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PHYSICS AND APPLICATIONS OF MICROFLUIDICS IN BIOLOGY

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■ **Abstract** Fluid flow at the microscale exhibits unique phenomena that can be leveraged to fabricate devices and components capable of performing functions useful for biological studies. The physics of importance to microfluidics are reviewed. Common methods of fabricating microfluidic devices and systems are described. Components, including valves, mixers, and pumps, capable of controlling fluid flow by utilizing the physics of the microscale are presented. Techniques for sensing flow characteristics are described and examples of devices and systems that perform bioanalysis are presented. The focus of this review is microscale phenomena and the use of the physics of the scale to create devices and systems that provide functionality useful to the life sciences.

CONTENTS

1. INTRODUCTION

Microfluidics has the potential to significantly change the way modern biology is performed. Microfluidic devices offer the ability to work with smaller reagent volumes, shorter reaction times, and the possibility of parallel operation. They also hold the promise of integrating an entire laboratory onto a single chip (i.e., lab-on-a-chip) (1). In addition to the traditional advantages conferred by miniaturization, the greatest potential lies in the physics of the microscale. By understanding and leveraging microscale phenomena, microfluidics can be used to perform techniques and experiments not possible on the macroscale, allowing new functionality and experimental paradigms to emerge.

Two examples of devices commonly considered microfluidic are gene chips and capillary electrophoresis. Whereas gene chips take advantage of some of the benefits of miniaturization, they are not technically microfluidic devices. Chip-based capillary electrophoresis devices are now commercially available and reviews are available elsewhere (2, 3). The focus here is on the physics of microfluidics, construction methods for making microchannels, and components and applications that make use of the unique properties of the microscale to address problems in biology.

An overview of the physics of microfluidics is given that highlights the important characteristics of the microscale. Certain fluid phenomena are dominant at the microscale and affect how devices can be made and used. Current techniques for making the devices are outlined and examples are given. Components of microdevices capable of actuating, sensing, and measuring within microfluidic systems are discussed. Finally, complete systems developed to perform functions in biology are presented.

2. THE PHYSICS OF MICROFLUIDICS

In order to understand and work with microfluidics, one must first understand the physical phenomena that dominate at the microscale. Other microfluidic reviews have been published (4–8), but none contain a comprehensive look at the physics of the microscale and how it makes certain devices possible. In this section, the physics of microfluidics are reviewed with references to more complete treatments.

Microfluidics is the handling and analyzing of fluids in structures of micrometer scale. The creation of microfluidic devices began by using technology originally developed for the microchip industry but has now grown into a field of its own (9–11).

At the microscale, different forces become dominant over those experienced in everyday life (12). Because of scaling, shrinking existing large devices and expecting them to function well at the microscale is often counterproductive (13). New designs must be made to take advantage of forces that work on the microscale.

The effects that become dominant in microfluidics include laminar flow, diffusion, fluidic resistance, surface area to volume ratio, and surface tension.

2.1. Reynolds Number

The Reynolds number (Re) of a fluid flow describes its flow regime—laminar or turbulent. Laminar flow is described in detail below. Turbulent flow is chaotic and unpredictable (i.e., it is impossible to predict the position of a particle in the fluid stream as a function of time). The Reynolds number can be calculated by

$$\mathrm{Re} = \frac{\rho v D_h}{\mu}, \tag{1}$$

where ρ is the fluid density, v is the characteristic velocity of the fluid, μ is the fluid viscosity, and D_h is the hydraulic diameter. The hydraulic diameter is a computed value that depends on the channel's cross-sectional geometry.

$\mathrm{Re} < 2300$, as calculated by the above formula, generally indicates a laminar flow. As Re approaches 2300, the fluid begins to show signs of turbulence, and as Re becomes greater than 2300 the flow is considered to be turbulent. The literature reports that the Re for transition from laminar to turbulent in microfluidic channels might be different than that predicted by theory. However, recent work indicates that the transition to turbulence in microchannels does follow theory and that reported differences are likely due to experimental error (14).

2.2. Laminar Flow

Laminar flow is a condition in which the velocity of a particle in a fluid stream is not a random function of time. Because of the small size of microchannels, flow is almost always laminar (15). One consequence of laminar flow is that two or more streams flowing in contact with each other will not mix except by diffusion (Figure 1*a*). [However, under certain conditions the diffusion between two streams is nonuniform through the height of the microchannel (16, 17).] Diffusion between laminar streams in a microdevice has been used for performing assays and sorting particles by size (18, 19). Another technique allowed by laminar flow is the creation of packets of fluid that, except for diffusive effects on either side of the packet, stay relatively well formed (Figure 1*b*). These packets can be moved around in a controlled manner and allow for many possibilities in cellular analysis.

2.3. Diffusion

Diffusion is the process by which a concentrated group of particles in a volume will, by Brownian motion, spread out over time so that the average concentration of particles throughout the volume is constant (Figure 1*a*). Diffusion can be modeled in one dimension by the equation $d^2 = 2Dt$, where d is the distance a particle moves in a time t, and D is the diffusion coefficient of the particle. Because distance varies to the square power, diffusion becomes very important on the microscale. For example, hemoglobin ($D = 7 \times 10^{-7}$ cm^2 s^{-1}) in water takes 10^6 sec to diffuse

