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Introduction: The Origin, Current Status, and Future of Microfluidics

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1.1 Introduction

Microfluidic device/system is generally defined as a component that handles a small quantity (micro- or nanoliter) of fluids (liquid or gas). Because most of the applications required for handling fluids relate to biomedical and chemical analyses, microfluidics has been currently realized as miniaturized analytical technology for biomedical and chemical applications. Conventional macroscopic equipment processing in wet laboratory can be miniaturized into microscopic devices. One of the objectives of the development of microfluidic systems is to provide a total solution from the sample application to the display of analytical results. Hence, microfluidic system is also called lab-on-chip (LOC), biochip, or micro-total analysis system (μTAS). Because of the miniaturization, a number of advantages can be achieved including less sample/reagent consumption, reduction of contamination risk, less cost per analysis, reduction of tedious operations, enhancement of sensitivity and specificity, and increase of reliability.

In the beginning of the development of microfluidic technology, fabrication of microfluidic devices was based on the microelectronic manufacturing infrastructure and microelectromechanical systems (MEMS) technology. Microfluidics was realized as a branch of MEMS technology specializing in handling fluids. Silicon was the major material for the substrate of these microfluidic devices. The typical microelectronic fabrication processes include photolithography, thin-film deposition, and etching. These processes are called surface-micromachining processes that can treat silicon surface of 1–2 μm in thickness at most. However, microfluidic devices require to fabricate high-aspect-ratio microstructures and bond multi-substrates. Bulk micromachining processes and substrate bonding techniques originally

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developed for MEMS were used to construct closed-volume microfluidic devices. An example of classical silicon-based microfluidic devices is ink-jet printer head. It has a large number of high-precision microscopic nozzles that eject ink onto paper. Generally, these nozzles are 10 μm in diameter and are fabricated by silicon material. However, silicon substrate is relatively expensive and is not optically transparent. It may limit the applications of optical detection, especially for biomedical and chemical analyses. Therefore, glass and polymer materials were introduced, and microfluidic technology became a specific research area in the 2000s [1–4]. Compared with silicon substrate, glass and polymer materials are less expensive and optically transparent. Polymer materials such as polymethylmethacrylate (PMMA), polystyrene (PS), polycarbonate (PC), and polydimethylsiloxane (PDMS) were used to demonstrate the fabrication of microfluidic devices [1–7]. Among these, PDMS is one of the most commonly used materials for fabricating microfluidic devices in current research laboratories. The advantages of using PDMS material include easy replication, optical transparency, biocompatibility, and low cost. To fabricate PDMS layers with microstructures, it is generally based on soft lithography, which is a nonphotolithographic strategy of replica molding [2]. Typically, microfluidic devices can be constructed by binding glass substrates and PDMS layers [3, 4]. Glass-/PDMS-based microfluidic devices were widely demonstrated on various biomedical applications such as DNA analysis [8–15], immunoassays [16–19], and cell-based assays [20, 21]. Recently, paper substrate has been proposed to be an alternative material used for fabricating microfluidic devices [22]. The use of a paper substrate has a number of advantages including being inexpensive, thin, light in weight, and disposable. Aqueous solution can be transported by wicking and a passive pumping is realized. Paper substrate is biocompatible with various biological samples and can be modified by a wide range of functional groups that can be covalently bound to proteins, DNA, or small molecules. The original idea of paper-based microfluidics was to suggest a new class of point-of-care diagnostic device for developing countries and remote environments [23]. Since then, various diagnostic applications were demonstrated [24–31], for example, paper-based enzyme-linked immunosorbent assay (ELISA) was shown to be completed within an hour, whereas conventional ELISA requires at least 6 h [30, 31]. Moreover, biological cells were also reported to be cultured on paper substrates for more advanced analyses [32–36].

The above discussions briefly introduce different materials used for the fabrication of microfluidic devices including silicon, glass/polymer, and even paper substrates. It is noticed that the design and material used by microfluidic devices are flexible and unlimited. In this chapter, the development from the origin and current status to the future prospect in microfluidics is discussed, including: (i) development of microfluidic components; (ii) development of complex microfluidic systems; and (iii) development of application-oriented microfluidic systems. An updated and systematic in-depth discussion is provided in this chapter.

