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FUNDAMENTALS OF MICROFLUIDICS DEVICES

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1.1 INTRODUCTION

Microfluidics is a term that describes the research discipline dealing with transport phenomena at microscopic length scales (typically 1–500 μm) and components and techniques used to control and actuate the fluids.

The science of miniaturization was initially fueled by the microelectronics industry during the development of miniature silicon-based electronic devices. Techniques for silicon microfabrication and miniaturization were then extended to the fabrication of mechanical devices that became known as microelectromechanical systems (MEMS).[1] A later trend in MEMS technology was the development of devices for applications in medical and life science areas. The term biological microelectromechanical systems (BioMEMS) was coined to describe such devices and systems, although subsequently they did not all necessarily have the components normally found in traditional MEMS devices. Hence, a broad definition of BioMEMS would include some devices and applications made using the modern implementation of microfluidics, which was developed by Manz and coworkers[2,3] in the early 1990s.

With the emergence of the field of nanotechnology (roughly defined as the understanding and control of matter at dimensions of 1–100 nm), the field of

Microfluidic Devices in Nanotechnology: Fundamental Concepts, Edited by Challa S. Kumar

nanofluidics has recently emerged. The main difference between microfluidics and nanofluidics is primarily a matter of scale, as defined by the volume of fluids handled in the system.

1.1.1 State-of-the-Art Commercial and Scientific Aspects

The fields of MEMS and microfluidics have extended beyond the traditional area of development of inkjet head and pressure sensors to areas such as drug delivery, chemical synthesis, protein crystallization,[4] cell culture,[5] point-of-care diagnositics,[6] genetic sequencing, drug discovery, genomics, and proteomics. Microfluidics has the potential to dramatically change the way in which the pharmaceutical industry screens for drugs and targets with a significant increase in performance due to the ability to do fast, high-throughput, parallel experiments with very little reagents on a single chip. This capability is not possible with current benchtop techniques.

Microfluidics has also found much use in the scientific community. It is now a common tool for chemists, physicists, biologists, and most engineering disciplines and has thus become a multidisciplinary platform advancing many frontiers in science and engineering. It has helped to advance understanding the theory and modeling of fluid dynamics, including the transition from continuum-based theory to discretized models. Life scientists are using microfluidics to explore phenomena at the single-cell level and in confined well-defined environments. Chemists and biophysicists are able to grow and analyze protein crystals and sequence DNA in a reagent and time efficient manner. The overall influence of microfluidics in the scientific community is evidenced by a large and sustained growth in the number of publications in journals and conferences from the mid-1990s to today.

1.1.2 Organization of the Chapter

The remaining chapter is divided into five sections, each of which is outlined as follows:

- Section 1.2 discusses aspects of the physics and modeling of microfluidics. We examine continuum and molecular-level models, and also introduce some of the dimensionless numbers relevant in the physics of microfluidics; since our discussion is not an exhaustive treatment, we direct readers to other publications with more in-depth analysis.[7]
- Section 1.3 deals with technological components used in microfluidics. The section is divided into subsections that deal with fluid switching, fluid flow and actuation, and fluid mixing. This section also includes a section on droplet microfluidics.
- Section 1.4 discusses aspects of fabrication of microfluidic devices. This section is divided into four subsections that examine techniques (based primarily on the nature of the material) for fabricating a microfluidic system.
- Section 1.5 discusses applications of microfluidics.

- Section 1.6 discusses future directions and probable techniques and applications that will drive the cutting edge of research in microfluidics.

1.2 PHYSICS AND MODELING

Attempts toward understanding the physics behind fluid flow at the microscale have been made to account for a different dominant type of force at microscale dimensions. At larger dimensions, inertial forces play a dominant role, whereas as the dimensions decrease surface forces become increasingly important. The Reynolds dimensionless number is used to compare the body forces and surface viscous forces. Moreover, surface area relative to volume increases as the characteristic dimension decreases.[8]

As the size scale decreases, the assumption of continuity is increasingly challenged. In general, approaches for modeling fluids can be divided into the two major categories of continuum models and molecular-level models.[9] In continuum models (the most common of which are formulated using Navier–Stokes equations), the discontinuity that exists among discrete molecules is discarded. This assumption is valid when the dimensions of the system are much larger than molecular dimensions, but the assumption breaks down when the dimensions of the system become comparable to molecular dimensions. For simulating systems with small dimensions, molecular-based models can be deterministic (e.g., some forms of molecular dynamics simulations), statistical (e.g. Monte Carlo), or a hybrid. A dimensionless number, the Knudsen number, can be used to evaluate whether the continuum flow or discrete molecular model is more appropriate.

1.2.1 Dimensionless Numbers

The above two examples hint at the importance of dimensionless numbers in microfluidics. Since it is the interplay of different phenomena that lead to specific flow patterns at the microscale, dimensionless numbers are defined to compare the relative importance of each of these phenomena. Some important dimensionless numbers in microfluidics are described briefly below:

- *Reynolds Number (Re)*: As the characteristic length in microfluidic devices is decreased, inertial forces (which are dominant in macroscale systems) decrease significantly compared to viscous forces. Since inertial terms are the cause for creating turbulence, flow regimes tend toward laminar flow at small dimensions. The Reynolds number ($\rho UL/\eta$) compares the relative magnitudes of inertial force ($\rho U^2/L$) and viscous force ($\eta U/L^2$). In microfluidics, Re is typically less than 1.
- For $Re \ll 1$, nonlinear terms (such as convective acceleration) can be neglected, such that fluid flow switches to the Stokes regime.
- *Knudsen Number (Kn)*: The validity of continuum model is normally assessed using the measure of free path length, as embodied by the Kn, which is the ratio of the mean free path length to the characteristic dimension. The assumption of

continuum model is valid as long as the *Kn* number is small ($Kn \ll 1$). On the basis of various magnitudes of Knudsen numbers, flow regimes change from continuous flow to slip flow, and finally free-molecule flow regime.[10]

- *Pèclet Number (Pe)*: When Reynolds number is small (which is generally the case in microfluidic systems), flow is not turbulent, and hence fluid mixing by convection is not significant. In such cases, mixing through diffusion becomes important. To define a measure of diffusive versus convective transport, the Pèclet number (Uh/D) is defined as the ratio of convective transport (Uh) to diffusive transport (D).[7]
- *Damköhler Number (Da)*: Extensive development of microfluidic applications has led to the incorporation of chemical reactions on surfaces. For instance, in immunoassay diagnostic devices, functionalized surface of the microchannel serves as a reacting surface, and the relative speed of reaction versus diffusion is one of the key factors in determining efficiency of the assay. The Damköhler number ($k_{on}Ch/D$) compares the relative speed of diffusive transport (D) and surface reaction ($k_{on}Ch$).
- *Capillary Number (Ca)*: In a microscale environment, surface effects are extremely important due to the high surface area-to-volume ratio. As such, surface tension plays a significant role in many microfluidic applications, including droplet formation. The relative magnitude of surface tension (γ) with respect to viscous effects (ηU) is compared by defining the dimensionless capillary number ($\eta U/\gamma$). One use of *Ca* is to make droplets with different dimensions (by choosing appropriate capillary numbers) to study chemical reaction kinetics.[11]
- *Mach Number (Ma)*: The Mach number is defined as the ratio of flow velocity to the velocity of sound in that medium; this number serves as a measure of fluid compressibility. For Ma less than 0.3, the fluid can be considered incompressible.

1.2.2 Modeling Approaches

Continuum models are defined in terms of independent variables (velocity, pressure, and density) that are solved for all fluid element nodes at each time step. For finite element simulations, smaller elements provide better accuracy at the expense of higher computational effort. Thus, the conservation of mass and momentum could be written as

$$\frac{\partial p}{\partial t} + \nabla, (\rho u) = 0$$

$$\rho\left(\frac{\partial u}{\partial t} + u, \nabla u\right) = -\nabla p + \nabla T + f$$

Where ρ and u are fluid density and velocity, respectively, p is pressure, T is surface force, and f is body force. In the case of viscous flow, the Navier–Stokes equation is

a second-order partial differential equation that requires two boundary conditions. First, the normal component of velocity is normally set to zero at the impermeable boundaries. Additional tangential boundary conditions are required to solve the Navier–Stokes equation in the region of interest. For continuum flow, the no-slip boundary condition is a second boundary condition, although this assumption breaks down for higher Knudsen numbers ($Kn > 0.1$).