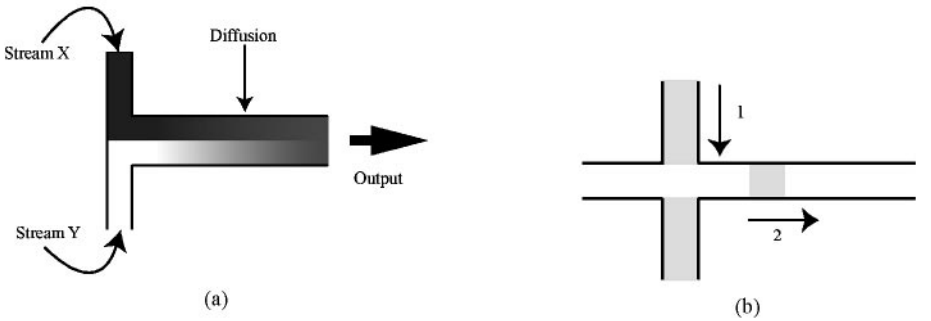

Figure 1 (*a*) Two streams flowing in contact will not mix except by diffusion. As the time of contact between two streams increases, the amount of diffusion between the two streams increases. (*b*) Fluid can be flowed in direction 1 with minimal leakage into the perpendicular channel. Fluid is then flowed in direction 2 to move a packet out of stream 1 and down the channel.

1 cm, but only 1 sec to diffuse 10 μm. Therefore, in a 1-cm wide tube, diffusion of hemoglobin is not usually an important consideration, but in a microchannel 10-μm wide, the distance travelled due to diffusion becomes important.

Because diffusion times can be short at the microscale, microchannels can be used to create concentration gradients having complex profiles (20, 21). Mixing schemes at the microscale must find ways to maximize the interfaces between solutions to allow diffusion to act quickly (22, 23).

2.4. Fluidic Resistance

Fluidic resistance in microchannels is governed by a set of equations whose solutions are well known (15). The flow rate within a microchannel is given by $Q = \Delta P/R$, where Q is the flow rate, ΔP is the pressure drop across the channel, and R is the channel resistance. The most common channel geometry, because of its presence in blood transport, is the circular tube. The resistance of a circular geometry can be calculated using the formula

$$R = \frac{8\mu L}{\pi r^4}, \tag{2}$$

where μ is fluid viscosity, L is the channel length, and r is the channel radius. For a rectangular microchannel with a low aspect ratio (i.e., $w \approx h$), the resistance can be found by

$$R = \frac{12\mu L}{wh^3}\left[1 - \frac{h}{w}\left(\frac{192}{\pi^5}\sum_{n=1,3,5}^{\infty}\frac{1}{n^5}\tanh\left(\frac{n\pi w}{2h}\right)\right)\right]^{-1}, \tag{3}$$

where w is the channel width and h is the channel height. The resistance of a rectangular microchannel with a high aspect ratio (i.e., $w \ll h$ or $h \ll w$) can be

found by

$$R = \frac{12\mu L}{wh^3}. \tag{4}$$

Other channel geometries and their resistances can be found elsewhere in References (10) and (15).

2.5. Surface Area to Volume Ratio

Surface area is another factor that becomes important at the microscale. As an example, a 35 mm diameter petri dish half full of water, a 2.5 mL volume, has a surface area to volume (SAV) ratio of 4.2 cm^{-1}, whereas a microchannel 50 μm tall, 50 μm wide, and 30 mm long, a 75 nL volume, has a SAV ratio of 800 cm^{-1}. When going from the macroscale to the microscale, an increase in the SAV ratio by orders of magnitude is not uncommon. A very large SAV ratio makes capillary electrophoresis (CE) more efficient in microchannels by removing excess heat more rapidly. Unfortunately, when transporting fluids using electrokinetic flow (24), the large SAV ratio allows macromolecules to quickly diffuse and adsorb to channel surfaces, reducing the efficiency of pumping (25).

2.6. Surface Tension

Surface tension forces at the microscale are also significant. As an example, consider that a water spider can easily walk on the surface of water, whereas a human cannot (Figure 2).

Surface tension is the result of cohesion between liquid molecules at the liquid/gas interface. The surface free energy of a liquid is a measure of how much tension its surface contains.

The height water will travel through a capillary is directly related to the water's surface free energy and inversely related to the radius of the capillary. When

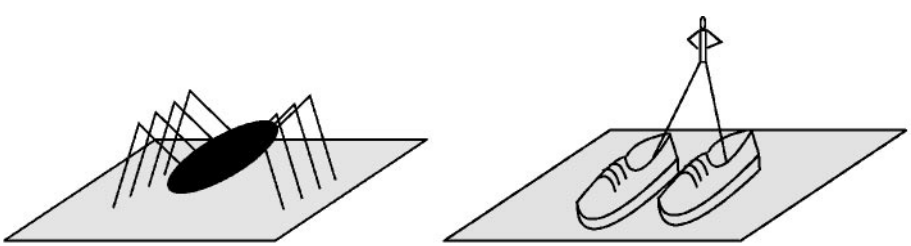

Figure 2 (*a*) A spider's weight is distributed over eight legs. Each leg by itself does not exert enough force to break the water's surface tension. (*b*) Humans have only two legs to distribute their weight. The force from each leg is far too great for the water's surface tension to support. If each leg's force was distributed over an area 1 mile long and 1/3 mile wide, humans could walk on water too (26).

microchannels with dimensions on the order of microns are used, the lengths liquids will travel based on capillary forces alone are significant. Surface energies have been exploited in microfluidics by creating virtual walls (27) as well as pumping mechanisms (28–30; G. Walker, submitted manuscript).

The pressure generated by a liquid surface with perpendicular radii of curvature R_1 and R_2 can be calculated with the Young-LaPlace equation:

$$\Delta P = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2} \right), \tag{5}$$

where γ is the surface free energy of the liquid. In the case of virtual walls (Section 4.1.1), the R defining the length of the wall goes to infinity and the equation reduces to

$$\Delta P = \frac{\gamma}{R}, \tag{6}$$

which gives the pressure present at the liquid boundary between two infinitely large parallel plates separated by a distance $2R$. If the surface is spherical, and $R_1 = R_2$, then the equation reduces to

$$\Delta P = \frac{2\gamma}{R} \tag{7}$$

and allows the calculation of the pressure contained within a spherical drop of liquid.