1.2 Development of Microfluidic Components

The development of microfluidics originated from MEMS technology, which was defined as a microscopic system integrating with electronic and mechanical components. Its objective is to miniaturize conventional macroscopic devices for measuring physical quantities into microscopic devices. Because of strong capital promotions from both government and industry, development of MEMS technology was rapid and that made the sensing components small and inexpensive. A typical example of MEMS is accelerometer, which currently is embedded in nearly every cell phone to sense gravity for identifying the orientation of the cell phone. Along with this concept, conventional macroscopic equipment processing in wet laboratory could be miniaturized into microscopic devices. These microscopic devices were designed to handle sub-milliliter fluids, so they were called microfluidic devices. In the beginning, most of the developments were focused on miniaturization of fluidic components such as pumps [37–39], mixers [40–42], and valves [43, 44]. These individual components were the fundamental elements of fluidic systems. The objective of the development was to demonstrate the capability of fluidic manipulation, but not for specific biomedical applications. For example, a silicon-based bidirectional micropump was reported and its schematic drawing is shown in Figure 1.1 [38]. The micropump was actuated by electrostatic diaphragm and two passive check valves. It was constructed by multisilicon substrates and fabricated by bulk micromachining technology. The maximum pump rate could be 850 $\mu l\,min^{-1}$ and back pressure was 31 000 Pa. Alternatively, microfluidic mixing in a continuous flow was demonstrated by ultrasonic vibration [41]. Illustration of the design of the micromixer is shown in Figure 1.2. It was constructed with a glass substrate and a silicon substrate. The glass substrate was etched and anodically bonded with the silicon substrate to form the flow channel. The silicon substrate was etched from the backside to form the oscillating diaphragm. A piezoelectric disk was then attached to the oscillating diaphragm. Laminar flows were mixed

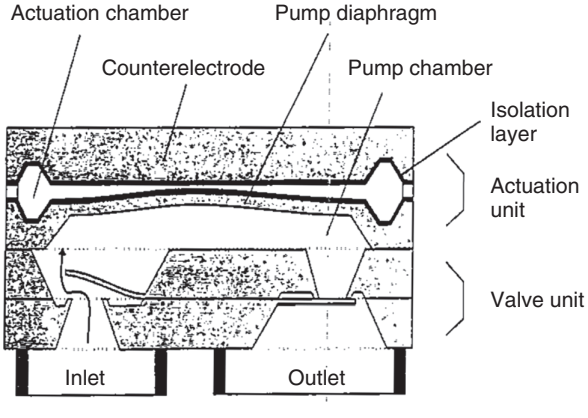

Figure 1.1 Silicon-based electrostatically driven diaphragm pump. (Zengerle *et al*. 1995 [38]. Reproduced with permission of Elsevier.)

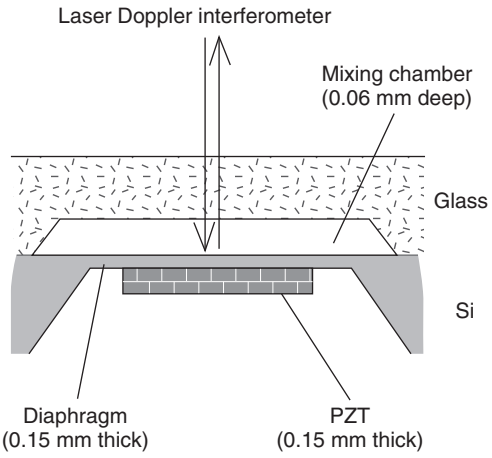

Figure 1.2 Schematic drawing of the cross-section of the micromixer. (Reprinted with permission from Ref. [41]. Copyright (2001) Elsevier.)

continuously and effectively by the ultrasonic vibration from the diaphragm actuated by the piezoelectric disk.

The above examples showed the focus of the early development of microfluidic technology in the 1990s. Fluidic manipulation and handling in microenvironment was the key issue to be solved at that moment. Because of limited substrate materials, that is, silicon and glass, microfluidic components were mainly fabricated by bulk micromachining technology. However, silicon substrate is relatively expensive and is not optically transparent. It may limit the applications of using optical detection, especially for biomedical and chemical analyses.

