of microfabricated cell sorters compared to conventional FACS include the low throughput of sorting (less than 100 cells per second, compared to thousands of cells per second for conventional FACS), and a low recovery of viable cells.

4.3.2 Magnetic sorting

Magnetic cell sorting is a technique commonly used for enriching one cell population from a mixture of cells. In this technique, target cells are labelled with antibody-coated superparamagnetic beads (50 nm or 3 μm). The mixture is then passed through a separation column, typically containing ferromagnetic collection elements to act as field concentrators, in the presence of a strong magnetic field; the labelled cells are retained on the column, the column is washed, the magnetic field is removed, and the retained cells are eluted.

In a miniaturized format, a permanent magnet can be placed next to the microchannel to effect magnetic separation. We developed a magnetic filtration system consisting of 15 μm diameter nickel posts which act as magnetic field concentrators in the presence of an external magnetic field (Fig. 4B) [64]. The device separated 4.5 μm paramagnetic from diamagnetic beads with 95% efficiency. We believe that this system can be extended to separate and sort magnetically tagged cells, although it has not so far been used for this purpose.

4.3.3 Manipulation of motile cells

Takayama *et al.* [65] built a PDMS-based device that sorted motile sperm from nonmotile sperm, a procedure important for choosing viable sperm for *in vitro* fertilization. The microscale sperm sorter made use of the ability of motile sperm to cross streamlines under laminar flow conditions. Nearly 100% of the sorted sperm was motile. Compared to conventional sorting methods (such as hand sorting), the microscale sperm sorter was quicker, simpler to use, and produced a comparable yield (the ratio of the number of motile sperm sorted and the total

number of sperm in the sample). Beebe *et al.* [66] have also developed a microfluidic device for transporting ova and sperm in microchannels and for culturing embryos. This device has the potential to automate *in vitro* fertilization and to increase its rate of success. The technology is now being commercialized (Table 1).

Table 1. PDMS-based microfluidic devices in the private sector

Company	URL	PDMS-based product
Cellectricon	www.cellectricon.se	*Parallel patch clamp, ion channel drug screening
Fluidigm	www.fluidigm.com	*Screen for protein crystallization
Surface Logix	www.surfacelogix.com	*Biosystems for drug discovery
Vitaellc	www.vitaellc.com	Microfluidics for assisted reproduction

* Product is on the market.
We list only companies that use PDMS in their final products (many companies use PDMS for prototyping). For a general summary of microfluidic products in the private sector (fabricated in any material), see [1, 81].

4.4 Cell biology using laminar flow

4.4.1 Laminar flow over single cells

The flow of fluids in a microchannel is normally laminar. We have used laminar flow in PDMS microchannels to deposit proteins and cells onto a solid substrate [67] (in general, microfluidic channels can be used to deposit proteins and cells onto substrates [12, 68, 69]). Using laminar flow in microchannels, we can also deliver molecules to different regions of live cells with subcellular precision [70]. Specifically, we placed a PDMS microchannel over live capillary endothelial cells, and controlled the flow in a way that caused solutions containing mitochondrial dyes of different colors to contact different parts of a cell; sub-populations of mitochondria inside the cell were labelled with different dyes. It was possible to follow the movement of the different populations of mitochondria; they mixed throughout the cell after 2.5 h. Also, by flowing a membrane-permeable, actin-disrupting molecule over specific locations, we disrupted actin filaments at targeted portions inside a cell.

As a tool to study subcellular biology, this method has the advantages over microinjection and microperfusion in that it involves no complicated micromanipulation, and that it works for any type of cell that can be grown on solid

support. Laminar flow was used to address the fundamental question of how signaling is transmitted in a cell after stimulation by a ligand [71]. Specifically, epidermal growth factor (EGF) receptors in localized areas of a COS cell were stimulated by a flow stream containing EGF, and the propagation of EGF signals was followed by fluorescent indicators (using genetically engineered proteins that fluoresced upon tyrosine phosphorylation or Ras activation). The authors concluded that the signals spread over the entire cell in cells overexpressing the EGF receptor, but that the signals were localized to the stimulated regions in cells expressing only a basal level of EGF receptors.