3. MANUFACTURING METHODS

The current techniques used for fabricating microfluidic devices include micromachining, soft lithography, embossing, in situ construction, injection molding, and laser ablation. Each technique has advantages and disadvantages, and the most suitable method of device fabrication often depends on the specific application of the device (6).

3.1. Micromachining

Silicon micromachining is widely used in microelectromechanical systems (MEMS) and was one of the first techniques to be applied to microfluidics. Complex systems can be manufactured out of silicon (31) (Figure 3). Recent advances in nanotechnology can also be used to create nanometer structures for microfluidic applications (32). Although micromachining techniques are widely used, silicon is often not the ideal material for microfluidic applications due to optical opacity, cost, difficulty in component integration, and surface characteristics that are not well suited to biological applications. The needs of many microfluidic applications do not require the precision that micromachining can offer. In addition, micromachining techniques are costly, labor intensive, and require highly specialized skills, equipment, and facilities. Silicon- and glass-based microfluidic devices are, however, well suited to some chemistry applications that require strong solvents, high

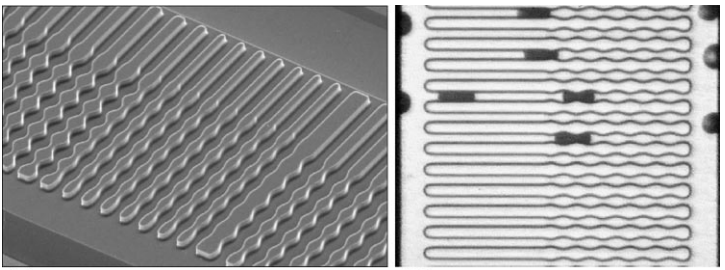

Figure 3 An SEM image of a glass silicon device is shown (*a*) and with erythrocytes flowing through the channels (*b*), which contain parallel-walled and varying cross-section elements for observing static and dynamic cellular deformation. Flow is from left to right. From Ref. 173, © 2001 IEEE. Reprinted with permission.

temperatures, or chemically stable surfaces. Chip-based CE is still largely within the domain of glass machining because of the surface properties provided by glass.

3.2. Soft Lithography

In order to promote widespread use of microfluidic devices in biology, a faster, less expensive, and less specialized method for device fabrication was needed. Elastomeric micromolding was first developed at Bell Labs in 1974 when researchers developed a technique of molding a soft material from a lithographic master (33). The concepts of soft lithography have been used to pattern surfaces via stamping and fabricate microchannels using molding and embossing. Several advances were made in Japan in the 1980s that demonstrated micromolded microchannels for use in biological experiments (34, 35). More recently, Whitesides (36–39) and others (40, 41) have revolutionized the way soft lithography is used in microfluidics.

Soft lithography typically refers to the molding of a two-part polymer (elastomer and curing agent), called polydimethylsiloxane (PDMS), using photoresist masters (Figure 4). A PDMS device has design features that are only limited by the master from which it is molded. Therefore, techniques used to create multidimensional masters using micromachining or photolithography can also be used to create complex masters to mold PDMS microstructures. A variety of complex devices have been fabricated, including ones with multidimensional layers (23, 42). Soft lithography is faster, less expensive, and more suitable for most biological applications than glass or silicon micromachining. The application of soft lithography to biology is thoroughly reviewed by Whitesides (39).

The term soft lithography can also be used to describe hot embossing techniques (43, 44). Hot embossing usually refers to the transfer of a pattern from a micromachined quartz or metal master to a pliable plastic sheet. Heat and high pressure allow the plastic sheet to become imprinted. The micromachined masters can be used many times to form plastic printed surfaces that can then be bonded to plastic tops to form microchannels (45). The plastic most commonly used for

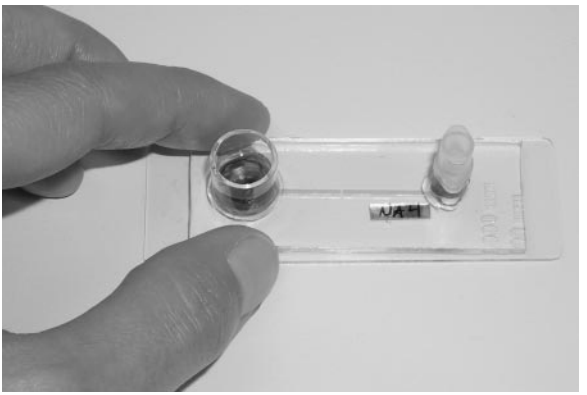

Figure 4 An example of a hybrid glass/PDMS device with a well and single channel for embryo culture. The micromolded PDMS slab has been permanently bonded to the glass slide. Photo courtesy of Vitae, LLC.

this purpose is polymethylmethacrylate (PMMA), which is the least hydrophobic of most common plastics (46). Hot embossing offers low cost devices, but does not offer a timely method for changing designs. In order to create new features or channel sizes, a new micromachined master is required, which is costly and time consuming. Hot embossing is appropriate for device designs that do not have to undergo changes and offers more material options than the elastomeric-based soft lithography techniques described above.

