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Fundamental principles and applications of microfluidic systems

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1. ABSTRACT

Microelectromechanical systems (MEMS) technology has provided the platform for the miniaturization of analytical devices for biological applications. Beside the fabrication technology, the study and understanding of flow characteristics of fluid in micrometer or even nanometer scale is vital for the successful implementation of such miniaturized systems. Microfluidics is currently under the spotlight for medical diagnostics and many other bio-analysis as its physical size manifested numerous advantages over lab-based devices. In this review, elementary concepts of fluid and its flow characteristics together with various transport processes and microchannel condition are presented. They are among the fundamental building block for the success in microfluidic systems. Selected application examples include biological cell handling employing different schemes of manipulation and DNA amplification using different microreactor arrangement and fluid flow regime.

2. INTRODUCTION

The downscaling of laboratory-based analysis systems has created a whole new class of devices for molecular analysis. Microfluidics, micro total analysis systems (μTAS) or lab-on-a-chip, integrate analytical process like sampling, sample treatment, reaction, detection and data analysis onto a micrometer-scale chip. Microfluidics research has boomed over the past decade or two, and will continue to flourish in the future. The attractiveness of microfluidic-based systems can be in general considered to be its size-effect. Smaller analysis platform means low consumption of reagent and power and of course the portability. They are also able to perform separations and detections with high resolution and sensitivity at low cost and much faster time. The field of microfluidics has four parents: molecular analysis, biodefence, molecular biology and microelectronics (1). The first origin of microfluidics is the microanalytical methods in capillary format (e.g. high-pressure liquid

chromatography, capillary electrophoresis). The success in these methods prompted the development of more advance and more compact formats for applications in microscale dimensions for chemistry and biochemistry. The second motivation for the development in microfluidic systems came from the realization of threats posed by chemical and biological weaponries. The development of field-deployable microfluidic systems for detection is of essence in countering such threats. These developments have created major advancement in academic microfluidic technology. The field of molecular biology offered the third motivation force. The sudden advancement in genomics in the 1980s coupled with the availability of microanalysis technologies (e.g. DNA sequencing) related to molecular biology generated a huge requirement for analytical methods with much greater throughput and higher sensitivity and resolution. The final contribution came from the technologies offered by the field of microelectronics. Photolithography and associated technologies provided a platform solution for the fabrication of microfluidic systems. Although microfluidic devices have not developed as clones of silicon microelectronic devices, microelectronic technologies are indispensable for the development. These four aspects have provided the motivation behind the development microfluidic systems. Microfluidics exploits both its physical scale and the characteristics of fluids in microchannels (e.g. laminar flow) to offer new capabilities in the control of molecules in space and time.

The interest in scaling laws and dimensionless groups for downscaling purposes is revived (2) by the field of microfluidics. However, the scaling laws are generally not valid on the nano- or even micrometer scale (3). Fluid behavior under such scale can be totally different. Here, we describe the elementary concepts of fluid flow, transport processes and surface conditions of microchannels, which are fundamental principles for the function of microfluidic systems. Microfluidics for biological cells studies and analyses have attracted significant attention (3-16), and play important role in cell biology, neurobiology, pharmacology and tissue engineering. In this review, we survey the area of biological cell handling, which is a crucial aspect in cell-based assays in microfluidics, and also the most successful analysis method to be miniaturized into a microfluidic platform - polymerase chain reaction (PCR).

3. FLUID FLOW

A fluid can be defined as a material that deforms continually under shear stress or more specifically, with the application of an external force attempting to displace part of the fluid elements at boundary layer (i.e. surface). The fluids we encounter in everyday life are gases (air or its components) and liquids (water, oil, etc.). Other complex systems consisting of several phases can also be classified as fluids (blood, suspensions, etc.). Elementary fluid mechanics will be discussed in this and the following section (Section 4), largely based on published monographs (17-21).

3.1. Fluid characteristics

The three important parameters characterizing a liquid are its density, ρ, pressure, P, and viscosity, η. The density is defined as the mass, m, per unit volume, V.

Pressure in the liquid only depends on the depth (i.e. pressure increases when going from the surface to the bottom). It is the same at every instance having the same elevation, and is not affected by the shape of the vessel containing the liquid. In planar microsystems with channel depths of micrometer range, pressure differences because of different depths can be ignored. Nevertheless, these channels are not closed systems; they have inlets and outlets; so any pressure difference induced externally at these openings is transmitted to the liquid, thereby inducing the liquid to flow.

If we were to set the liquid into motion, there will be a resistance to the effort. This internal friction is viscosity. Using a classic example; two parallel plates are set on top of each other with a liquid film of thickness L between them. The lower plate is stationary, and the upper plate can be set into motion by a force, F, resulting in velocity, v. The movement of the upper plate first sets the immediately adjacent layer molecules into motion; this layer transmits the action to the subsequent layers underneath it due to the intermolecular forces between liquid molecules. In a steady state, the velocities of these layers range from v (the layer closest to the moving plate) to zero (the layer closest to the stationary plate). The applied force acts on an area, A, of the liquid surface (surface force), inducing a shear stress (F/A), while the displacement of liquid at the top plate, Δx, relative to the thickness of the film is the shear strain ($\Delta x/L$). The coefficient of viscosity, η, can then be defined as the ratio of the shear stress to the shear rate:

$$\eta = \frac{F/A}{v/l} \quad (3.1)$$

And, in more general terms, Eq. 3.1 can be rewritten as

$$\eta = \frac{F/A}{dv/dy} \quad (3.2)$$

From Eq. 3.2, if the shear stress of a fluid is directly proportional to the velocity gradient, the fluid is called Newtonian fluid. For example, water, oil, glycerin are Newtonian fluids. In the other fluids, however, the viscosity changes with the shear stress; these liquids are hence termed non-Newtonian. An example of non-Newtonian fluid is blood. The viscosity is very dependent on temperature in liquids in which cohesive forces play a dominant role. The viscosity of liquids usually decreases with increased temperature.

The use of non-Newtonian fluids as compared to Newtonian fluids is more promising for the development of microfluidics devices. In non-Newtonian fluids, the viscosity either grows (shear thickening) or decreases

(shear thinning) with increasing shear rate. There can be turbulent-like instabilities in flows of such fluids at low Reynolds number. These "elastic turbulence" could be generated in microfluidic channels to act as efficient mixers (22).

3.2. Flow characteristics

The fluid flow conditions in microsystems can be determined based on the relation between the magnitudes of the inertial and viscous forces. Expression given in Eq. 3.3 is the dimensionless Reynolds number, *Re*:

$$\mathrm{Re} = \frac{\rho d v}{\eta} \quad (3.3)$$

Where *d* is the typical length scale (e.g. diameter or channel depth), and *v* is the average velocity of the moving liquid. From empirical studies, Reynolds numbers larger than about 2300 correspond to turbulent flow. Under this regime, inertial forces are dominant. The region in which Reynolds number is below about 2000 is referred to as the laminar (or creeping) flow regime. From Eq. 3.3, it is obvious that low Reynolds numbers are attained at lower velocities, smaller dimensions, smaller densities, or higher viscosities. Therefore, in microchannels, laminar flow regime is dominant due to the small dimensions, where the velocities of flow would have to exceed the speed of sound before the onset of turbulence.