4.4.2 Molecular gradients

Laminar flow can be used to generate stable gradients of molecules in solution and bound to the surface (Fig. 1C). This technology enables the quantitative investigation of cellular phenomena involving molecular gradients over a distance of several hundred μm. In one study, Toner *et al.* [72] used a PDMS microfluidic gradient generator to produce stable solution gradients of IL-8, an important cytokine in inducing chemotaxis of neutrophils to the site of infection during the inflammatory response (Fig. 5A). Flow of IL-8 gradients over surface-bound neutrophils made possible the measurement of the migratory response of the neutrophils as a function of the steepness and shape of the gradient. With gradual gradients, neutrophils migrated past the area of maximum chemoattractant before reversing direction; with a steep gradient, the neutrophils halted migration at the boundary. Overall, the technique provided an assay to study the behavior of chemotactic cells with a quantitative precision and control not possible in earlier studies.

Neurons are another important class of cells that respond to extracellular gradients. In particular, gradients of solution and surface-bound chemoattractants and chemorepellants are important in determining the behavior of axon growth, a key step in brain development. We fabricated a network of PDMS microchannels to generate linear gradients over hundreds of microns of surface-bound laminin (Fig. 5B) [73]. Hippocampal neurons were cultured on the immobilized laminin gradients, and the growth of processes from the neurons followed by microscopy. Of the several processes formed from neurons in the first days of culture, one process (designated the axon) elongates much more rapidly than the others. We found that after 24 h in culture, gradients of surface-bound laminin meeting a threshold slope oriented axonal specification in the direction of increasing laminin concentration for 60% of the neurons, com-
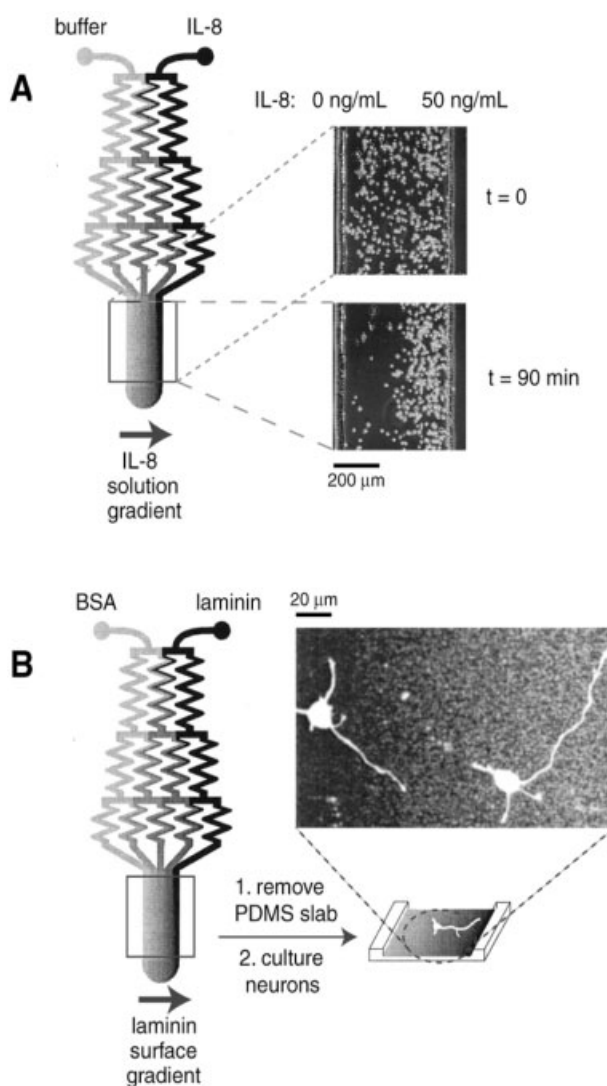

Figure 5. Studying the responses of cells to molecular gradients in microchannels. (A) Study of chemotaxis of neutrophils in a solution gradient of IL-8. In a linear gradient of IL-8 from 0 ng/mL to 50 ng/mL, neutrophils migrated to the region of highest IL-8. Figure adapted from [72]. (B) Dependence of axonal specification of hippocampal neurons on surface-bound gradients of laminin. A surface-bound gradient of laminin is formed from a solution gradient. Neurons cultured on the gradients exhibit preferential axonal specification in the direction of increasing laminin concentration. Figure adapted from [73].

pared to 33% (random orientation) in the absence of the gradient. Cremer *et al.* [74] used a simple laminar flow setup in a PDMS microchannel to create a solution gradient of chemoeffectors for bacterial chemotaxis. The microfluidic assay for chemotaxis showed greater sensitivity than conventional capillary assays; for exam-

ple, the microfluidic assay showed that *E. coli* chemotaxis was sensitive to a chemoattractor, L-Asp, at concentrations three orders of magnitude lower than previously reported.