1.2.3 Particle Imaging Velocimetry

Fluid flow models can be verified with particle imaging velocimetry (PIV). Methods for qualitative visualization of fluid flow are insightful but are not sufficient for determining the flow structures in microchannels. Traditionally, intrusive methods using pressure tubes or hot-wire anemometer, and nonintrusive methods such as laser Doppler velocimetry, have been used to determine single-point velocities in the fluid, but are ill-suited to determine the velocity field across all fluid elements.[12] In particle imaging velocimetry, tracer particles are added to the fluid, and a single cross-sectional plane of the fluid is visualized. A CCD camera is used to record a sequence of images (with the frequency of the recording determined in part by fluid velocity), and postprocessing algorithms help determine the velocity field. PIV has proven to be a powerful method in the visualization fluid flow in microfluidic devices.[13]

1.3 COMPONENTS OF MICROFLUIDICS

Since around 1990, when the modern implementation of microfluidic systems originated,[3] researchers have developed a diverse range of microfluidic components, ranging from micropumps to micromixers. Such components allow precise control and manipulation of fluids. The design and operation of these components are based on the volume of fluids handled, the type of fluids, and the particular application.

1.3.1 Fluid Actuation

In this section, we examine methods and techniques that are used to drive fluids through the microfluidic system. We discuss only systems where the pumping is done by components on-chip, although syringe pumps (which are external to the chip) are often used. We discuss traditional mechanical pumps as well as two pumping schemes that have been enabled by the microscale. Important parameters that are considered in design and fabrication of pumps include the maximum flow rates that can be achieved, power consumption, efficiency, and back pressures that can be sustained while maintaining appreciable pumping.

1.3.1.1 Mechanical Displacement Pumps

Most pumps in this category use a diaphragm or a flexible membrane. Displacement of the diaphragm applies a force to the fluid, thus causing it to be displaced. In the reciprocating pumps, the deflection is done in a periodic manner. Materials for the

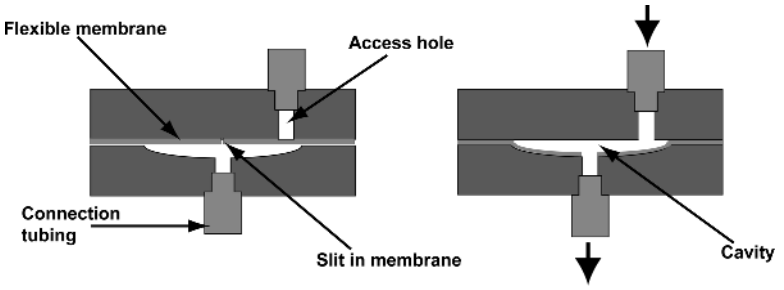

FIGURE 1.1 Schematic showing the mode of operation of a passive membrane type valve.

diaphragms include thin silicon, glass films, elastomeric polymers such as thin layers of silicone or polydimethylsiloxane (PDMS), and thin films of other polymers such as polyimide and parylene C. Such pumps normally have passive check valves (Section 1.3.2.2) that regulate the flow of fluid into and out of the pump chambers. During a single pump cycle, movement of the diaphragm first increases the volume of the pump chamber (during the suction or expansion stroke), thereby drawing fluid into the chamber. A subsequent decrease in the volume of the pump chamber during the alternate stroke of the diaphragm (during the contraction or discharge stroke) forces the fluid out (Figure 1.1). In some cases, more than one pumping chamber is connected in series to form a peristaltic pump.[14–16]

Alternatively, electrostatically driven micropumps can be driven by both AC and DC current. Materials include parylene (multilayer peristaltic pump)[17] and a combination of glass, silicon, and polysilicon with gold electrodes.[18]

The use of piezoelectrics to drive microfluidic micropumps was initially demonstrated in inkjet printer heads in the early 1980s.[19] Piezoelectrically driven micropumps have become more common because they are relatively inexpensive to fabricate especially when the diaphragm or flexible membrane is made with cheap elastomeric materials like PDMS instead of thin glass or silicon membranes. The literature on piezoelectric-driven micropumps is extensive, with many recent designs reported in the literature.[20–24]

Electromagnetically driven micropumps use Lorentz forces generated by interaction of electromagnetic fields (when an electric current is passed through a coil that surrounds a permanent magnet). The electromagnet is designed with a movable part that deflects an attached membrane upon turning on the electromagnet. The advantage of this actuation mechanism over others (such as electrostatic) is a lower requirement of voltage. Furthermore, by incorporating the coils and permanent magnet directly into the device, electromagnetically driven micropumps have been fabricated with improved diaphragm deflection and reduced sizes.[25,26] Modifications to this technique include the use of composite diaphragms in which magnetic material is incorporated into the diaphragm material such as PDMS.[27]

Thermally actuated micropumps depend on expansion of materials or changes in stress due to applied heat for actuation. Common thermal techniques include the use of thermopneumatic (e.g., indium tin oxide (ITO) coated on glass with a PDMS

diaphragm)[28] and shape memory alloy (e.g. thin-film TiNi shape memory alloy (SMA)[29] or bimetallic strip actuators. Drawbacks for such systems include slow response times and low pumping rates. A variation of a thermopneumatic pump (from Ahn's group) encloses nitrogen gas in plastic capsules. Release of the gas using localized heaters as detonators drives the fluids through the plastic chip.[30,31] However, these microfluidic chips are designed primarily for single use.

Pneumatic pumps use changes in gas pressure to deflect the membrane. As described in Section 1.3.2.1, Quake's group has used external gas sources to drive pneumatic PDMS-based membranes, which can also act as pumps, fabricated using soft lithography techniques.[32–34] Takayama's group also uses PDMS-based membranes for microfluidic pumps, but rather than external gas sources to drive the membranes, uses Braille pins that move in a sequential manner.[35–37]

Other pumping methods are based on displacement but not a diaphragm for driving fluids. For example, a micropump has been developed that consists of two serial check valves that convert the periodic motion of a ferrofluidic plug into a pulsed quasi-continuous flow (using an external NdFeB permanent magnet).[38]

Phase change pumps also involve mechanical displacement of the fluid interface. Instead of a diaphragm, these methods take advantage of a change in volume in a liquid–vapor transition (e.g., isopropyl alcohol) to drive fluids. Other examples of this technique include bubble pumps and electrochemical pumps.[39,40]

1.3.1.2 Nonmechanical Pumps

Certain micropumps operate by continuously adding momentum or directly imparting Lorentz-type forces in the fluid volumes. They require the conversion of nonmechanical energy to kinetic energy to supply the fluid with momentum. These phenomena are practical only on the microscale. We briefly discuss two methods as follows:

- *Electroosmotic Micropumps*: Electrosmosis is an electrokinetic phenomenon (i.e., in which fluids are moved by applying a voltage) that can be leveraged to pump electrolytic fluids. Electroosmotic pumps use instantaneous surface charges that develop on a solid surface (fused silica becomes negatively charged when it comes into contact with aqueous solutions); these surface charges attract ions of opposite polarity from the bulk of the fluid. A portion of the counterions in the liquid phase can be set into motion by applying an electric field parallel to the wall, with the mobile ions dragging bulk liquid in the direction of the electric force. Advantages of electroosmotic pumps include the absence of any moving parts and the ability to switch fluid flow direction quickly by switching the electric field. Some drawbacks include the requirement for high voltages and electrically conductive fluids. Electroosmotic pumps based on silica monoliths[41] as well as other examples have been demonstrated.[42,43]
- *Electrohydrodynamic Micropumps*: Electrohydrodynamic (EHD) micropumps[44,45] use interactions of electrostatic fields with ions in dielectric fluids. Three different mechanisms are associated with electrohydrodynamic pumps, and hence three different types exist: *induction*-type EHD pumps, where charge is induced in an inhomogeneous working fluid through the application of

a potential difference across the fluid; *conduction* EHD pumps, which rely on ion drag associated with this bipolar conduction; *injection* EHD micropumps, which are based on the injection of ions into the bulk fluid by applying high electric fields at the interface between electrodes and fluids and in which Coulomb forces act on the ions to generate a pressure gradient and motion of fluids between the electrodes.[46] The main disadvantage with EHD-driven pumps is the requirement for extremely high voltages (50–600 V) that may not be suitable for certain applications.

1.3.2 Fluid Switching

On a chip, fluid switching and control are normally accomplished using either electrokinetics or microvalves. Fluid control is often one of the most important elements for the realization of a fully integrated microfluidic system. In pressure-driven systems, microvalves (i.e., small valves that are built into the chip itself) can switch and regulate flow, as well as physically isolate different parts of a microfluidic system. Microvalves can generally be classified into two main categories: active and passive. In both categories, they can be either mechanical, nonmechanical, or, in the case of active valves, externally actuated. Important considerations for designing microvalves include leakage, power consumption, response time, and reliability. The valves can be designed to be normally open, closed, or bistable (in which case the resting state can be adjusted).