Very often, microchannels do not have a cylindrical cross-section. Therefore, in order to determine the Reynolds number in channels with other symmetry, hydraulic diameter, *Dh*, can be used, which can be defined as

$$D_h = \frac{4A}{P_{wet}} \quad (3.4)$$

Where A is the cross-sectional area and P_{wet} is the wetted perimeter, which is the entire perimeter that is in contact with the liquid.

The theoretical framework developed to analyze fluid flow is often referred to as Navier-Stokes formalism. It is based on the fundamental laws of conservation (mass, momentum, and energy), and combining them with consecutive equations for fluids governing viscosity and thermal conductivity to arrive at a set of equations commonly referred to as Navier-Stokes equations. The Navier-Stokes equations have more unknown parameters than equations, thereby making complete analytical solution impossible. Typically, several boundary conditions and/or equations of state are adopted to solve the Navier-Stokes equations under particular conditions. The most important and most-used boundary condition is the no-slip condition, which states that the velocities at phase boundaries (e.g. wall-liquid) must be equal. This means that for flow of a liquid inside capillary or a channel, the fluid velocity at the wall must be zero.

An important solution to the Navier-Stokes equations is the Poiseuille (or Hagen-Poiseuille) flow, which applies when a pressure gradient is used to drive a liquid through a capillary or channel. For a capillary with cylindrical cross-section the following expression for the volume flow, Q, exists:

$$Q = \frac{\Delta V}{t} = \frac{\pi R^4}{8\eta L}\Delta P \quad (3.5)$$

Where R is the radius of the capillary, L is its length and ΔP is the pressure drop across this length (also called hydraulic pressure). The term, $8\eta L/\pi R^4$, of which the reciprocal appears in Eq. 3.5, is also called the fluidic resistance. The dependency on $1/R^4$ implies that the fluidic resistance increases drastically as the channel dimensions are reduced. Consequently, higher pressure drops are necessary to move liquid through smaller conduits. For channels with noncylindrical cross sections, expressions similar to those in Eq.3.5 can be found, but with different terms for the fluidic resistance.

4. TRANSPORT MECHANISMS

Generally, there are two different types of transport in microfluidic systems – directed transport and statistical transport. The difference lies mainly in the nature of the driving agent behind the transport. Directed transport is controlled by exerting work on the fluid. The work results in a volume flow of the fluid, where the flow can usually be characterized by a direction and flow profile. The work is often generated mechanically by a pump or electrically by a voltage. Flow that is driven mechanically is called pressure-driven flow, and flow driven by a voltage is called electro-osmotic flow.

Statistical transport, on the other hand, is an entropy-driven transport, meaning transport only occurs if, after transport, the fluid is more disordered than before. A typical situation is the statistical transport of molecules from the side with high concentration to the side with zero concentration (i.e. by the presence of a concentration gradient). The concentration will in time be equal in both liquids; the liquids are hence evenly mixed, and the situation is less ordered than before. This statistical transport is called diffusion.

Normally, a mixture of these types of transport is more realistic in actual flow of a fluid. Mixed transports occur when a directed transport meets a gradient of some kind: a temperature gradient or a concentration gradient. The molecules in the liquid follow the direction of the exerted outer flow, while at the same time the gradient is being equalized. A good example is forced-heat convection, in which there is a directed flow of molecules alongside a surface, with a heat-driven diffusion of molecules away from the surface. The physics behind each mechanism are similar to macro systems, except that effect of physical size is taken into consideration.

4.1. Convection

Convection in physics means heat transfer by mass transport. In everyday life, convection is experienced where warm layers of fluids move into colder regions due to the density difference that is caused by a temperature difference. This phenomenon is also called free convection or natural convection. Forced convection can also be exerted by external forces to create a directed flow of the fluid.

There are several ways in which forced convection can generate directed flow in a microsystem. For example, capillary flow where a working liquid is filling up the microsystem. In a similar fashion, other forces, such as gravity, a pressurized air bladder, or the centripetal forces in a spinning disk, create a single instant pressure difference in the microsystem. There are also facilitators like mechanical and electro-osmotic pumps. An ingenious way of convection-based transport with no external forces is discussed in a latter section.

4.2. Migration

Migration is the directed transport of molecules in response to a certain driving force. For example, the moving molecules are ionized in a polar solvent. Put in an electric field, these electrically charged molecules experience a coulombic force due to the electric field. The charged molecules accelerate towards one of the electrodes, but will slow down and reach a terminal velocity, as there is a drag caused by friction with the liquid. The coulomb force, F, is given by

$$F = qE \quad (4.1)$$

Where q is the charge on the molecule and E the strength of the electric field. Once the molecules reach their terminal velocity, the coulombic force is balanced by a drag force, also called the Stokes force:

$$F = 6\pi\eta r v \quad (4.2)$$

Where η is the viscosity of the liquid, r is the hydrodynamics radius of the molecule, and v is the speed of the molecules. The terminal speed of the molecules is reached when both forces are equal and opposite, so that

$$qE = 6\pi\eta r v \quad (4.3)$$

From Eq. 4.3 the terminal speed is calculated as

$$v = \mu E \quad (4.4)$$

Where μ is the mobility of the molecules, given by

$$\mu = \frac{q}{6\pi\eta r} \quad (4.5)$$

The relation in Eq. 4.4 is also true in three-dimension, in which both electric field and the velocity are vectors. The electric field strength and the speed form the magnitudes of these vectors.

4.3. Diffusion

Diffusion is a statistical transport phenomenon. Diffusion occurs when there is a concentration gradient of one kind of molecule within a fluid. For example, when a concentration gradient of large molecules in water is present, there will be a net movement of large molecules away from areas of high concentration. The main reason for this is that there are more large molecules in areas of high concentration than in areas of low concentration, so that many molecules move randomly in one direction, but only a few molecules move randomly backwards.

The statistical movement of a single molecule in a fluid can be described as random. The movement is characterized by Einstein-Smoluchowski relation:

$$x = \sqrt{2Dt} \quad (4.6)$$

Where x is the average distance moved after an elapsed time t between molecule collisions and D is a diffusion constant that is characteristic for the given molecule. From Eq. 4.6, we can notice that the moved distance is proportional only to the square root of time, in contrast to directed movement, where the distance is directly proportional to time. Eq. 4.6 also shows that a larger diffusion constant means faster movement. In general, it is true that the larger the molecule is, the smaller is its diffusion constant.