3.3. In Situ Construction

Recently, a new method for in situ construction of microfluidic devices using photodefinable polymers, called microfluidic tectonics, was introduced (47). The concept uses liquid phase photopolymerizable materials, lithography, and laminar flow to create microfluidic devices. The liquid prepolymer is confined to a shallow cavity and exposed to UV light through a mask. The prepolymer polymerizes in less than a minute. Channel walls are formed by the exposed polymer, which is a hard, clear, chemically resistant solid. Any unpolymerized monomer is flushed out of the channel (48). Once the walls have been formed, other types of photopolymerizable materials can be flowed into the channel and polymerized through masks to form components such as valves (49) and filters (50). The process is fast, typically requiring only a few minutes to create a simple device (Figure 5). Also, there is no need for cleanroom facilities, specialized skills, or expensive equipment. This method may prove to be useful for researchers wanting to enter the field of microfluidics without investing in expensive equipment or cleanroom facilities. The method also eliminates the bonding step (often the yield limiting step in manufacturing) associated with other methods. Although this method provides a

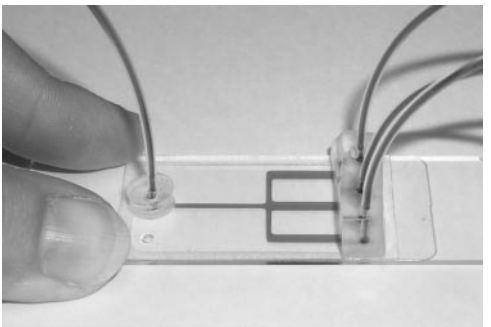

Figure 5 A device constructed using in situ construction techniques shows a channel network with external fluidic connections.

reasonably low-cost alternative, the device dimensions are limited by the resolution of the mask and polymerization effects of the polymer. Several materials have been used for in situ construction, including an isobornyl acrylate (IBA)–based polymer (47), as well as other UV-curable polymers (51, 52).

3.4. Micromolding

Injection molding is a very promising technique for low cost fabrication of microfluidic devices (53). Thermoplastic polymer materials are heated past their glass transition temperature to make them soft and pliable. The molten plastic is injected into a cavity that contains the master. Because the cavity is maintained at a lower temperature than the plastic, rapid cooling of the plastic occurs, and the molded part is ready in only a few minutes. The only time-consuming step is creating the master that shapes the plastics. This master, often referred to as the molding tool, can be fabricated in several ways including metal micromachining, electroplating, and silicon micromachining. The methods of fabricating the molding tool are similar to those used for making the master for hot embossing and thus, the same issues of cost apply. However, the injection molding process is considerably faster than hot embossing and is the preferred method, from a cost perspective, for high volume manufacturing. Limitations of injection molding for microfluidics include resolution and materials choices.

3.5. Other Methods

Another method of forming microfluidic devices is laser ablation of polymer surfaces (54, 55) with subsequent bonding to form channels. The process can easily be adapted to create multi-layer channel networks. Limitations include throughput due to the “writing” nature of the cutting process.

4. COMPONENTS

The main component in any microfluidic device is the channel network. The fabrication techniques described earlier have been used to make channels out of many different materials. Typically the cross-sectional shapes of microchannels are square, rectangular, or trapezoidal, although circular channels have been fabricated (56). Although a simple channel network (i.e., t or T junction) can be useful in some applications (i.e., CE), one must add components to increase the functionality of the system for other applications. In this section, components for use in microfluidic systems are discussed. The examples given focus on components that leverage the unique properties of the microscale to achieve the desired function.

4.1. Actuators

4.1.1. VALVES The ability to manipulate fluid flow using valves is essential in many microfluidic applications. There are two types of valves: passive valves that require no energy and active valves that use energy for operation. The type of valve used in a device depends on the amount and type of control needed for the application.

Active valves often use external macroscale devices that control the actuation and provide energy. Some recent designs include an electromagnetically actuated microvalve (57) and an air-driven pressure valve (58). Other active valve designs use energy from the driving fluid, eliminating the need for external power or energy from direct chemical to mechanical conversions. Rehm has demonstrated a hydrogel slug valve in which the driving force of the fluid moves a passive hydrogel slug to open or close an orifice (59). Others have used stimuli-responsive hydrogel materials that undergo volume changes through direct chemical to mechanical energy conversion. A variety of responsive hydrogel post valves (60) have been demonstrated. A responsive biomimetic hydrogel valve resembling the check valves found in veins has also been fabricated (49) (Figure 6) as well as a hydrogel-based flow sorter device (61) that directs flow autonomously based on the pH of the stream. These types of valves offer autonomous actuation capabilities due to the pH responsiveness of the hydrogel material. The hydrogel valves described here are practical due to the physics of the microscale. Because diffusion determines

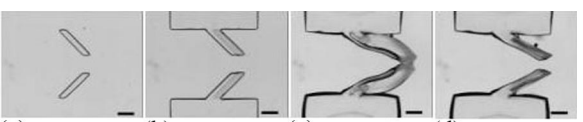

Figure 6 Fabrication of the bio-mimetic hydrogel check valve. (*a*) After polymerization of the pH-sensitive hydrogel strips. (*b*) After polymerization of the non-pH-sensitive strips to form the bi-strip hydrogel valves with anchors. (*c*) When exposed to basic solution, the bi-strip hydrogel expands and curves to form a normally closed valve. (*d*) When exposed to acidic solutions, the valve is deactivated, returning to the permanently open state. Scale bars, 500 μm.

the response of the hydrogel, scaling effects make the hydrogel respond faster (on the order of seconds) when constructed with smaller dimensions and larger surface area to volume ratios.