1.3.2.1 Active Microvalves

Actively driven mechanical microvalves are designed based on a number of different actuation mechanisms. In many designs, a membrane is deflected when the actuating source is turned on. These types of valves can be built using the traditional bulk and surface micromachining techniques employed in the MEMS industry.

Magnetically actuated microvalves can use permanent magnets,[47] electromagnets, or a hybrid attached to a membrane. Instead of using membranes, valves using magnetic balls have also been reported, such as a design with three polymer layers that form the base and casing for the valve and where the fluidic layer is made of metal.[48] Other designs of magnetically actuated ball valves can be scaled down to the nanometer length scale, which is important for nanofluidic applications.[49]

Electrostatically actuated microvalves have also been built. Because of high voltage requirements, however, this type of valve is often used to control gas flow rather than liquids (which can undergo electrolysis at high voltages). Electrokinetics has been used to move particles and liquids in microfluidics and has also been used for fluid switching and control. For example, electrokinetically driven flow can be used to move polymer monoliths photopatterned with lasers to open and close fluidic channels made in glass. The valves hold off pressures as high as 350 bar.[50]

Piezoelectric-driven microvalves are quite common. Although piezoelectric disks are able to generate appreciable deflection forces, their stroke is limited due to the high Young's modulus of the materials used. This limitation has been addressed by a number of groups using the hydraulic amplification principle.[51,52]

Actuation mechanisms that depend on transfer of thermal energy include thermopneumatic, bimetallic strip actuated valves (thermomechanic) and valves that use shape memory alloy. In the case of thermopneumatic actuation, energy derived from a change in volume of sealed liquid, solid, or gas due to thermal loading is used to drive a membrane or diaphragm that controls fluid flow. A two-chamber thermopneumatic valve with a PDMS diaphragm sandwiched between fluidic layers built on silicon and glass has been demonstrated. Air is used as the sealed fluid.[53]

Bimetallic strips are two bonded solids that have different coefficients of thermal expansion. They bend or morph when the metal is heated: this change in shape is used to deflect a flexible membrane. A bimetallic strip-driven microvalve with an 8 μm thick silicon membrane and a 5 μm thick aluminum layer that provides fully proportional control of flows has been demonstrated.[54]

Another actuation mechanism that depends on transfer of thermal energy uses shape memory alloys that allow for compact designs and can generate large forces. Valves using these alloys can also be built using thin films of the alloys on membranes.[55]

One problem with microvalves that are actuated using transfer of thermal energy is that designs cannot be used in applications where high temperatures cannot be tolerated.

Hydrogel-based microvalves can act as active nonmechanical valves. The valve structures can be fabricated *in situ* by directly photopatterning the liquid phase of the gel.[56] Contraction and expansion of the hydrogel due to changes in pH[56] or temperature[57] of the surrounding fluids are used to open and close the valves. A modified three-dimensional (3D) hybrid hydrogel microvalve where the expansion of the hydrogel is used to deflect a membrane acting as the valve has also been demonstrated.[58] The modified design allows physical separation of the sensing and regulated streams. A drawback with hydrogel-based valves is the slow response time (thermal responsive hyrogels provide faster actuation than the pH-sensitive gels).

Paraffin-based microvalves are also active nonmechanical valves. A plug of paraffin wax is used to seal the fluidic channel, and melting of the plug (by heating) opens up the channel. Reversible opening and closing can be achieved using a combination of pressurized air and vacuum system that drive the melted paraffin wax to either close or open the valve.[59] A variation of this design uses a latching mechanism to reduce the power necessary to actuate the valves.[60] Irreversible paraffin valves have also been demonstrated.[61,62]

Other active microvalves include those actuated using pneumatics or external vacuum sources. Such externally actuated valves have flexible membranes made of thin silicon layers, silicone, or PDMS that deflect due to the applied pressure and open or closed channels depending on their resting state. Quake's group developed a microfabricated two-layer normally open PDMS membrane valve actuated using external pneumatics.[4,32–34,63] This valve design is robust and scales very well and hence has been adapted and used by other groups.[5,64,65] One drawback of this valve design operating in a normally open configuration is that energy has to be expended to keep it closed. Mathies' group has developed a valve that is normally closed and opens

upon application of vacuum. The device consists of three layers: a PDMS membrane and two glass layers that form the fluidic and control layers. One caveat of this design is that external power consumption (in the closed state) is reduced at the expense of simplicity of fabrication.[66,67]

1.3.2.2 Passive Microvalves

This section will briefly look at various types of passive microvalves both mechanical and nonmechanical. Passive mechanical valves are normally flaps, spherical balls, membranes with slits, or other mobile structures that are displaced by motion of the fluid due to forward pressure. Figure 1.2 shows the general mode of operation of the membrane type with a slit. These types of valves can also act as check valves.

Surface micromachined check valves in parylene C have been demonstrated. The valves are able to regulate the pressure and flow rate of air and liquid without external power consumption.[68] Parylene is used as a structural material for check valves because it is more flexible than other traditional materials such as thin membranes of metal or silicon.

Passive valves can also be ball check valves[69] or moveable structures created *in situ* within glass microfluidic channels using laser polymerization.[70] The latter design has the advantage of making moving parts without the limitations and difficulties of sacrificial layers or mechanical assembly. Another passive valve system uses surface tension properties and changes in flow resistance due to changes in channel dimensions to control the flow of fluids through different sections of a fluidic device: for example, Ahn's group has successfully demonstrated this technique in plastic chips with multiplexing capabilities.[30,31]

1.3.3 Fluid Mixers

Mixing of samples and fluids in microfluidics is important especially in biological and chemical applications where different reagents have to be combined. On the macroscale, mixing of fluids is easily accomplished via turbulence in flow. On the

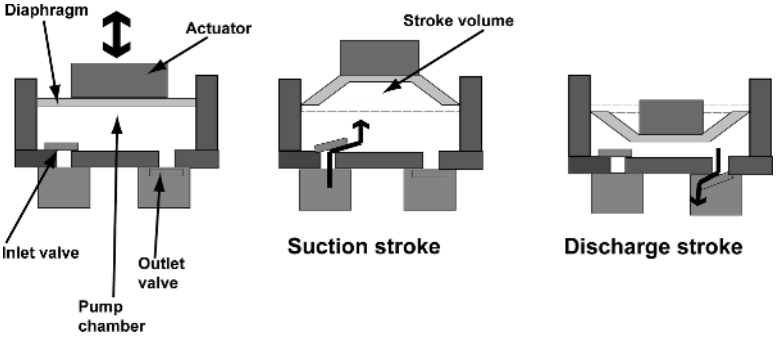

FIGURE 1.2 Schematic showing the mode of operation of mechanical displacement pumps.

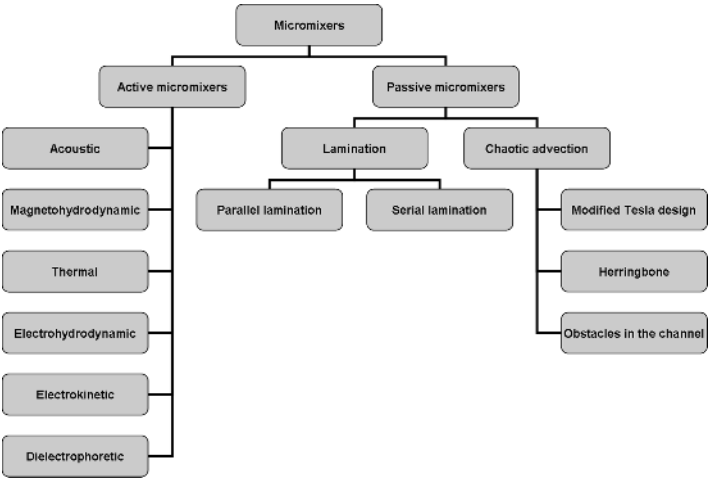

FIGURE 1.3 Classification of micromixers.

microscale, mixing is more difficult because flows are laminar. Microfluidic mixers are designed to reduce the time for mixing to take place. Similar to the microvalves discussed in Section 1.3.1, micromixers can also be classified as either passive with no moving parts or active with moving parts. A broad classification of microfluidic mixers is shown in Figure 1.3. Passive mixers are generally cheaper and easier to fabricate than active mixers.