4.3.1. Temperature dependence of diffusion

A relation between the macroscopically observed diffusion behavior in the form of the diffusion constant D and a microscopic property of the molecules in the form of the hydrodynamic radius r can be stated. This is the Stokes-Einstein relation:

$$D = \frac{kT}{f}, f = 6\pi\eta r \quad (4.7)$$

Where f is a frictional constant known from the Stokes equation (Eq. 4.2), k is the Boltzman constant, and T is the temperature. Eq. 4.7 shows the temperature dependence of the diffusion constant and allows us to estimate the diffusion constant of molecules when hydrodynamic radius is known.

4.3.2. Peclét number

Under different circumstances and for different geometries of microfluidic systems, one or the other of the flow types (directed transport or statistical transport) dominates, or both flow types might be of equal importance. To evaluate the various flow situations, we can examine the ratio between the mass transport due to directed flow and that of diffusion. This ratio is a dimensionless number, the Peclét number (Pe), given as

$$P_e = \frac{vd}{D} \quad (4.8)$$

Where d is a characteristic length of the microfluidic system. If the Peclét number is much smaller than 1, then diffusion dominates the microfluidic flow, and directed flow is secondary importance. If the Peclét number is much larger than 1, then the molecules of interest flow mainly according to the externally applied driving force, and diffusion has only a minor influence. In microsystems, the flow velocities are usually comparatively small. The crucial variable that determines the Peclét number is therefore the channel lengths d. For long enough channels, the Peclét number is always larger than 1, and the flow is thus directed.

Both Peclét number and Reynolds numbers are typical of the field of hydrodynamics and allow statement to be made about the importance of a given type of flow phenomenon in a system. In microfluidics, the most important geometric dimensions, the operating conditions and the properties of the involved molecules are often unknown. Under most circumstances, the length and width of a microchannel, the average speed of the working fluid as well as the diffusion constants of the molecules of interest are therefore known. The Peclét number of the microchannel under question can be calculated with these data and the effect of diffusion compared to the directed flow can be evaluated. The obtained information is vital for the design of microfluidic systems.

5. SURFACE OF MICROCHANNELS

The heart of a microfluidic device is often the microchannel, in which reaction, separation, or detection takes place. It is therefore apparent that the surface properties of these microchannels are critical for proper functioning. In a micrometer-scale channel, capillary force dictates the flow of the fluid. It is largely influenced by the surface energy of the channel walls and/or the surface tension of fluid itself.

The surface free energy for any particular surface can be readily determined from contact angle measurement. It relies on a relation which has been recognized by Young (23) in 1805. The contact angle of a liquid drop on a solid surface is defined by the mechanical equilibrium of the drop under the action of three interfacial tensions: solid-vapor, γ_{sv}, solid-liquid, γ_{sl}, and liquid-vapor, γ_{lv}. This equilibrium relation is known as Young's equation:

$$\gamma_{lv} \cos\theta = \gamma_{sv} - \gamma_{sl} \quad (5.1)$$

Where θ is the contact angle measured. Several surface tension component approaches have been developed to determined the solid surface energy γ_{sv}; they are Fowkes approach (24), Owens-Wendt-Kaelble approach (25) and Lifshitz-van der Waals/acid-base (van Oss) approach (26).

At the liquid-solid surface interface, if the molecules of the liquid have a stronger attraction to the molecules of the solid surface than to each other, i.e. the adhesive forces are stronger than the cohesive forces, wetting of the surface occurs. Such a surface having high surface energy is termed hydrophilic. Alternately, if the liquid molecules were more strongly attracted to each other than the molecules of the solid surface, i.e. the cohesive forces are stronger than the adhesive forces, the liquid beads-up and does not wet the surface. The surface has a lower surface energy, and such a surface is hydrophobic. The capillary force on the fluid in the microchannel is due to air/liquid/solid interface interactions and can be calculated using

$$F = 2\pi r \gamma_{lv} \cos\theta \quad (5.2)$$

Where r is the channel radius. For liquid flow in microchannels, this effect is represented as an equivalent pressure or capillary pressure, P,

For circular tubes: $$P = \frac{F}{area} = \frac{2\gamma_{lv}\cos\theta}{r} \quad (5.3)$$

For rectangular tubes: $$P = \gamma_{lv}\cos\theta\left(\frac{1}{h} + \frac{1}{w}\right) \quad (5.4)$$

where h and w are the depth and width of the microchannel respectively. These expressions demonstrate that a larger contact angle (hydrophobic surface) means decreased force or pressure and therefore a decrease in the flow rate (27).

From above, it is apparent that the wetting behavior of the microchannel material greatly influences the flow characteristics of the fluid. Zhao *et al.* (28) demonstrated surface-directed flow inside microchannels. Self-assembled monolayer chemistry (for modifying wetting properties) was used in combination with either multi-stream laminar flow or photolithography to pattern surface free energies inside microchannel networks. Aqueous liquids introduced into these patterned channels are confined to the hydrophilic pathways, while the hydrophobic regions act as virtual walls in which liquids cannot cross when pressure is maintained under a critical value. Liquid flow inside microchannels is laminar, meaning that multiple liquid streams can flow side-by-side without turbulent mixing, allowing good spatial control (Figure 1). Chen *et al.* (29) proposed a nanoliter mixer for microchannels, which is power-free with the surface tension-capillary pumping the only mechanism for driving liquids. The microchannel is designed to have no sidewalls for reducing the frictional force, with the fluid being confined to the flow between the bottom hydrophilic stripe and the fully top-covered hydrophobic substrate. Once the fluid is pipetted into the entrance, the capillary pumping generated by the liquid meniscus at the hydrophilic interface will drag the fluid into the microchannel. The top cover cannot be hydrophilic because then once the fluid is introduced to the entrance, it will spread over and fill in the space between the top and the bottom substrates, thereby causing ineffective mixing.

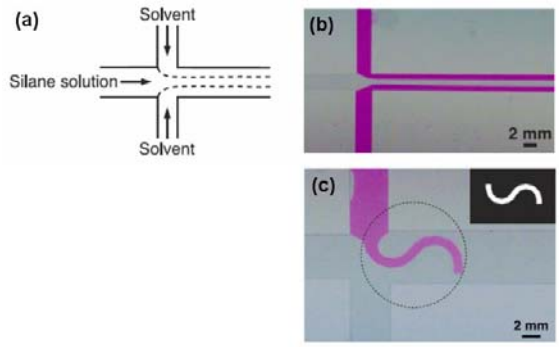

Figure 1. (a) Schematic illustration of multistream laminar flows and the corresponding images of aqueous flow inside channel after surface patterning (b). The liquid is a dilute solution of Rhodamine B dye (0.057 w/w %) in deionized water. (c) A dilute Rhodamine B aqueous solution was added into the channels by a syringe, and a pressure was applied to push the solution slowly through the hydrophobic region. When the aqueous solution reached the edge of the irradiated area, it wetted the hydrophilic region spontaneously and formed patterns identical to the photomasks, shown at a reduced size in the upper right corner. "Reproduced with permission of 28".