Passive valves can be used to limit flow to one direction, to remove air, or to provide a temporary flow stop. Passive one way valves (similar to the responsive bi-strip valve described above) have been constructed from both silicon and elastomers (62). An alternative method of constructing passive valves involves the use of porous hydrophobic materials or surface treatments to create selective vents or flow stops, respectively. Vents control fluid movement by allowing air to pass, but not the liquid being moved (63). Hydrophobic surface patterning can also be used to create a valve by making a section of channel hydrophobic (64). Such a hydrophobic valve is used in iSTAT's® blood gas measurement cartridge (65). Once the pressure to break the hydrophobic barrier has been reached, the valve breaks down allowing fluid flow. Surface patterning can also be used to create "virtual walls" that use hydrophobic regions to contain the liquid. By patterning different regions of the channel with different surface energies, a pressure switch can be formed that breaks down the wall once the maximum pressure of that surface has been reached (27) (Figure 7). The maximum pressures that the walls can sustain are proportional to the liquid surface free energy, the angle of curvature, and the inverse of the channel height. These parameters constrain the use of the virtual walls to the microscale. The resistance of the fluid channel can also be used to control flow. By changing the fluid resistance (i.e., the geometry) the pressure

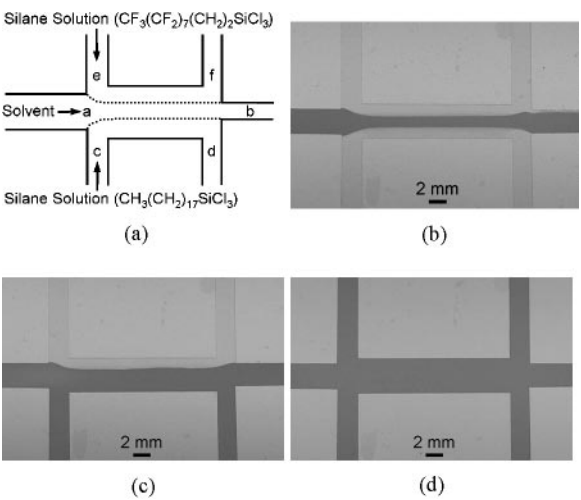

Figure 7 Demonstration of a pressure switch. (*a*) The laminar flow scheme for patterning two different surface energies in a microchannel. Images of Rhodamine B solution at various water-column heights: (*b*) 10 mm, (*c*) 26 mm, and (*d*) 39 mm. Reprinted with permission from Ref. (27). Copyright 2001 American Association for the Advancement of Science.

required to introduce fluid varies (66). The development of microfluidics on a CD format uses changing resistances and changing pressures (via centrifugal force) to program fluid flow (67, 68).

4.1.2. MIXERS Mixing is a basic process required for many biological applications. At the microscale, laminar flow conditions prevent mixing except by diffusion. However, diffusion does not happen fast enough to provide an adequate means of mixing in some microfluidic-based assays, particularly those that require relatively large particles (i.e., cells) to mix. In a microfluidic device, there are two ways of mixing fluid streams. Passive mixers use channel geometry to fold fluid streams to increase the area over which diffusion occurs. Examples of passive mixing include a distributive mixer (69–71), a static mixer (72, 73), a T-type mixer (74), and a vortex mixer (75). The Coanda effect is used to make an in-plane micromixer that splits the fluid streams and recombines them to induce mixing (76) (Figures 8*a* and 8*b*). A design for passively inducing chaotic advection in a

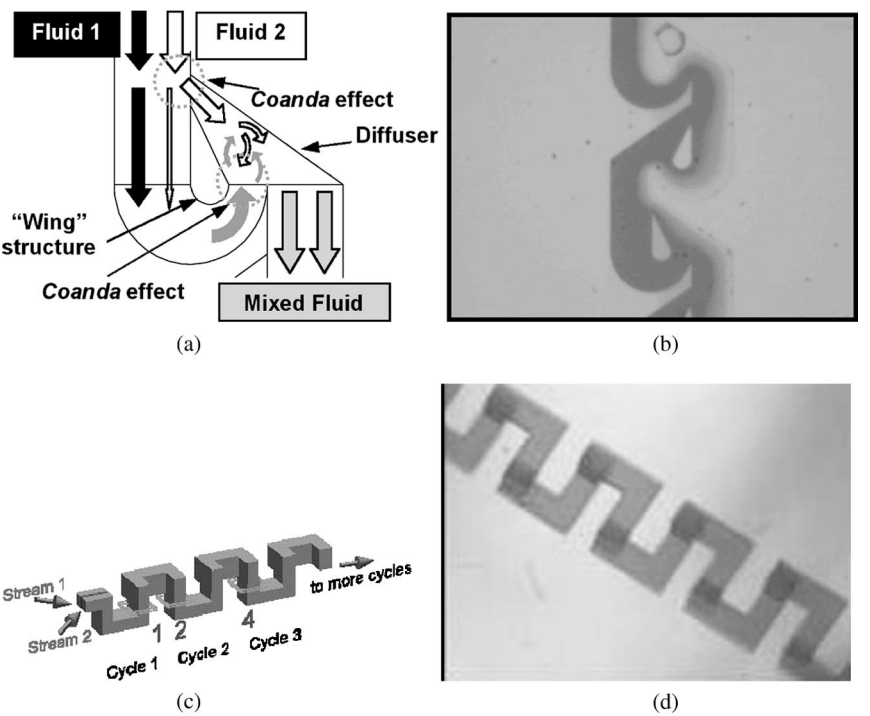

Figure 8 Two examples of passive micromixers. (*a*) A schematic of a mixer using the Coanda effect, which splits the fluid streams and then recombines them, and (*b*) a picture of the Coanda effect mixer. (*c*) A schematic of a 3-D serpentine micromixer, which induces chaotic advection, and (*d*) a device which shows a serpentine channel for mixing. From Ref. (76) reprinted with permission from Kluwer Academic Publishers © 2001.

microchannel uses a three-dimensional serpentine microchannel (23) (Figures 8*c* and 8*d*).