1.3.3.1 Active Mixers

Active mixers use disturbances to promote mixing in laminar flows. Several techniques have been used to introduce the disturbance. In electrohydrodynamic-aided mixing, electrodes are placed along the fluidic channels perpendicular to the flow direction. Changing the voltage and the frequency of an alternating electrical signal applied to the electrodes introduces disturbances in the fluid that promotes mixing.[71,72] The magnetohydrodynamic (MHD)-based mixer uses a varying magnetic field that generates Lorentz forces in the fluid when a DC voltage is applied to electrodes that span the mixing channel.[73] Another method employed to introduce disturbances is the dielectrophoretic effect that uses movement of particles between electrodes due to induced dipole moments on the particles.[74]

Acoustic mixing uses focused acoustic energy to induce disturbances in the fluidic channel. One design uses surface acoustic waves (SAW) to achieve mixing. The microfluidic device is acoustically coupled to a piezoelectric substrate and the SAW is excited on this substrate and propagated into the microfluidic device.[75] Another design uses bubbles induced by acoustics for mixing.[76]

Quake's group has developed a mixer based on a circulating pump. This design consists of a circular loop in which a microfabricated pneumatic two-layer pump[33] is used to drive fluids. The mixer achieves complete mixing within a minute.[32] This

circulating mixer has been used for a number of applications including mixing of reagents for synthesis of radioimaging probes.[34]

1.3.3.2 Passive Mixers

Passive mixers, which are generally simple to fabricate, do not require active disturbance to improve mixing. They are designed to decrease the diffusion path, increase the interfacial area between the mixing fluids, or both to induce fluid mixing. Two common designs are the T- and Y-junction type of mixers[77–80] that mix parallel laminar flow streams. Another design uses splitting and joining of streams in an intersecting manner.[81] Other serial lamination designs enhance mixing through splitting and later joining of streams. The inlet streams are first split horizontally, and then split again vertically in the next stage before being joined again in the final stage.[82] Such designs are rather complex to implement and are normally fabricated in silicon because of the requirement for 3D fabrication.

Chaotic advection can be used to split, fold, or stretch fluid elements to induce mixing of laminar flow streams.[83,84] These mixers can be fabricated as part of the channel design (Figure 1.4a).[85,86]

Another technique that has been used in the past to improve mixing by chaotic advection is the introduction of obstructions in the form of ribs or grooves in the channel walls.[87,88] These structures twist the flows into helical streams to improve mixing. Whitesides' group has developed a similar design referred to as a staggered

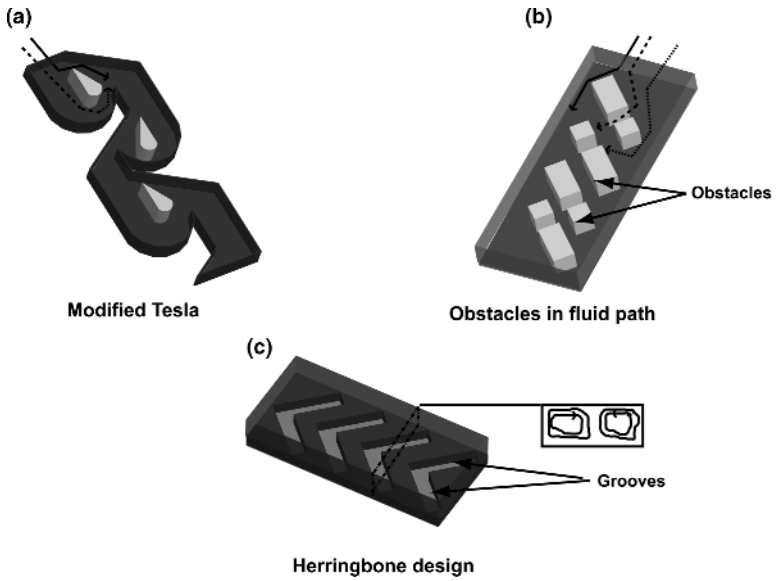

FIGURE 1.4 Schematics showing different kinds of passive mixers: (a) modified Tesla mixer (adapted from Ref [86]), (b) obstructions in channels (adapted from Ref. [94]), and (c) herringbone mixer (adapted from Ref. [89]).

herringbone mixer (Figure 1.4c).[89,90] The herringbone mixer has been thoroughly investigated by other groups and provides adequate mixing within reasonable times with very little footprints in terms of size.[42,91,92] Other improvements have also been made to this design with the inclusion of embedded barriers parallel to the flow direction generating hyperbolic instead of elliptical or helical streams.[93] A drawback for such designs is the need for complex 3D fabrication involving multiple steps.

To overcome the problem of complex 3D fabrication, other groups have introduced designs that involve placement of obstacles in the fluid path (Figure 1.4b). The fabrication process is simplified compared to the herringbone mixer. By optimizing the shape and position of the obstacles, they are able to achieve good mixing efficiencies.[85,94] One group has also demonstrated mixing in fabricated planar 2D microchannels by simply introducing curvature and changes in width of the microchannels.[95]

1.3.4 Digital Microfluidics

In the previous sections, we mainly focused on continuous-flow systems. An alternative approach, digital microfluidics, is based on the manipulation of discrete fluid droplets under electrical control (without the use of pumps, valves, or fixed channels). Digital microfluidic systems[96] can be designed to perform many microfluidic procedures, including precision dispensing, analyte detection, fluid transport, fluid mixing, and filtering. An advantage is that the system can easily be reconfigured during an experiment.

1.3.4.1 Working Principles and Architecture of Digital Microfluidics

In digital microfluidics, droplets can be manipulated through various mechanisms, including electrowetting,[97,98] thermocapillary transport,[99] dielectrophoresis,[100] optical methods,[101] and surface acoustic wave transport.[102] Here, we mainly focus on electrowetting-based digital microfluidics. The term electrowetting was first introduced for optical applications.[103] From a mechanical point of view, the phenomenon of electrowetting can be understood by considering the interfacial forces at the air–liquid–solid interfaces (Figure 1.5). Application of a voltage between liquid and electrode lowers the interfacial tension between the liquid and the insulator material surface, and as a result, the liquid tends to spread on the insulator surface, thus lowering the contact angle. Although both alternating current (AC) and direct current (DC) can be applied between the liquid and the electrode for liquid actuation, AC is preferred over DC due to increased reliability in avoiding insulator charging and breakdown.

In practice, liquid droplets are often sandwiched between two parallel plates. The top and bottom plates are separated by a spacer, and silicone oil is used between the plates to prevent droplet evaporation.[92] Both plate surfaces are coated with a hydrophobic layer to enable smooth actuation of aqueous droplets. Recent innovations in digital microfluidic device infrastructure include coplanar designs (where both electrodes are located at the bottom surface) and the use of substrates that are mechanically flexible facilitating integration of different physicochemical environments on a common platform.[104,105]

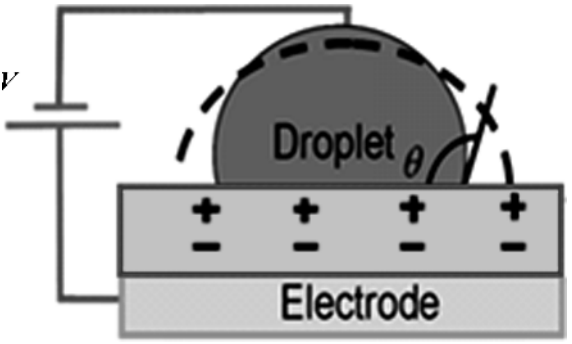

FIGURE 1.5 Fabrication of microchannels with silicon bulk micromachining and silicon surface micromachining. (a) Examples of bulk micromachined channels fabricated using wet etching. (b) DRIE used to micromachine a buried channel. (c) Fabrication process for making surface micromachined polysilicon channel.

1.3.4.2 Characteristics of Digital Microfluidics

Digital microfluidics offers a number of advantages over continuous-flow-based microfluidics. Unlike continuous-flow-based microfluidics, digital microfluidics works under programmable electronic control, eliminating the need for mechanical tubes, pumps, and valves. Although an external potential is required for droplet control and manipulation, the electrical consumption is small as there is no current flow in the chip. The volume of the liquid droplet is small, which not only reduces reagent consumption and costs, but also makes it possible to mimic chemical reactions and molecular processes at the nanoliter scale. Also, multiple droplets can be simultaneously controlled to perform multiplexed analysis. Digital microfluidic chips are usually fabricated on transparent substrates, which are compatible with conventional optical detection instruments. Digital microfluidic systems have been demonstrated with physiological fluids, including whole blood, serum, plasma, urine, and saliva, for biomedical and clinical applications.[106]

1.3.4.3 World-to-Chip Interface and Biofouling Problems

Loading samples and reagents onto a microfluidic device requires an interface between the device and the loading system, sometimes referred to as the "world-to-chip interface." Significant progress has been made to address this challenge. In digital microfluidics, direct dispensing of nanoliter and picoliter droplets from pipettes becomes impractical. An alternative for parallel sample loading is using an electrowetting-based translator device.[98,106] The liquid is loaded into a narrow channel via a hole in the top plate. By applying pressure through a pipette or actuation using a buried electrode, the liquid in the narrow channel is transferred to a microscale reservoir. This delicate droplet-dispending control allows nanoliter scale droplets to be transferred to the chip.