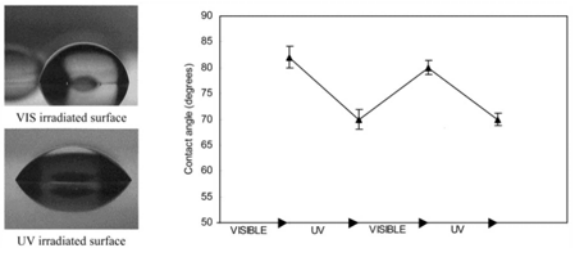

Figure 2. (a) Examples of water drops on a photosensitive surface after visible and UV irradiation. (b) Light-induced change in water contact angle on coated slides. "Reproduced from ref. 31, with permission from American Chemical Society"

Flow instabilities are induced to enhance mixing by the carefully designed asymmetric staggered grooved cavities on the bottom hydrophilic substrate.

Although surface energy is an intrinsic property of a material, it is however possible to modify the surface to attain favorable flow conditions and/or interaction with biological fluids. For example, Wu *et al.* (30) grafted an epoxy-modified hydrophilic polymers onto polydimethylsiloxane (PDMS) microfluidic chip to resist nonspecific protein adsorption. Surface adsorption of lysozyme and bovine serum albumin was reduced to less than 10% relative to that on the native PDMS surface (which is hydrophobic). Instead of a passive modification of the surface energy, a dynamic way is also possible. Rosario *et al.* (31) use a coating of photo-responsive spiropyran molecules covalently bound to a glass surface along with a mixture of silanes to induce reversible wettability changes when irradiated with UV and visible light (Figure 2). Water in capillary tubes coated with the photosensitive layer was observed to rise when the light source was switched from visible to UV. Contact angle hysteresis in the system was found to prevent the water from moving down the capillary when the light was switched from UV back to visible. Although surface modification can be done, in handling biological fluids, the channel materials must be biocompatible. For example, handling blood is very tricky, as thrombus formation is highly possible if the material is not compatible. Surface energy has shown to be one of the main aspects in affecting blood-compatibility. Zhang *et al.* (32) has conducted the study on blood-compatibility study on amorphous carbon, which has also shown potential to be a candidate for microchannels (33). The internal bonding of the amorphous carbon has been changed through post-deposition annealing. The change in the bondings alters the surface energy of the film. The results show that blood platelet adhesion increases when the polar/dispersive component ratio decreases (Figure 3). The analysis is based on Lifshitz-van der Waals/acid-base (van Oss) approach (26). The adhesion of platelet inside a microchannel is undesirable as it has detrimental effect on the biological fluid flow.

6. BIOLOGICAL CELL MANIPULATION

Depending on the force used, various classes of microfluidic devices for cell manipulation can be fabricated. Here, we describe the four main categories of cell manipulations that employ different manipulating forces.

6.1. Mechanical manipulation

The main application for on-chip mechanical manipulation is the separation of target cells for assay or culture purposes. Many configurations of constriction microstructures have been designed and fabricated to allow cell separation within microchannels. These structures include microgripper (34), microwells (35-38), microfilters (39-42), and dam (43).

The microgripper in (34) is fabricated from SU-8 (Figure 4a). It can manipulate single cell in physiological ionic solutions without exerting any force in closed position. A simple cell docking method induced by receding meniscus to capture non-adherent yeast cells onto microwells inside a microfluidic channel by lateral capillary force created at the bottom of the meniscus has been demonstrated (Figure 4b, c) (35). Khademhosseini *et al.* (37) fabricated poly(ethylene glycol) microwell arrays within microchannels using a novel two-step process. Crowley and Pizziconi (39) demonstrated the use of passive, operating entirely on capillary action, transverse-flow microfilter devices for the microfluidic isolation of plasma from whole blood. Efficiency of these microfilters is approximately three times higher than the separation efficiencies predicted for microporous membranes under similar conditions. The cells flowing through the microchannels can be docked and analyzed within pre-defined location. Moorthy and Beebe (42) fabricated

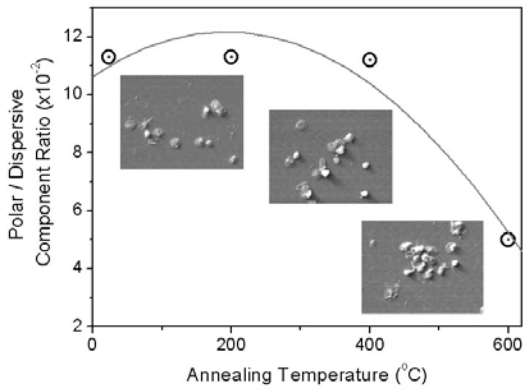

Figure 3. Ratio of polar to dispersive surface energy component on silicon containing amorphous carbon film annealed at different temperatures. From Lifshitz-van der Waals/acid-base (van Oss) approach, the polar component can further be differentiated into base (electron donor) and acid (electron acceptor) components. It is shown that the base components for all the films are very much larger than the acid component, indicating the polar component of the surfaces is predominantly negatively charged. This is beneficial towards haemocompatibility as the platelets and proteins tend to have a net negative zeta-potential of -8 to -13 mV. In other words, there will not be any preferential adsorption of any of the proteins and platelets due to electrostatic interaction if the polar component is increased. "Reproduced with permission of 32".