Active mixers use external sources to increase the interfacial area between fluid streams. Examples of active mixing include a PZT-based mixer (77), electrokinetic mixers (78, 79), a chaotic advection mixer (80), and magnetically driven mixers (50, 81). The type of mixer preferred generally depends on the type of reagents that need mixing and the fluid regime of operation (i.e., the Re number). Some mixers are more efficient with faster flow rates, whereas others work more efficiently with slower flow rates. One difficulty in assessing micromixers is the lack of agreement on how to quantify mixing at the microscale (82).

4.1.3. PUMPS Pumping schemes incorporate many different physical principles (83). The different types of pumps have drastically different features including flow rate, stability, efficiency, power consumption, and pressure head. A few examples of pumping schemes that use external control include a shape memory alloy micropump (84), a valve-less diffuser pump (85), a fixed-valve pump (86) that uses piezoelectric actuation, and a self-filling pump based on printed circuit board technology (87). Pumps can also be injection molded (88, 89) to form inexpensive disposable pumping chambers that are externally actuated. Magnetically driven pumps include a magnetically embedded silicone elastomer (90, 91), a magnetohydrodynamic micropump (92), and pumps driven by ferrofluidic movement (93, 94) (Figure 9). A micromotor that can valve, stir, or pump fluids was also developed that was controlled by external magnetic forces (95).

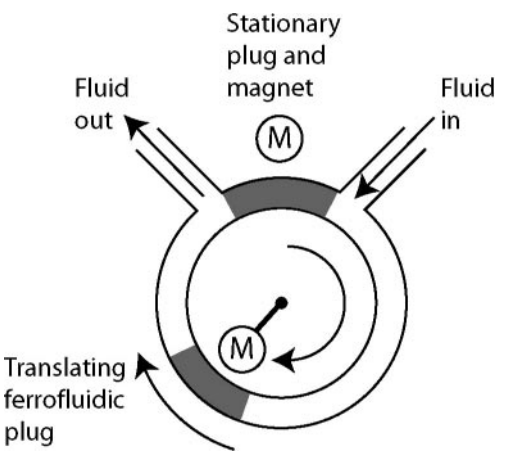

Figure 9 The ferrofluidic pump works by moving a plug of ferrofluid around the fluid-filled circle with a rotating magnet. Because the plug is immiscible with the fluid in the circle, fluid is pumped as the plug moves. The plug merges with the stationary plug as the arm swings past the stationary magnet. A new plug is created as the arm moves past the stationary plug and begins a new cycle.

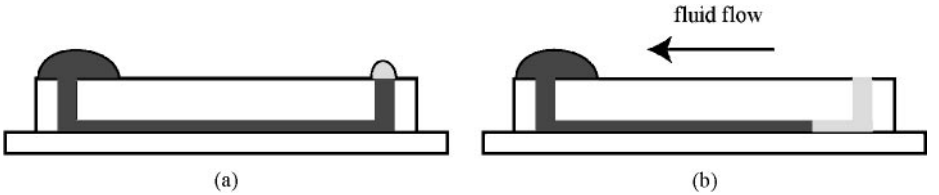

Figure 10 The passive pump relies on the surface tension of a drop of water to push fluid through a microchannel. A small drop has a higher internal pressure than a large drop (*a*). The difference in pressure will cause fluid to flow towards the larger drop (*b*).

The physical processes that dominate at the microscale allow the creation of pumps that are not feasible on the macroscale. Some designs require no moving parts like a bubble pump (96) that relies on the formation of a vapor bubble in a channel, an osmotic-based pump (97), and an evaporation-based pump that relies on a sorption agent to wick fluid through the channel (98). The surface tension present in small drops of liquid can also be used to pump fluid (Figure 10). The passive pumping technique provides a means of moving fluid by the changes in internal pressure of liquid drops (G. Walker, submitted manuscript). A smaller drop has a higher internal pressure than a larger drop. When a small drop is fluidically connected to a larger drop (i.e., through a microchannel), the fluid in the small drop will move towards the larger drop. In this manner, fluid can be passively pumped through microchannels simply by controlling the size of the drops on top of the microchannels.

4.2. Sensors

The development of microchannels resulted in the need for sensing and measuring capabilities at the microscale. The need for sensing in microfluidics falls into two categories.

First, one needs to measure the output of the device or system. Reducing volumes for chemical or biological assays to the microscale is of little use if there is no way to determine results quantitatively as in the macroscale. Reducing the sample size means reducing the amount of material to detect and increases the need for greater sensitivity. Creating sensors or sensing capabilities that are more responsive and smaller in size is an ongoing challenge at the microscale.

Second, one needs to measure the physics and chemistry of flow in microfluidic devices in order to understand and improve device and system designs. Quantifying both electrokinetic and pressure driven flow characteristics inside micro channels is critical to providing a basic science foundation upon which the field of microfluidics can grow (99). This section focuses on methods and techniques developed to quantify characteristics of fluids in microchannels.

The most straightforward method for measuring fluid flow (flow rate) in microchannels is to collect fluid at an output, measure the volume, and divide by the time over which the sample was collected. The method is normally quite accurate

for obtaining a bulk flow rate measurement. When dealing with small quantities, issues of collection, evaporation, and volume measurement must be carefully controlled to retain good accuracy. For electrokinetic flow, the current monitoring method is widely used for measuring flow rate (100). However, these methods do not provide any spatial information about flow inside the microchannel. Currently one of the most useful methods of measuring chemical and physical parameters in microchannels is fluorescence. Measuring fluorescence intensity is very sensitive, and fluorescently labelled chemicals are widely available. Fluorescence is used to measure such parameters as temperature (101, 102), cell function (103), flow velocity (104), flow profiles (16), and polymer dynamics (105). The development of μPIV (micro particle imaging velocimetry) has enabled researchers to quantify the flow patterns inside micro channels with high spatial resolution (Figure 11).