Another important problem for digital microfluidics is that of biofouling, especially when it comes to biological applications. In digital microfluidics, the plates that

droplets contact are hydrophobic. Most proteins in the physiological liquid tend to adsorb irreversibly to those hydrophobic surfaces and contaminate them. To prevent the contact of droplet with the hydrophobic surface, fluids with low surface tension such as silicone oil is widely used in digital microfluidics although its stability appears to decrease with lowering of the interfacial tension between the liquid and the oil.

1.3.4.4 The Application of Digital Microfluidics

There is currently great enthusiasm for applying digital microfluidics to a wide range of applications including protein sample preparation, polymerase chain reaction (PCR), cell-based assays, and enzyme assays. Examples include an ultralow volume system for DNA ligation,[107] and a cell-based assay that outperformed an identical assay in a well plate while exhibiting 30-fold lower reagent consumption and a 20-fold increase in sensitivity.[104] Point-of-care testing can also be achieved in a magnetic bead-based immunoassay on a digital microfluidic cartridge in less than 8 min using whole blood sample.[108] While digital microfluidics is promising in that it represents an alternative for miniaturization of chemistry, biology, and bioengineering applications, improvements have to be made to fully exploit the advantages of digital microfluidics for biomedical and clinical applications.

1.4 FABRICATION TECHNIQUES

The most common materials used for fabrication of microfluidic devices are silicon, glass, elastomeric polymers, and hard plastics. Lithography is required for fabricating microfluidic systems for most of these materials. We also describe the microfabrication techniques for different materials.

1.4.1 Lithography

Lithography and related processes form the backbone of the microelectronics and MEMS industries. It is an important technique for the fabrication of micro- and nanostructures.[109] Lithography techniques can be divided based on the kind of energy beam used for illumination: electron beam (e-beam) lithography,[110–113] X-ray lithography for the LIGA technique (German acronym of "Lithographie Galvanoformung Abformung"),[114,115] ion beam lithography,[116,117] normal I-line photolithography,[118] and deep UV lithography[119] are some of the major subcategories. Of these subcategories, photolithography and X-ray lithography are still predominantly used for fabrication of microfluidic devices. However, there is now a trend where some of the other techniques such as e-beam and deep UV are being used especially in cases when submicron fluidic structures are desired.

In the common mode of projection-based photolithography, a photoresist and a transparent mask are required. Photolithography involves three main steps: a positioning step between the photolithography mask and the substrate that normally has a photoresist material coated on it, an exposure step of the photoresist, and finally a

development process that involves etching or dissolution of the photoresist pattern. Depending on the substrate material used for the final microfluidic device, further processing steps may be required.

1.4.2 Silicon-Based Methods

Silicon-based materials (such as Si or SiO_2) are still the primary material used in MEMS and microfluidics. The fabrication techniques used are well established and involve either bulk removal of silicon material (bulk micromachining) or deposition and patterning of polysilicon (surface micromachining).

1.4.2.1 Bulk Micromachining

This process is a subtractive technique that selectively removes silicon material to leave the desired structure. Normally, a patterned photoresist serves as a barrier against removal of other portions of the silicon. The photoresist is stripped off the surface of the silicon after bulk removal to yield the final structure. Two methods are used for this process. Wet etching uses chemicals to remove silicon material based on its crystalline structure.[120,121] In this case, the etch process is normally anisotropic, and depending on the silicon crystal orientation, different channel geometries can be obtained (Figure 1.6a). The other is known as dry reactive ion etching (Figure 1.6b) and it does not depend on the crystal orientation of silicon for removal of material. Dry etching can be done at cryogenic temperatures,[122] or it can use a process of alternate etching and chemical vapor deposition at standard temperatures (the Bosch process).[123,124] There are many examples of microfluidic devices built using bulk micromachining.[125,126]

1.4.2.2 Surface Micromachining

Surface micromachining was originally developed for making thin-film microstructures. Silicon surface micromachining is more of an additive than subtractive process.[127] It generally involves deposition of polysilicon, single crystal silicon, or other materials such as phosphorus silicate glass (PSG) used as a sacrificial layer. The sacrificial layer is later etched to release the final silicon structures. The basic process can be divided into four main steps: substrate passivation, sacrificial layer deposition and patterning, structural polysilicon deposition doping and stress annealing, and final microstructure releasing through etching of sacrificial layer.[128]Figure 1.6c shows a schematic of a typical process using surface micromachining techniques for fabrication of microfluidic devices.

1.4.3 Glass-Based Methods

Glass is used as a material for microfluidics because of optical transparency, good fluorescence properties, stability at high temperatures, chemical resistivity, controllable surface chemistry and high electrical resistivity (making it possible to carry out electrophoresis), and good biological compatibility necessary for medical and biological applications. Glass-based methods for fabrication of microfluidic channels are

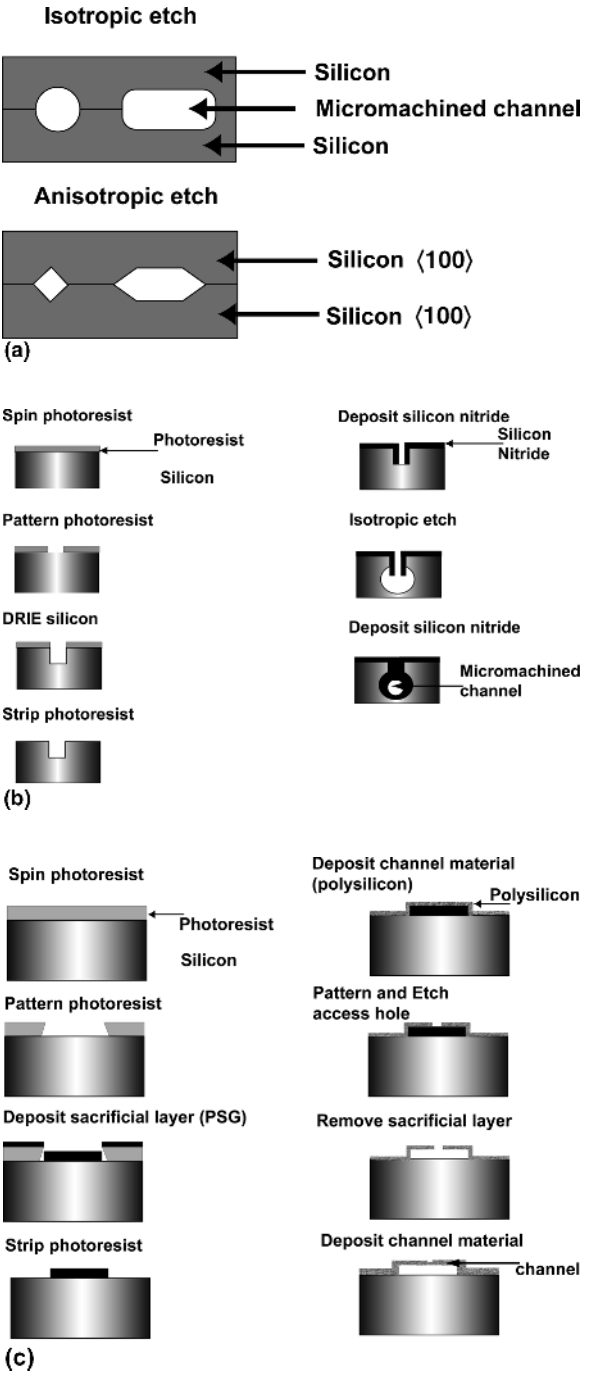

FIGURE 1.6 Schematic diagram that illustrates the phenomenon of interfacial forces at an air–liquid–solid interface.

similar to the wet chemical bulk micromachining techniques used for silicon. The common mask material used for micromachining microfluidic channels in glass is an evaporated metal film such as chromium, gold, or other materials like polysilicon or nitride that is patterned with photoresist and then etched.[2,129] Once the mask layer is patterned, the glass is then etched using a buffered oxide etch (BOE, made of ammonium fluoride and hydrofluoric acid) that etches the glass in an isotropic process. Access holes are drilled through another piece of glass that is used as a cover for the patterned glass. The two pieces are aligned and then bonded together to obtain the final microfluidic chip. A more recent approach reported in the literature uses hardened photoresist for the etch mask, thus simplifying the fabrication process and eliminating the expensive and time-consuming evaporative metal step.[130] The photoresist used as an etch mask is applied to the glass substrate and then patterned using standard lithography. The patterned layer is then hard baked to make it more resistant to the harsh chemicals used in the BOE process for the glass. The photoresist is then stripped using a diluted KOH solution. Figure 1.7a shows a schematic of the method that uses evaporated metal as an etch mask. The process flow for the alternate cheaper and faster method is shown in Figure 1.7b.[130] A downside for using glass as a structural material for microfluidic devices is that the etch process does not produce smooth surfaces.