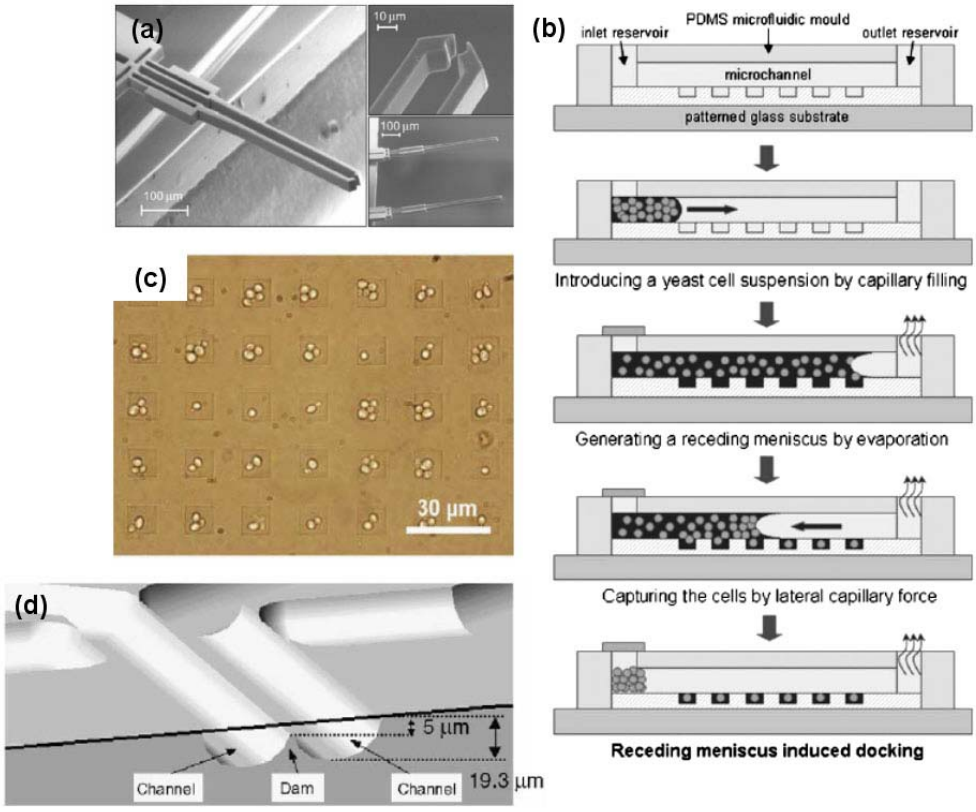

Figure 4. (a) SEM micrographs of the overhanging SU-8 microgripper "reproduced from ref. 34, with permission from Institute of Electrical and Electronics Engineers"; (b) A schematic diagram of the receding meniscus induced cell-docking method. A yeast cell suspension was flowed into the microchannel by surface tension-driven capillary filling and subsequently a receding meniscus was generated at the evaporating front "reproduced from ref. 35, with permission from Royal Society of Chemistry"; (c) Brightfield image of the captured SG3 yeast cells in the 10 µm well using the receding meniscus docking technique "reproduced from ref. 35, with permission from Royal Society of Chemistry"; (d) Cross section of the microchip showing the dimensions of the channels and the dam structure. "Reproduced with permission of 43".

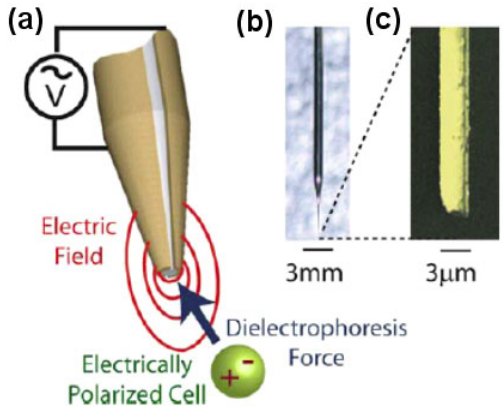

Figure 5. DEP tweezers for cell manipulation. (a) Schematic of DEP tweezers in operation. A voltage across two electrodes on either side of a sharp glass tip creates an electric field which polarizes a cell and pulls the cell into the field maximum at the end of the tip. (b) Photograph of DEP tweezers and SEM image of the tweezers tip. Electrodes appear light while the insulating gap between electrodes is dark. "Reproduced with permission of 52".

porous filters inside a microchannel based on emulsion polymerization. Studies with rabbit whole blood demonstrated that separation of blood cells from serum by the porous filter is comparable to traditional centrifuge techniques with the added advantage of the elimination of an external power source. Yang *et al*. (43) fabricated a dam structure in parallel to the fluid flow for docking and alignment of biological cells (Figure 4d). The fragile cells are allowed to move in the microfluidic channels and to be immobilized with controllable numbers in desired locations. The cells docked on the parallel dam structure are exposed to minimal stress caused by fluidic pressure.

6.2. Electrical manipulation

Electrical manipulations in microfluidics are mainly based on dielectrophoresis (DEP). It is the translation of neutral particles caused by polarization effects in a non-uniform electric field (44). Traditionally, a DEP trap can be formed by creating an electric field gradient with an arrangement of planar metallic electrodes either directly connected to a voltage source ([4]5,46) or free-floating (47,48) in presence of an AC field. Parallel electrical manipulation of micro- and nano-sized objects (e.g. proteins, cells, DNA, etc.) is possible using DEP techniques. In more recent development, an electrodeless DEP (EDEP) has been reported (49,50). A constriction or channel in an insulating material instead of a metallic wire is used to squeeze the electric field in a conducting solution, (e.g. ionic buffer), thereby creating a high field gradient with a local maximum. In this EDEP technology, no metal evaporation during the fabrication is needed; the structure is mechanically robust and chemically inert; and a very high electric field may be applied without gas evolution.

Zou *et al.* (51) has fabricated a 2D-MEMS DEP device to investigate a range of effects of DEP on osteoblasts. The experiments show that the osteoblasts can experience both positive dielectrophoresis (p-DEP) and negative dielectrophoresis (n-DEP) when the frequency of the AC field is changed (p-DEP at 5 MHz frequency, n-DEP at 0.1 MHz). And the viability values for cells exposed to DEP are nearly three times higher than the control values, indicating that dielectrophoresis may have an anabolic effect on osteoblasts. Manipulating and positioning single cell is very important in areas of biomedical research. Hunt *et al.* (52) developed a DEP tweezers; a sharp glass tip with electrodes on either side, capable of trapping single cells with electric fields (Figure 5). Mounted on a micromanipulator, dielectrophoresis tweezers can position a single cell in three dimensions, holding the cell against fluid flow of hundreds of microns per second without the cell escaping. This simple technique would greatly facilitate *in vitro* fertilization and single cell transfection studies. The rapid formation of reproducible 3D cellular structures within a photo-polymerizable hydrogel using DEP forces has also been demonstrated (53). A chip designed with enhanced field-induced DEP trap has been demonstrated (54).By constructing the geometric shape and the distribution of stellate tips, the DEP electrodes enhance the desired spatial electric-field gradients to guide and snare individual cells to form the desired biomimetic pattern.

The above mentioned EDEP technique can probe the response of DNA molecules well below 1 kHz, revealing a huge increase in dielectric response at low frequencies (below 1 kHz), which is difficult to observe using metal electrodes as trapping structures (49). Barrett *et al.* (55) demonstrated the trapping and deflection of particles and continuous concentration and separation of *Bacillus subtilis* from a two-component sample mixture. Making use of the insulating properties of glass and the 3D ridge-like structures in microchannels, the DEP force that results from the electric field gradient near the ridges is used to affect particle motion parallel to the ridges in the absence of a bulk pressure-driven flow. EDEP has also been employed to manipulate live and dead *Escherichia coli*, were concentrated and selectively released by applying stepped DC voltages across a microchannel containing an array of insulating posts etched in glass (56).