4.5 Combinatorial screening

An exciting application of microfluidics is combinatorial screening by the use of many microchannels on a single chip. Quake *et al.* [75] built a PDMS-based microfluidic device that rapidly screened conditions for protein crystallization. Because PDMS is gas-permeable, large numbers of microchannels in a complex architecture could be filled with solutions with no trapped air bubbles [76]. The chip consumed less than 3 μL of protein sample, and tested for 144 different crystallization conditions in parallel using free interface diffusion. Compared to the conventional vapor diffusion method using a sparse matrix for sampling crystallization conditions, the microfluidic chip detected more conditions that generated crystals (of a variety of qualities), and consumed two orders of magnitude less protein sample. A number of different protein samples were crystallized (including the bacterial 70S ribosome, a large protein-RNA complex), and crystals extracted from the chip diffracted X-rays. The technology is now commercialized (Table 1).

Temperature is another important variable in biochemical assays. The temperatures in different parts of a PDMS-based chip can be varied by using heating devices. For example, by placing tungsten heaters at different parts of a circular channel, a PCR device consuming only 12 nL of sample was constructed [77]. Cremer *et al.* [78] fabricated a device that exhibited a temperature gradient either parallel to or perpendicular to the microchannels. The temperature gradient was used to construct a melting curve of double-stranded DNA, which could distinguish perfectly complementary DNA strands from those containing single mismatches. The temperature gradient can also be combined with another variable (such as sample concentration) in a 2-D format, in order to screen for optimal conditions for bioassays (such as protein crystallization, biochemical reactions, or cell behavior).

Large-scale microfluidic chips have many biological applications. Quake *et al.* [22] constructed a two-dimensional microfluidic array of 256 individually addressable chambers by integrating thousands of micromechanical valves. In one biological application of this array, *E. coli* expressing cytochrome *c* oxidase was identified in each chamber and noninvasively purged from the microfluidic chip. Potential uses of large-scale microfluidic arrays

include high-throughput analysis of proteins and DNA, and manipulation of cells (such as high-throughput cell fusion [79]).

5 Conclusions

Microfluidics offers a set of exciting tools for studying biology. It reduces the time and cost of common bioanalytical assays, and enables technologies to study cells in detail. As the material of choice for microfluidic systems, polymers such as PDMS exhibit advantages over silicon and glass, because they are easy to fabricate, and compatible with the requirements of many bioassays [80]. PDMS-based microfluidic systems can be used as a useful step to test new designs, or as a final product, as shown by a number of functional devices developed in academic institutions and private companies (see Table 1 for work done in the private sector). Some disadvantages of PDMS include: hydrophobicity of its surface, which resists wetting by aqueous solutions and is prone to nonspecific protein adsorption (necessitating in some cases surface modification); incompatibility with high concentrations of some organic solvents, which may otherwise be useful in some assays (*e.g.*, liquid chromatography) (Lee *et al.,* submitted); and limitations of feature geometries (its elasticity limits the aspect ratios of the features due to shrinking or sagging).

The technology for microfluidics is growing but still immature. Several key challenges must be met in order to build a functional "lab-on-a-chip". These challenges include the following: building a seamless world-to-chip interface; developing methods for pretreating "real-world" samples (from the laboratory, body or field), for handling fluids on-chip, and for minimizing clogging of microchannels due to small particles of dust or sample precipitation; integrating multiple microfluidic components and assays (each with different requirements for buffer and running conditions). Also, the use of small amounts of liquids, while a virtue in many ways, also brings some disadvantages: it requires a sensitive method of detection (which imposes a significant limitation for dilute samples) and has a very limited capacity for preparative work. Nonetheless, potential applications are many, including miniaturized bioanalytical instruments, clinical diagnostics, and perhaps, high-throughput methods for drug screening, gene-expression profiling, proteomics and combinatorial assays. We believe that polymeric systems will play an important role in the developments of miniaturized microfluidic devices, by lowering the barrier to entry for new researchers through simple fabrication procedures, and maintaining high compatibility with bioassay requirements.

6 References

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