1.4.4 Polymer-Based Methods

Polymers are cheaper than glass or silicon and some of the techniques (injection molding and hot embossing) can be easily adapted for mass production. This section discusses the use of polymers as structural material, templates, and molds for making microfluidic devices. The use of injection molding and hot embossing techniques for making structures in hard plastics is discussed in the next section. The techniques used in polymer-based methods have steps that are common to the silicon and glass methods. Also, some of the polymer-based methods depend on other polymer-based methods. A typical example is the dependence of elastomeric micromolding techniques on thick photoresist lithography.

1.4.4.1 Soft Lithography-Based Techniques

Another technique that has gained widespread use was introduced by Whitesides.[131–133] This process is commonly referred to as soft lithography because of the use of PDMS, an elastomeric polymer. This process also uses thick polymer photoresists (such as SU-8) that form master molds for the microfluidic networks. Liquid prepolymer of PDMS is poured on the master mold and cured in an oven. This procedure produces a complementary relief pattern of the thick polymer photoresist in the PDMS. This PDMS is then peeled off the master mold and bonded to a flat piece of glass to form the final microfluidic device (Figure 1.8). Depending on the relief structures formed on the surface, it can also be used for further processing to make the final microfluidic devices. These further techniques include microtransfer molding, micromolding in capillaries, and solvent-assisted micromolding.[133] In microtransfer molding, prepolymer of PDMS is poured on the relief structures

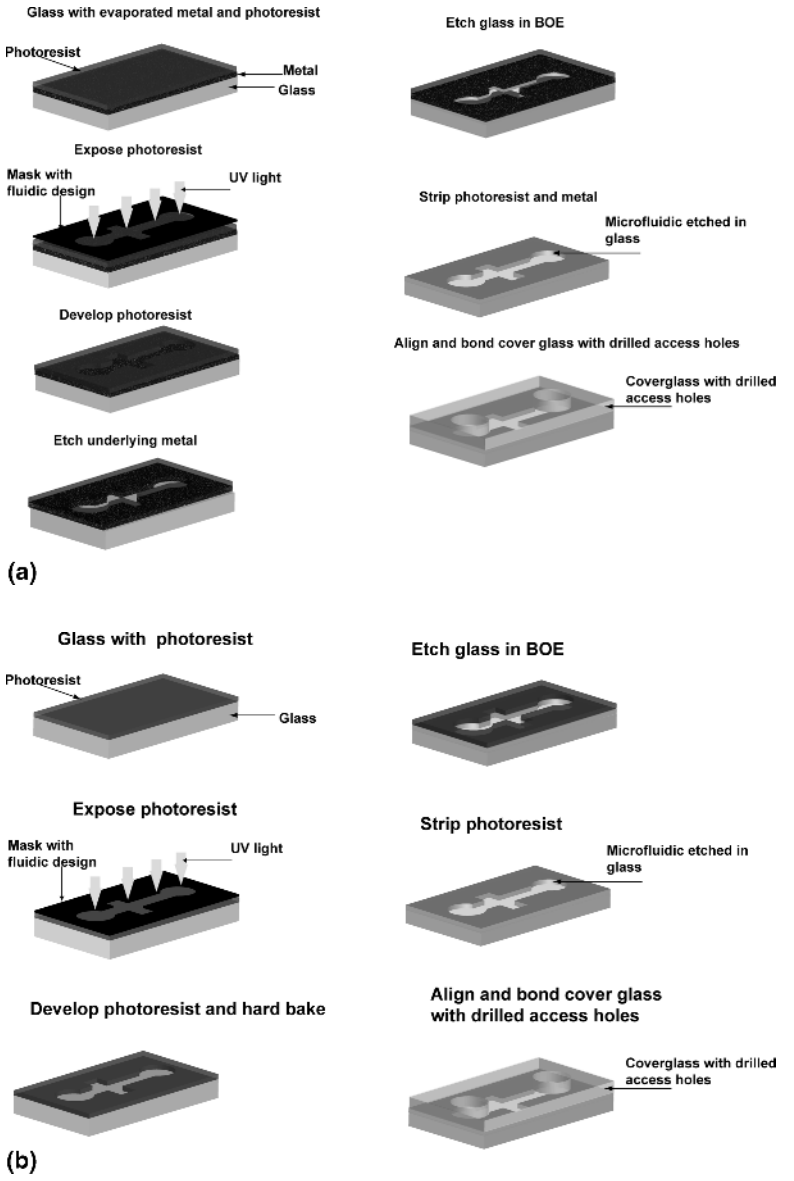

FIGURE 1.7 Fabrication of microfluidic structures in glass. (a) Common technique that uses a patterned metal layer as an etch mask. (b) A modified technique that uses a hard baked photoresist. (Adapted from Ref. [130]).

obtained from the first molding step using the thick polymer photoresist (PDMS master mold). Excess prepolymer is removed from the surface of the PDMS master mold. The base substrate for the final microfluidic device (normally a flat glass piece) is then placed on the PDMS master mold with the trapped prepolymer

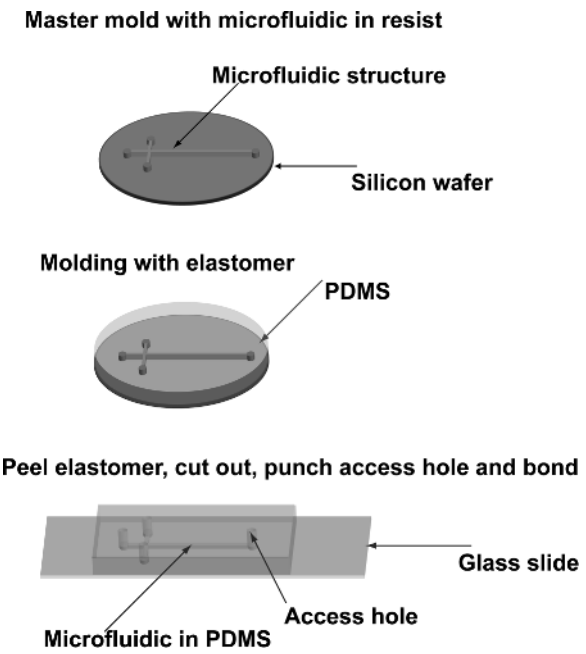

FIGURE 1.8 Fabrication of microfluidic networks in PDMS elastomer based on the soft lithographic process. In some cases, the peeled PDMS template obtained is used as a master mold for further processing steps to obtain the final microfluidic.

between the relief structures. The whole device is then placed in an oven to cure the prepolymer. The final microfluidic device is obtained by peeling off the PDMS master mold from the second set of poured prepolymer. Normally, to facilitate mold release, the surface of the PDMS master mold is treated with fluorinated or chlorinated silanes such as (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane.[118,134]

In the case of micromolding in capillaries, the PDMS master mold is placed on another flat surface such as glass and then pressed down. This process causes the walls of the relief structure to form capillary channels on the flat surface. Prepolymer of the desired material in liquid form is placed at the ends of the channels and these wicks into the channels by capillary forces. After curing the prepolymer, the master mold is peeled off leaving a complementary relief pattern of the PDMS master. The mold can then be sealed with another flat substrate to obtain the microfluidic device. This technique can also be used to pattern glass or silicon with different materials.

1.4.4.2 Thick Polymer Photoresist Techniques

Polymethylmethacrylate (PMMA) commercially available under different trade names such as Perspex, Acrylic, and Plexiglas is a thick photoresist material that is primarily used in the LIGA technique. Application to the substrate is achieved by spin coating or bonding of preformed sheets.[135] Formation of microfluidic structures

directly in PMMA using lithography requires the use of collimated X-rays, available at synchrotron facilities. In addition, the mask material (such as beryllium and titanium) must absorb X-rays. The X-rays alter the properties of the exposed regions of the PMMA polymer and make them susceptible to etching by the developer. The LIGA process is able to generate very high aspect ratio structures unlike the other thick photoresists. However, due to a limited number of synchrotron facilities coupled with the added expense of the mask materials, PMMA and the associated LIGA technique are not normally used to make actual final fluidic structures.