6.3. Magnetic manipulation

In order to use magnetic fields to manipulate and apply forces to biological components, the desired cells should be attached to magnetic particles ([5]7,58), as most biological cells have zero magnetic susceptibility. Forces can be created with magnetic fields generated by planar micro-electromagnets integrated in a micro-chamber rather than using the external magnetic field produced by a permanent magnet (59). The high selectivity and the long range force possible with the magnetic manipulation make it the preferable method for biological-cell handling compared to other techniques such as electrostatic, optical, and physical means. Typical magnetic particles studied

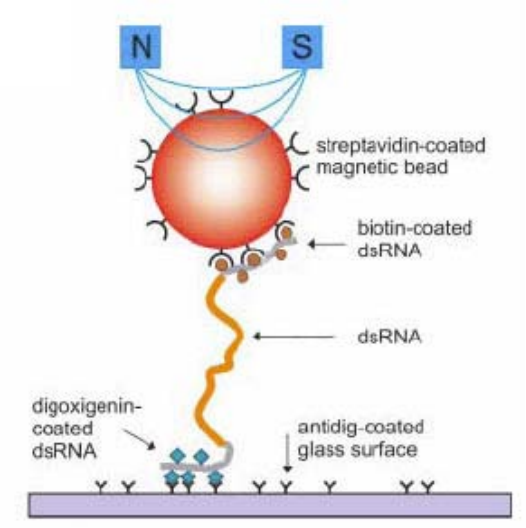

Figure 6. Illustration of a dsRNA molecule as placed in the magnetic tweezers. The double-stranded RNA molecule is tethered to a magnetic bead by a biotin-streptavidin linkage and to the bottom glass surface of a flow cell using an antidigoxigenin-digoxigenin linkage. Magnets are placed above the flow cell to exert a force on the magnetic bead, and hence on the double-stranded RNA molecule. "Reproduced with permission of 64".

comprise of a magnetic core (usually magnetite Fe_3O_4, or maghemite γ-Fe_2O_3) with a biocompatible coating ([6]0,61). These clusters of single-domain magnetic core make it "superparamagnetic", which means that the bead responds to the magnetic field, but it demagnetizes completely when the field is removed (62).

Yeung *et al.* (63) has demonstrated the capturing of genomic DNA directly from *E. coli* culture by avidin-coated magnetic particles through DNA–DNA hybridization in a silicon/glass-based micro-reactor patterned with a platinum heater and sensors. After washing under an external magnetic field, the genomic DNA anchored on the functionalized magnetic particles are ready for polymerase chain reaction and electrochemical detection by gold labeling and silver deposition/stripping. Abels *et al.* (64) has introduced two new approaches for the study of double-stranded RNA persistence length. One of which is a magnetic tweezers consisting of streptavidin-coated magnetic particles (Figure 6). They deduce a mean persistence length for long dsRNA molecules of about 63.8 nm from force extension measurements with the magnetic tweezers and found the result to be consistent with that determined from atomic force microscopy, which is about 62 nm.

In microfluidic system, the mixing of magnetic particles and biological cells becomes crucial. The flow is in almost all cases laminar due to its low Reynolds number (as mentioned earlier). Conventional mixing principles utilizing turbulence or flow separation cannot be achieved. Since the molecular diffusion is the only way to mix, it takes considerable time and thus limits the performance of the device. A simple yet efficient magnetic force driven mixer has been developed to facilitate the mixing of magnetic particles and biomolecules in a microchannel (65). With appropriate temporal variations of the force field, chaotic mixing can be achieved. The mixing device consists of embedded microconductors as a magnetic field source and a microchannel that guides the streams of working fluid. It is demonstrated that a pair of integrated microconductors provides a local magnetic field strong enough to attract nearby magnetic particles. Mixing of magnetic particles is accomplished by applying a time-dependent control signal to a row of conductors, at the Reynolds number of as low as 10^{-2}. There are also a number of interesting reports on magnetic manipulation over the last few years (66-72).

6.4. Optical manipulation

After the first use of gradient forces from optical beams to trap particles and exert controlled forces on small beads, viruses and bacteria (73,74), the technique has came to known to be "optical tweezers". It has become a powerful tool for individual cell manipulation (75-78). There is great interest of using this technique to manipulate biological species on microfluidic systems because of its non-contact and contamination-free characteristics. Furthermore, it can be used in fluids, and hence can be used for biological-cell in a natural hydrated and functioning condition. Therefore, optical tweezers is an attractive mean of particle manipulation in microfluidic systems.

Akselrod *et al.* (79) have assembled three-dimensional heterotypic networks of living cells in hydrogel without loss of viability using arrays of time-multiplexed, holographic optical traps. They have demonstrated the ability to manipulate hundreds of *Pseudomonas aeruginosa* simultaneously into two- and three-dimensional arrays. Keyser *et al.* (80) developed a novel experimental technique that combines optical tweezers with ionic current detection and solid-state nanopores. They are able to arrest DNA molecules in nanopores and measure the force on this DNA molecule. It is believed that their new combination of nanopores and optical tweezers can be used for new experiments in biophysics like the theoretically proposed unzipping of RNA hairpins (81,82) and the detection of proteins bound to DNA (83). Arai *et al.* (84) demonstrated the rapid separation and immobilization of a single cell by concomitantly utilizing laser manipulation and locally thermosensitive hydrogelation. A single yeast cell was successfully separated, positioned, and immobilized in the thermal gel, and then cultured. Enger *et al.* (85) developed a microfluidic system to move cells between reservoirs filled with different media by means of optical tweezers. The cells can be moved from one reservoir to another in a few seconds without the media being dragged along with them (Figure 7). MacDonald *et al.* (86) demonstrate an optical sorter for microscopic particles that exploits the polarizability of the particles with an extended, interlinked, dynamically reconfigurable, three-dimensional optical lattice. They demonstrated both sorting by size (of protein microcapsule drug delivery agents) and sorting by refractive index (of other colloidal particle streams), and

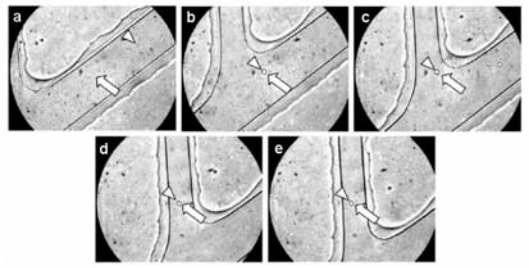

Figure 7. The figure shows five video frames from a sequence when a yeast cell is trapped with the optical tweezers and moved into the collection channel, whereas the other cells continue straight forward into the drain channel. The arrow indicates the position of the tweezers, and the triangle marks the selected cell. "Reproduced with permission of 85".

the sorting efficiency is 100%. Terray *et al.* (87) has demonstrated the use of optical trap in the construction of linear structures within microfluidic channel with colloidal microspheres to create particle or cell directing devices.