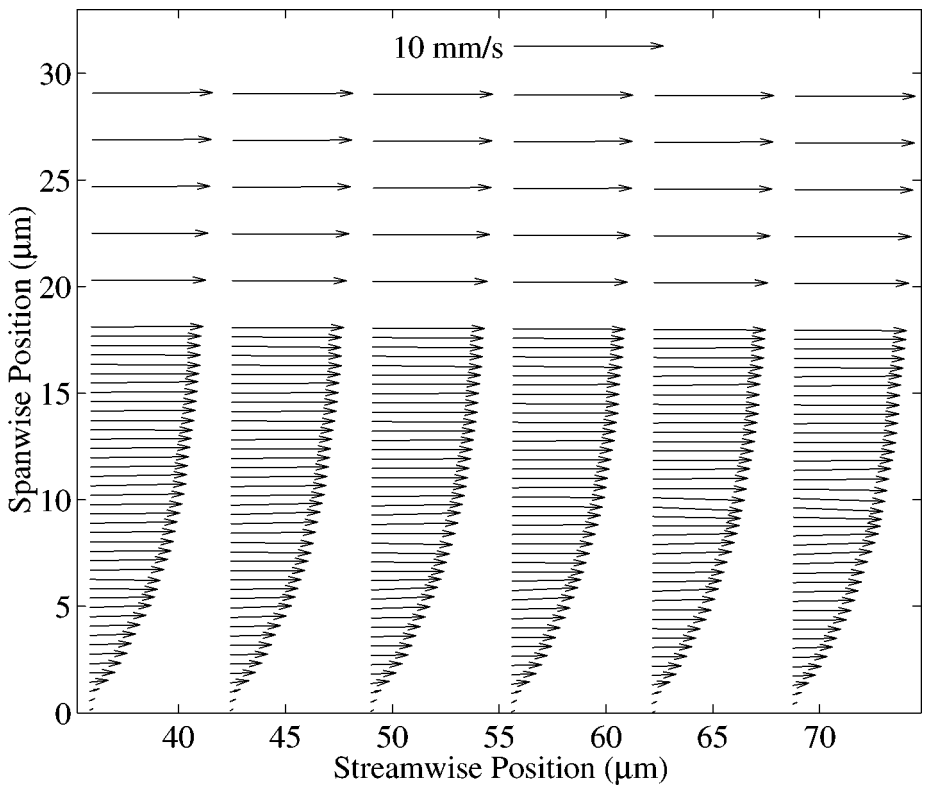

Figure 11 Ensemble-averaged velocity-vector field measured in a 30-μm deep $\times$ 300 μm-wide $\times$ 25 mm channel. The spatial resolution, defined by the interrogation spot size of the first interrogation window, is 13.6 μm $\times$ 4.4 μm away from the wall, and 13.6 μm $\times$ 0.9 μm near the wall. A 50% overlap between interrogation spots yields a velocity vector spacing of 450 nm in the wall-normal direction near the wall: a near-wall view of the lower 30 μm of vector field. From Ref. 174. Reprinted with permission.

Chemical reactions can also be monitored via fluorescence (106) or chemiluminescence (107–110) in microchannels. The fluorescence typically comes from labeled chemicals or beads added to the system. Although it is very useful to see a fluorescence reaction occurring in a microchannel, the presence of labeled chemicals passively serving the purpose of measurement may also interfere with the process under investigation. In addition, not all chemical, physical, or biological sensors are amenable to fluorescenct tags.

5. SYSTEMS

Microfluidic systems have become more popular in industry and academia over the past several years. Most microfluidic analysis devices are simply miniaturized versions of macroscale systems. However, the number of devices that take advantage of properties unique to the microscale is slowly growing. The ultimate goal of microfluidic systems is a "lab-on-a-chip" (111, 112)—the incorporation of multiple aspects of modern biology or chemistry labs on a single microchip.

5.1. Macromolecular Analysis

5.1.1. DNA ANALYSIS Next to CE, the polymerase chain reaction (PCR) is the most studied DNA analysis technique at the microscale (113–119). Other demonstrated applications of microfluidic devices for DNA analysis are a device that mixes DNA and a restriction enzyme and then separates the fragments (120) and a device that performs sample preparation (121).

Several integrated DNA analysis devices have been reported (122–125). These devices are capable of performing biochemical reactions (e.g., PCR) and separation steps (e.g., electrophoresis). Reviews of additional devices can be found elsewhere (126–130).

5.1.2. ENZYME ASSAYS Integrated microfluidic systems capable of performing assays to determine an enzyme's reaction kinetics have been developed. Microfluidic devices benefit enzyme assays by decreasing assay times, reducing reagent requirements, and increasing sensitivity.

The first microfluidics-based enzyme assays were reported at approximately the same time. One system was used to measure the activities of liver transaminases (131). The other system was used to determine the reaction kinetics of the enzyme β-galactosidase (β-Gal) (132).

More recently, microfluidic enzyme assays were developed to analyze protein kinase A (133) and an essential nerve enzyme, acetylcholinesterase (134). A microfluidic CD-based assay has also been developed that can conduct 45 simultaneous reactions on one disk (135). The CD relies on surface tension and centrifugal force to manipulate fluids in each microfluidic device.

Another example of incorporating the physics of the microscale into a functional microfluidic assay has been reported (136). The microfluidic device performs cell lysis, protein extraction via diffusion, and detection by diffusion using a fluorogenic enzyme assay (Figure 12).