A more common thick photoresist that is used is SU-8. It is much cheaper than PMMA and requires only a UV-I line source for exposure. Making the masks for microfluidic structures in SU-8 involves printing of CAD-generated designs on transparencies using commercial printing services. Turnaround time is as short as overnight. However, there are limitations to the resolutions and the feature sizes that can be obtained.[136] SU-8 can be used to make open microfluidic channels that are sealed with other materials or can be used to fabricate fully functional microfluidic devices (Figure 1.9).[137–139] For the process shown in Figure 1.8, the first layer of SU-8 is flood exposed and baked on top of a silicon or glass wafer. A second layer of SU-8 is patterned, exposed, and developed. A third layer of SU-8 is applied to a glass wafer (bonding layer) and the two pieces are aligned and pressed together after a partial soft bake of the SU-8. The bonding layer is then exposed through the glass and the bonding

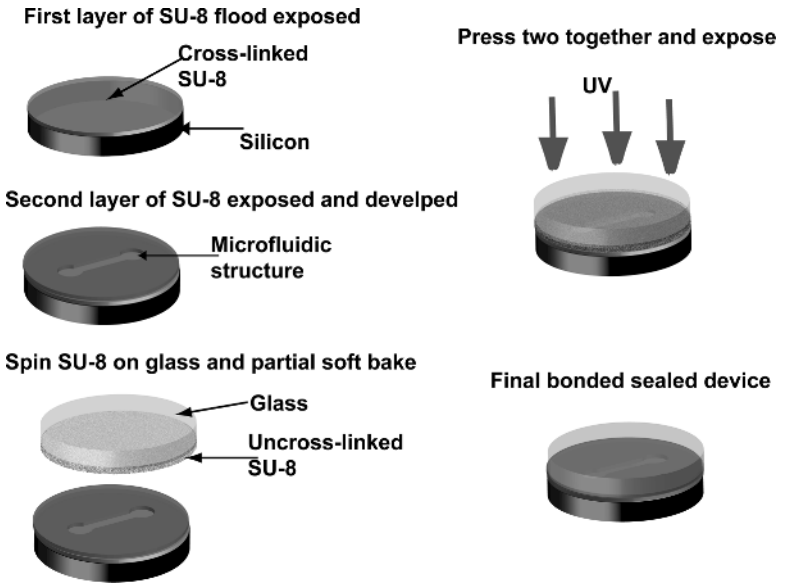

FIGURE 1.9 Process flow for fabrication of closed microchannels in SU-8. First layer of SU-8 flood exposed and baked on top of silicon wafer. Second layer of SU-8 is exposed and developed. Bonding layer of SU-8 is coated on glass slide and partially soft baked. Two layers are then pressed together and bonding layer is exposed through the glass slide that cross-links SU-8 and completes seal. (Adapted from Ref. [139]).

process is completed during postexposure bake when the two layers of SU-8 cross-link fully. Other common thick photoresists that are used include the AZ line of photoresists (AZ4562, AZ9260).

1.4.5 Plastics (Injection Molded and Hot Embossing Techniques)

The mechanical, electrical, and thermal properties of thermoplastics make them attractive candidates for use in microfluidic devices. Some of the common polymers used for thermoplastic micromolding include cyclic olefin copolymer (COC), PMMA, polystyrene (PS), and polycarbonate (PC).

Several techniques can be used to create microfluidic systems from hard plastics, including injection molding, reaction injection molding, hot embossing, injection compression molding, and thermoforming.[140,141] Of these techniques, hot embossing and injection molding are the most common methods used for fabrication of microfluidic devices. In injection molding, the mold insert determines the form of the final molded plastic. The insert is placed in a mold cavity that is evacuated and heated above the glass transition temperature of the polymer. An injection unit heats the polymer up above its glass transition temperature and injects the molten viscous polymer into the cavity. The polymer and mold are then allowed to cool below the glass transition temperature of the polymer and then the final molded polymer is demolded from the tool. Figure 1.10 shows a schematic of the injection molded process. Injection molding can be easily adapted for mass production once the mold is in place although the cost for setting up the system is high.

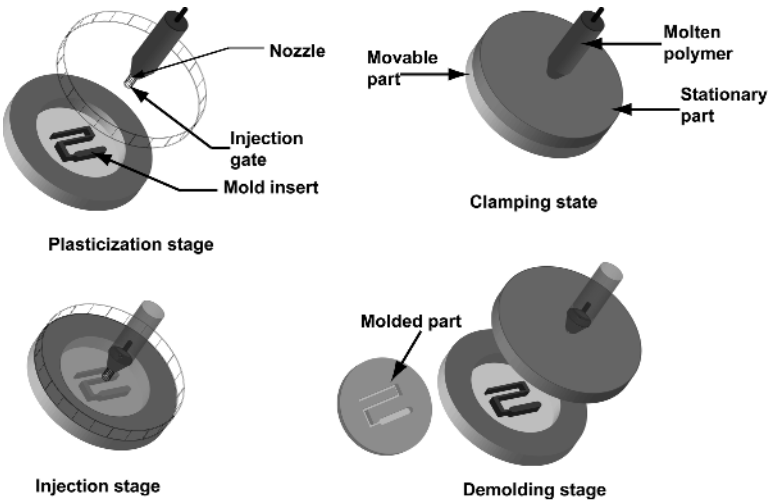

FIGURE 1.10 Schematic drawing of the injection molding process. The stationary part is shown as a wire frame in some cases for clarity. Image (adapted from Ref. [140]).

In hot embossing, a thermoplastic material is inserted between two plates in an evacuated chamber. One of the plates containing the mold insert with the required pattern is pressed into a polymer film that is heated above its softening temperature. This pressing causes the mold to be filled with the plastic material, thereby replicating the structures in the mold. The setup is allowed to cool, and the plastic is removed to yield the final product. In both techniques, the most critical component is the mold insert that typically has high aspect ratios and must be made with a high degree of precision. The LIGA technique (Section 1.4.1) is a favored technique for making the inserts.

Ahn's group have reported a modified injection molding technique (rapid thermal process for injection molding) that uses a replaceable mold disk and also IR radiation from a high-power halogen lamp to effect rapid heating of the mold (made of nickel).[31] They also employed hot embossing techniques to package the final microfluidic device.[30]

1.4.6 Submicron Techniques

New fabrication techniques have remerged to make submicron fluidic channels. The most common of these are subtractive techniques that involve removal of the bulk material to yield the final device.

Focused ion beam (FIB) micromachining uses a highly focused ion beam to scan the surface of a substrate and cuts or etches channels and holes by removing bulk material.[142] The ion source is ejected from a liquid metal ion source and focused and accelerated through a column. The spot size is determined by the acceleration voltage and the type of liquid metal, with typical resolutions of less than 10 nm. The scanning of an ion beam across the substrate is similar to scanning electron microscopy, and the removal of material occurs in a manner similar to removal of material from the metal target during the sputtering process.[143–145] Focused ion beam micromachining and focused electron beam micromachining should not be confused with e-beam lithography (Section 1.4.1). In the case of focused ion beam and focused electron beam micromachining, the use of a photoresist and the additional process steps of development and stripping are not required. The focused ion beam direct writing method has been used to fabricate nanochannels that have a width of about 40 nm, depth of 60 nm, and length of 50 mm for manipulation of DNA molecules.[146]

Another technique that is similar to the focused ion beam technique but is more amenable to nonsilicon-based fluidic applications is the use of laser ablation.[147,148] In this case, a high-intensity laser beam is focused onto the material and the concentrated energy of the beam evaporates the material at the focal point.[149] In an optional step, a mask can be used to define the final shape of the features. The final feature size is determined by the type of laser and whether it is used in a continuous or pulsed mode. Three-dimensional nanochannels in glass with diameters less than 700 nm have been fabricated using the precision of optics at critical intensity (OCI).[150] Some of the disadvantages of laser micromaching include roughness of the final features formed (especially in the case of the pulsed mode since several pulses are needed to achieve appreciable depths). Also, there is a need to find suitable methods to remove ablated materials.

1.5 APPLICATIONS

1.5.1 DNA Amplification

Miniaturization of the PCR has many advantages, such as decreased cost of fabrication and use, decreased time of DNA amplification, reduced consumption of reagents and samples for PCR, increased portability and integration of the PCR device, safe disposal of the PCR reaction vessel, and reduced cross-talk of the PCR reaction. In addition, large numbers of parallel amplification analyses on a single PCR microfluidic chip can be performed.