7. DNA AMPLIFICATION

DNA amplification is the production of multiple copies of a sequence of DNA. As quoted in Scientific American, Kary Mullis wrote, *"Beginning with a single molecule of the genetic material DNA, the PCR can generate 100 billion similar molecules in an afternoon. The reaction is easy to execute. It requires no more than a test tube, a few simple reagents, and a source of heat."* Polymerase chain reaction (PCR) is an *in vitro* technique which is used to enzymatically replicate DNA without using a living organism, such as *E. coli* or yeast. The technique allows a small amount of DNA to be amplified exponentially, in order to produce enough DNA to be adequately tested. This technique can be used to identify with a very high-probability, disease-causing viruses and/or bacteria, a deceased person, or a criminal suspect. Since the technique was invented in the mid-eighties by Mullis (88), PCR has made prominent impact on clinical medicine, genetic disease diagnostics, forensic science, and evolutionary biology.

PCR reaction process is divided into three steps: (1) denaturation – where the double-stranded DNA is heated at around 94-96°C such that it melts into single-stranded DNA; (2) annealing – after denaturation, the temperature is lowered to a degree specific for the primer (oligonucleotide), which generally lies between 55-70°C. The primer then takes its place at the specific DNA-recognition-sequence (according to their complementary) of the single-stranded DNA-template-sequence; (3) extension – the temperature is increased to 72°C, which corresponds to the optimal temperature for the Taq-polymerase enzyme to work. The DNA polymerase has to copy the DNA strands. It starts at the annealed primer and works its way along the DNA strand, until the double-stranded DNA-molecule is complete again.

PCR was one of the first diagnostic techniques to be successfully implemented in a microfluidic format. The miniaturization of PCR devices leads to many improvements like decreased cost of production and applications, decreased time for amplification, reduced consumption of biological sample necessary for test, and increased portability and integration of PCR devices. Effective and rapid PCR in volumes ranging from 1 picoliter (pl) to 50 microliter (µl) has been performed using various heating mechanisms, including infrared-mediated thermal cycling (89), microwave heating (90), Joule heating (91) and resistive heating (92). In addition, the high surface area-to-volume ratio also facilitates rapid heat transfer and smaller sample volumes have less heat capacity, thereby allowing rapid temperature change. Since the introduction of the first PCR chip (93,94), many PCR microfluidic technologies have been developed to facilitate DNA amplification with improved performances. For example, a single molecule PCR can be performed in PCR microfluidics, starting with a single-copy sequence in the mixture (95-99). Here, we give a general view on PCR microfluidics by reviewing the substrate material development used in PCR microfluidics, and the various PCR microfluidics designs.

7.1. Substrate for PCR microfluidics

It has been ten years since the first PCR chip are fabricated from silicon (93-95,100-104), or glass (98,99,105-110). Till now, most of the microchambers and microchannels are still so made. Employing silicon or glass as the substrate materials of the PCR chip reaction well is understandable as the standard photolithography and chemical etching techniques are well developed and thus can effectively fabricate the microfluidic networks for PCR.

Silicon has superior thermal conductivity, thereby allowing fast temperature ramping rates. In addition, integration with other components is possible when metal films (as heaters or sensors) can be patterned and deposited on its surface. However, the high thermal conductivity of silicon can have a detrimental effect, as thermal insulation of the substrate is usually needed to reduce the energy losses to the surroundings. The incorporation of such insulation will increase the complexity to the PCR microsystems. Furthermore, silicon is not transparent, thereby limiting real-time optical detection. And also, bare silicon will inhibit the PCR reaction by reducing the amplification efficiency. Glass on the other hand, possesses some beneficial characteristics such as well-defined surface chemistries, and superior optical transparence. However, the PCR microfluidics made from silicon or glass material are not disposable due to the high cost of fabrication.

There is no single substrate material that can cater to all restrictions like cost, ease of fabrication, optical transparency, disposability, or biocompatibility. But a new class of material – polymers, has been of interest to many research groups in recent years, and has shown to be superior to silicon or glass as material for PCR microfluidic substrate. Many polymer-based PCR microfluidics have

been synthesized. They include polydimethylsiloxane (PDMS) (111-114), polymethylmethacrylate (PMMA) (115,116), polycarbonate (PC) (116-119), polyethylene terephatha-late (PET) (120), even photo-resist like SU-8 (121,122), and others.

In order to take full advantage of the properties of silicon, glass and polymers, researchers have developed hybrid materials for PCR substrate, such as silicon/glass (123,124), polymer/silicon (122), and polymer/glass (125).

7.2. Types of PCR microfluidics

The main advantages for the miniaturization of PCR systems are shorter amplification time, higher throughput, and minimum world-to-PCR intervention. There are many designs of PCR microfluidics that allow such effectiveness. Until now, there are three main classes, namely, stationary PCR microfluidics, flow-through PCR microfluidics, and thermal convection driven PCR microfluidics.

7.2.1. Stationary PCR microfluidics

The working principle of this type of PCR microfluidics is the same as conventional PCR devices. The PCR solution is kept stationary and the temperature of the reaction chamber is cycled between three different temperatures. After the PCR reaction, the amplification products can be detected off-line or in an on-line way. There are the single chamber and multi-chamber stationary PCR microfluidics.

The first PCR microfluidics (93) has a reaction chamber fabricated by silicon anisotropic wet etching. The amplification was 20 cycles carried out in a 50 μl well. The time is four-fold faster as compared to conventional PCR system. In addition, it consumes less power. Single chamber stationary PCR microfluidics is the classic design, and has since been studied and improved by many groups (102-104,107,109,111,113,115,117,119,121,124). The fluidic and thermal controls in these microsystems are good, and the thermal and fluidic cross-talk between the reaction microchambers is minimal. However, a number of sequential PCR tests are needed for a quantity of DNA samples to be amplified to meet detection requirement. Hence, there exists the possibility of incomplete amplification if these amplifications are carried out in the same PCR microfluidics.

Therefore, in order to increase the PCR throughput and reduce the time for analysis, multi-chamber stationary PCR microfluidics on a single chip has been developed (95,97-99,106,110,114,120). Since now multiple reaction chambers work concurrently, special attention must be taken in the design to ensure temperature uniformity between chambers. The processing of small sample volumes in increased number of PCR reaction chambers however can result in loss of sample on the surface of transferring devices, and loss of sample through evaporation.

7.2.2. Flow-through PCR microfluidics

This type of PCR microfluidics is based on "time-space conversion" concept, in which the PCR solution is continuously and repeatedly flowing through three different temperatures zones that are necessary for DNA amplification. It is more flexible than chamber stationary PCR microfluidics as the reaction rate can be altered, giving an increased number of cycling and reduced heating time.

The simplest configuration of flow-through PCR microfluidics is the single straight capillary based flow-through design. This design consists of a capillary tube that transfers the PCR solution through different heating zones. This type of PCR microfluidics is reported by Chiou *et al.* (126), where three heating blocks define the denaturation, annealing and extension zones. 30 cycles of a 500 base-pair product can be performed in 2.5 min with 78% amplification efficiency. And more recently, a reusable kind of such PCR microfluidics has been developed for continuous monitoring of infectious biological agents, where the amplification efficiency is around 96.5% with a good elimination of carryover from sequential runs (96).