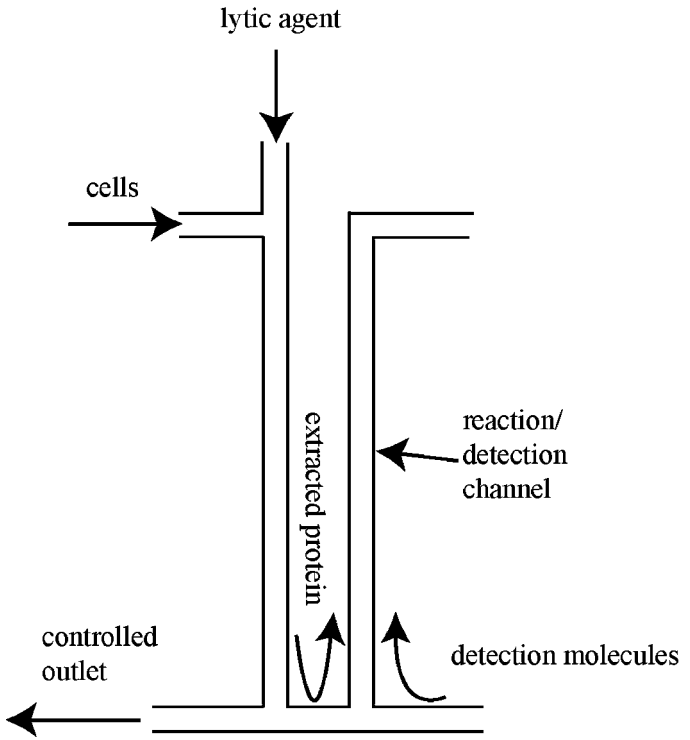

Figure 12 Lytic agent diffuses into the cell stream, lysing the cells and releasing the protein of interest. A small volume of the proteins are routed into the detection channel where molecules from a stream of detection reagent diffuse into the proteins, giving off a fluorescent signal.

5.1.3. IMMUNOASSAYS High-throughput screening (HTS) was first reported in 1962 (137). Since then, 96-well microtiter plates have become the fundamental technology for research and development in the pharmaceutical industry. Microfluidic devices are poised to supersede their less-functional microtiter plate counterparts.

Several immunoassays have been demonstrated in which only the separation of the product was performed within a microfluidic device (138–141). Other immunoassays have been performed in which bound fluorescent molecules were quantified instead of separated. In general, antigens were fixed to a surface within the microfluidic device and antibodies flowed past (142–145). One device even used *Escherichia coli* as its bound antigen (146). Microfluidic devices show promise for performing immunoassays because of their greatly increased SAV ratios.

In a departure from traditional immunoassays, a sensor using diffusion between two laminar streams, one containing antibodies and the other antigens, has been reported (19). Concentration of bound antibody/antigen was quantified using an inverted microscope and fluorescence source.

Another immunosorbent assay was reported in which secretory human immunoglobulin A was adsorbed onto polystyrene beads, which were then placed in a microchannel. The beads were held in place within the channel while the labelled antibody was flowed past (147).

More recently, immunoassays have been implemented in which all steps of the process (i.e., mixing, reaction, and separation) are incorporated into a single microfluidic device (148, 149).

5.2. Cellular Analysis

5.2.1. CYTOMETRY Macroscale flow cytometry systems have been in use for years to sort, analyze, and count cells. Methods have been reported for cell manipulation that could be incorporated into microfabricated flow cytometry devices (150–153). Some of these techniques have been implemented in a microfabricated fluorescence-activated cell sorter that is more sensitive and less expensive than conventional fluorescence-activated cell sorters (154). A flow cytometer has also been implemented on a microchip that is capable of counting particles of two different sizes—1 μm and 2 μm (155).

Cells can be identified based on the change in impedance they induce between a pair of electrodes (156). Single cells can be detected by measuring changes in capacitance at very high frequencies, thus eliminating the need for fluorescence tagging in flow cytometry (157, 158). A more thorough review of microscale cytometry work can be found elsewhere (159).

5.2.2. CELL-BASED ASSAYS Reports on current cell-based high-throughput assays and how they might be implemented in microfluidic chips can be found elsewhere (152, 159, 160).

5.2.3. CELLULAR BIOSENSORS Cell-based biosensors can provide more information than other biosensors because cells often have multifaceted physiological responses to stimuli. Cells ranging from *E. coli* to mammalian lines have been used as sensors for applications in environmental monitoring, toxin detection, and physiological monitoring, just to name a few. Several reviews have been published on the development and current state of cell-based biosensor research (161–163).

A complete device has recently been reported that uses a microfluidic cell-cartridge with a corresponding handheld electronics system for sample analysis (164).

5.2.4. CULTURING Microfluidic systems offer the ability to create cell-cell, cell-substrate, and cell-medium interactions with a high degree of precision (165, 166). To facilitate such studies, a method has been developed to investigate the properties of individual cells within their own environment (167).

As cell-based microfluidic assays become more popular, characterization of cell culture within microfluidic devices gains importance. Embryos cultured in microfluidic devices develop at more in vivo rates compared to traditional culturing methods (168). Insect cells have also been cultured in microchannels and grow at a much slower rate than reported at the macroscale (169). Different growth

characteristics in cultures at the macroscale and microscale highlight the fallacy in assuming biology scales down without consequence.

6. OUTLOOK AND CONCLUSIONS

Learning to think on an entirely different scale is a new and exciting challenge in microfluidics. The laws that govern the microscale are being exploited to control fluid in ways not previously available. Creating devices out of silicon, glass, and plastics has been accomplished by both traditional and nontraditional techniques. The fabrication technique and material chosen are dependant on the application. By learning to control fluid flow in microchannels, systems can be realized that perform the basic steps in common biological assays. Reducing sample size, decreasing assay time, and minimizing reagent volume are all advantages of the microscale. The greatest advantages will be seen where applications are identified that can only be performed due to the physics of the scale.

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