The first silicon-based stationary PCR chip was described several years after the introduction of PCR. Since then, many research groups began to develop chip-based PCR devices. Most of these devices are based on silicon and glass but more recently, many polymer materials are being used such as PDMS,[151,152] PMMA,[153] polycarbonate, SU-8, polyimide, poly(cyclic olefin), and epoxy. The three main design concepts for PCR microfluidics are chamber stationary PCR, flow-through PCR, and thermal convection-driven PCR.[154] A PDMS-based chamber stationary design similar to conventional PCR methods has been demonstrated.[155] In this design, the PCR fluid is kept stationary while the temperature of the reaction chamber is cycled between three different temperatures (melting, annealing, and extension phases). The biochip is based on a multichamber design with nanoliter volumes and the PCR products are detected via fluorescence.

Flow-through PCR microfluidics allow more flexibility in changing reaction rates and times due to a time–space conversion concept. This has been demonstrated in a work on continuous-flow microreactors having an annular microchannel for cyclical chemical reactions.[156] In this work, the devices were fabricated from silicon or SU-8 that allows MHD actuation. MHD actuation was used for fluid propulsion to push the PCR solutions through temperature zones to enable thermal cycling during each revolution. Therefore, the duration of each cycle could be controlled by the fluid velocity. This design allows rapid heat transfer and thermal cycling and the run time for such assays is in the order of minutes. Compared to chamber designs, flow-through designs also decrease the possibility of cross-contamination between samples.

Thermal convection-driven PCR microfluidics involves driving the reaction solutions through two temperature zones. This concept is similar to the flow-through design except that the driving force of the sample is buoyancy. In this manner, a temperature gradient is generated across a reaction vessel that drives thermal convection to circulate the solution between the hot and cold regions for PCR.[157]

As PCR is a temperature-controlled, enzyme-catalyzed biochemical reaction system, the method in which the different temperature zones are generated and maintained is crucial to the design of PCR microfluidics. Various heating methods have been employed and they can be broadly categorized into contact and noncontact heating methods. Contact heating has been implemented in PCR microfluidics using integrated thin-film platinum resistors as both the heating and sensing elements on these chips.[158] Examples of noncontact methods include hot air cycling,[159] IR light radiation,[160] and laser-mediated heating.[161]

As an alternative to PCR, isothermal amplification methods are also being miniaturized.[162]

1.5.2 Immunoassays

Immunoassays are biochemical assays used to measure concentration of analytes (typically proteins) in solutions using antibodies. Clinical immunoassays are traditionally based on 96-well microtiter plates. However, microfluidic devices are attractive for running high-throughput immunoassays, as it is possible to minimize sample volumes, reagent costs, and processing time.[163] The use of microfluidics allows the automation of fluid delivery and the analysis of many samples in parallel. Microfluidics also allows the development of low-cost, compact diagnostic devices for resource-poor settings based on immunoassays.[164] Although detection and quantification of the analytes can either be done off or on the microfluidic chip, current trends point toward fully integrated point-of-care microfluidic systems.

Typical methods for detection include optical methods such as fluorescence. However, the small cross-sectional path length in microchannels limits the sensitivity of such assays. A method using silver reduction has been reported to increase the sensitivity of such tests.[165] Different methods of detection, as opposed to traditional immunoassays, have been developed. For example, a biosensor using diffusion between two laminar streams (a solution of antibodies and another of antigens) has been developed.[166] The concentration of the bound antibody/antigen can then be rapidly quantified in the stream using an inverted microscope and fluorescence source. The use of DNA-directed self-assembly on the surface of microfluidics chips to immobilize the capture probes allowing a multiplexed immunological detection assay has also been demonstrated.[163]

1.5.3 Cell-Based Assays

Cell-based assays are used to assess the effects of chemical stimuli on biological cells to obtain data that may be reflective of higher level biological responses. The most common use for this type of assay is in the pharmaceutical industry, where cell-based assays provide information necessary in the drug discovery process. Another application of cell-based assays is in basic biological research such as ion channel research and chemotaxis studies.

Conventional cell-based assays have been miniaturized, with multiwell plates in common use. The use of microfluidics in cell-based assays not only allows a decrease in cost but also an increase in throughput as well as data quality.[165] Microfluidics also enables better control of the 3D culture environment of cells and allows the creation of multiplexed nanoliter arrays for improved biological analysis[167] Other advantages include the similarity of the dimensions of the cell and the microchannels, laminar flow in the channels, generation of highly resolved chemical gradients[168] subcellular delivery of stimuli, reduced dilution of analytes, and the favorable scaling of electrical and magnetic fields.[169]

A number of microfluidic devices have been developed for cell-based high-throughput screening.[170] In studies of ion channels, a microfludics approach to conventional patch clamp experiments[171] increases the throughput of such experiments. Cells are patch clamped in a microfluidic channel and delivered from sample reservoirs through microfluidic channels to an array of small channels. Subsequently, the cells are trapped and brought to whole-cell configuration by the application of a vacuum. Drugs are then delivered via the same microchannels that contain the cells.

The generation of chemical gradients in microfluidic chips allows precise spatial and temporal control of the chemical environment around cells in chemotaxis studies.[172,173] Gradient-generating devices can be easily incorporated into microfluidic chips, and the behavior of cells in response to the chemoattractants introduced into the chip can be studied.

Cell-based assays in a microfluidic format can also be used to study diseases by isolating cells infected with viruses and parasites of interest such as *Plasmodium falciparum*.[174] Also, culturing cells in a microfluidics system offers the ability to create cell–cell, cell–substrate, and cell–medium interactions to a high degree of precision. For example, embryos cultured in microfluidic devices develop at rates closer to the *in vivo* environment compared to traditional culturing methods.[175] Microfluidics can also be implemented in other cellular analysis methods such as cytometry and cellular biosensors.

1.5.4 Drug Delivery

There are many factors that limit the capabilities of conventional drug delivery technology such as long-term treatment, complex dosing schedules, combinational therapy, and unstable active ingredients.[176] Microfluidic technologies combined with biological science provides a powerful platform that can be used to precisely control drug release, deliver multiple doses, eliminate frequent injection, and protect labile active ingredients.[176] Microfluidic devices also present unique advantages for sample handling, reagent mixing, separation, and detection, as well as provide a platform to move particles, mix fluids, and control reaction rates.[177]

Microfluidic devices with micropumps and microvalves have been fabricated for drug delivery solutions.[178,179]

Microfluidics-based drug delivery devices can also improve drug therapeutics and efficiency by providing an accurate dose, employing complex release mechanisms, enhancing local delivery of drugs, and increasing drug stability.[180]

1.5.5 Diagnostics for Point of Care

Point of care (POC) diagnostics are improved by microfluidic technologies that offer many potential advantages including reduced reagent volume, low cost, short analysis times, and small size.[6,181–183] Improved and appropriate point-of-care diagnostics have potential roles in health care in both developed and developing world, such as providing suitable and prompt treatments, ensuring safe blood

banking, and improving clinical outcomes.[6] With a lack of portable and accurate diagnostic tests in developing countries, patients are treated based on clinical symptoms and predominance of disease, which may lead to incorrect diagnosis and treatment of disease.[184]

Potential uses of microfluidic systems for POC diagnostics include analysis of blood samples for HIV infection,[185,186] detection of cancerous cells in blood components,[187] and separation of blood constituents.[188]

1.6 FUTURE DIRECTIONS

In the past decade, microfluidics has generated enthusiasm in both academic and industrial communities due to the promise that laboratory processes can be miniaturized onto a chip. This miniaturization not only brings about many obvious advantages, such as small sample consumption, low cost, short turnover time, but also enables novel functionalities that cannot otherwise be accomplished in conventional fluidic systems. Although progress has been made in basic research of confined fluidic phenomena, as well as development of functional devices and systems for biomedical and clinical applications, on the whole microfluidics has yet to develop into a major new technology for the consumer. Nonetheless, new uses of microfluidic systems are appearing, including hydrogel-based microfluidics, as first described by Stroock's group,[189,190] as emerging tool for studying cells and tissues,[191] as well as for fabrication of materials, such as Doyle's group for solution particles[192] and our group for making multicomponent 3D structures.[193] As such, given the intense and sustained interest in this technology in both academia and industry, it is likely that new sets of microfluidics-based applications will emerge in areas as diverse as proteomics, genomics, organic synthesis in small channels, and point-of-care diagnostics in the years to come.

ACKNOWLEDGMENTS

We thank the Wallace H. Coulter Foundation, NSF (CAREER Award), and NIH (5R41NR010753) for financial support. Sau Y. Chin acknowledges the Agency for Science Technology and Research (A* STAR), Singapore, for financial support.

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