Most on-chip rectangular serpentine channel PCR microfluidics (105,112) has the following temperature zone arrangement: denaturation temperature $\rightarrow$ extension temperature $\rightarrow$ annealing temperature. Such an arrangement ensures smooth temperature gradient without the need of a cooling process (Figure 8). However, the melted single-stranded DNA sample might form double strands with the template strands or their complementary fragments when passing through the extension zone, which will result in decreased amplification efficiency. In order to avoid this problem, an alternative temperature arrangement is developed, which consists of a circular arrangement of the three temperatures zones to generate the sequence of denaturation, annealing, and extension (116,118,122,127-129). The microchannels of these PCR microfluidics can consists of capillary tubes (127,128) or an annular channel (122,129).

7.2.3. Convection-driven PCR microfluidics

This type of PCR microfluidics employs two fixed temperature zones to facilitate the convection-driven sample flow. There is no external force to drive the fluid through the different temperature zones as the sample fluid is driven by buoyancy forces. The convection-driven PCR microfluidics has simplicity in design, they are cheap, and have much faster temperature transition speed. They only require a cavity/loop and specific thermostable zones, and have no electronic control components for temperature cycling.

Krishnan *et al.* (130) reported an elegant microfluidic system for PCR that relies on the control of thermal convection in a Rayleigh–Bénard cell to provide thermal cycling conditions. PCR amplification was performed inside a 35-μL cylindrical cavity. The temperature cycling was achieved as the flow continuously shuttles fluid vertically between the two temperature zones of annealing/extension (top, 61°C) and denaturation (below, 97°C).

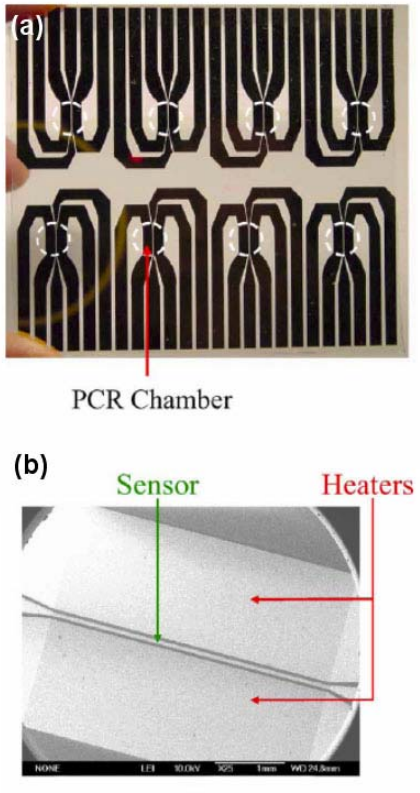

Figure 8. (a) Schematic of a chip for flow-through PCR. Three well-defined zones are kept at 95°, 77°, and 60°C by means of thermostated copper blocks. The sample is hydrostatically pumped through a single channel etched into the glass chip. The channel passing through the three temperature zones defines the thermal cycling process; (b) Layout of the device used in this study. The device has three inlets on the left side of the device and one outlet to the right. Only two inlets are used: one carrying the sample, the other bringing a constant buffer flow. The whole chip incorporates 20 identical cycles. Two precision syringe pumps deliver the PCR sample and the buffer solution onto the chip. Product is collected at the outlet capillary and then analyzed. "Reproduced with permission of 105".

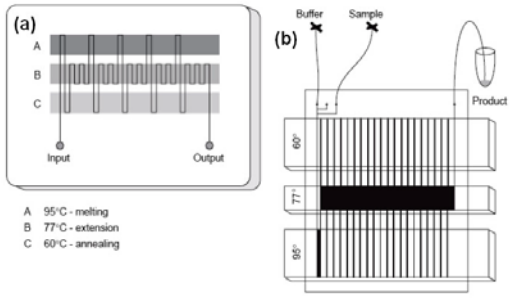

Figure 9. Convective PCR in a Rayleigh–Bénard cell. PCR in a restricted Rayleigh–Bénard convection cell is induced by heating from below. This leads to laminar convection that elegantly implements the temperature oscillation needed for PCR. "Reproduced with permission of 130".

Later on, a laminar convection PCR microfluidics was developed by Braun *et al.* (131-133). The PCR cycles a 20 μl sample and can achieve a million fold λ-DNA amplification within 25 min. Concepts of convective PCR based on Rayleigh–Bénard cell is illustrated in (Figure 9).

8. SUMMARY AND PERSPECTIVE

Among microfluidic systems, the polymerase chain reaction (PCR) received the most attention and development, though few chip-based systems have been commercialized. In recent years, polymer has been the center of attraction for microfluidic systems, mainly due to their biocompatibility, ease of fabrication, low cost and optical properties. Various PCR configurations like stationary chamber, flow-through and convection-driven have been developed. There are however no doubt plenty of rooms for advancement, especially when many researchers are focusing on throughput and efficiency at this stage of development. For example a multi-level PCR microfluidics might be developed for ultra-high throughput. Though substrate material selection and chip configuration are vital, the real challenge now and for some time to come is the integration of PCR microfluidics with sample preparation and/or product detection microdevices.

Cell handling is a vital aspect in microfluidic systems for cell-based analysis. There is tremendous development in manipulation techniques. The mechanical manipulation through constriction structures though is simple; this passive method suffers poor selectivity, which ultimately limits their applications. Dielectrophoresis manipulation demonstrated effectiveness and selectiveness in manipulating and separating cells and bacteria, but the survival rate of biological cells are low due to the use of electric field. Magnetic manipulation is a non-invasive method for cell handling. Development of new magnetic particles in terms of materials and surface modification can increase the efficiency of this technique in the future. Optical manipulation techniques like those in electrical manipulation require expensive instrumentation and complex setup. But the non-contact and contamination-free process will continue to receive great attention for development, especially because of its versatility in terms of the milieu in which biological cells are contained.

Surface modification or modifying surface conditions on microchannel walls remain an interesting area for study. New coatings and/or materials, organic or inorganic, will be studied to better the performances. The mechanics described in this paper is elementary. Huge progress has been made in understanding the characteristics of fluid and their flow in the modern days, especially with the help of complex modeling software. Exploitation of new phenomena occurring in fluids in micro- or even nano-scale level remains an important task. Development made from such studies can one day produce microfluidic lab-on-a-chip a household item for quick detection of illnesses, just like thermometer is for temperature.

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Abbreviations: MEMS: micro-electro-mechanical systems, TAS: total analysis systems, DEP: dielectrophoresis, EDEP: electrodeless dielectrophoresis, PCR: polymerase chain reaction, DNA: deoxyribonucleic acid, RNA: ribonucleic acid, PC: polycarbonate, PET: polyethylene-terephathalate PDMS: polydimethylsiloxane, PMMA: polymethylmethacrylate

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