1.3 Development of Complex Microfluidic Systems

In the 2000s, polymer materials were introduced to construct microfluidic devices/systems [1–4]. Currently, PDMS is one of the most commonly used materials for fabricating microfluidic devices/systems in research laboratories. Because of the advantage of easy replication, complicated microfluidic systems were successfully fabricated by integrating many fluidic components [3, 4]. A pioneer work was demonstrated constructing a microfluidic system integrating with on–off valves, switching valves, and pumps [3]. The system was entirely consisted of elastomer based on multilayer soft lithography. Another developed latching microfluidic valve structures controlled independently by using an on-chip pneumatic demultiplexer [45]. A microfluidic system was constructed by a four-bit demultiplexer for routing pressure and vacuum pulses from a single input connection to each of the 16 latching valves as shown in Figure 1.3. Because these valve assemblies can form the standard logic gates, it was expected to develop complex pneumatic microprocessors for handling fluids. Besides microfluidic operations in a continuous flow, manipulation of discrete microdroplets was introduced and called digital microfluidics [46–48]. Electrolytic droplets were actuated by direct electrical control of the surface tension through a pair of opposing planar electrodes that was based on electrowetting-on-dielectric

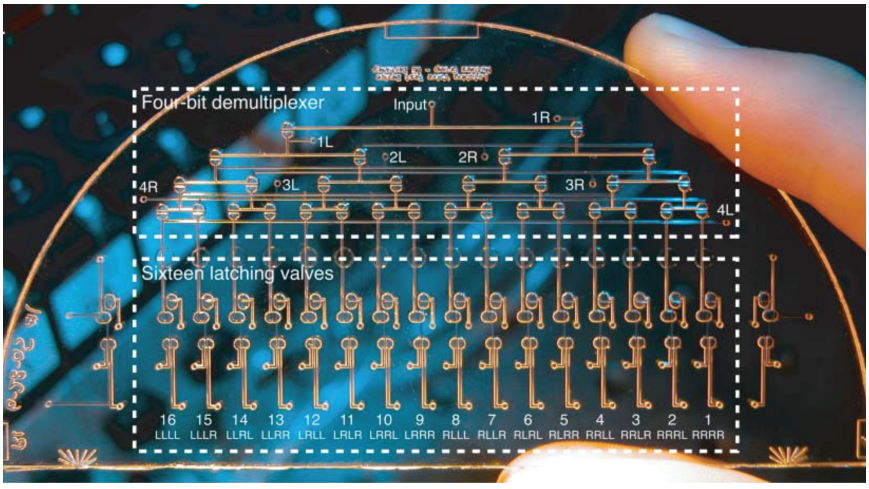

Figure 1.3 Photograph of the multiplexed latching valve system with a 4-bit demultiplexer and 16 latching valves. (Grover *et al*. 2006 [45]. Reproduced with permission of Royal Society of Chemistry.)

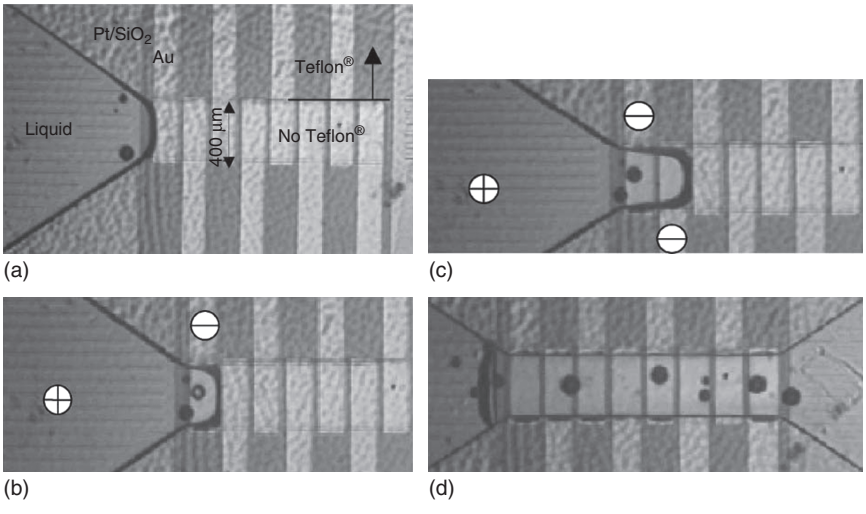

Figure 1.4 Manipulation of discrete microdroplets by digital microfluidics. (a) Liquid introduced, (b) first electrode biased, (c) first and second electrodes biased, and (d) all the electrodes biased. (Lee *et al*. 2002 [47]. Reproduced with permission of Elsevier.)

(EWOD) principle. By applying electrical potentials to sequential electrodes, a droplet can be dispensed from a reservoir, transported to any position on the array, merged with other droplets to perform reactions, and split into two droplets. An example of the manipulation of microdroplets is shown in Figure 1.4. Digital microfluidics was proposed to have several advantages over traditional counterparts, such as elimination of dead volume, enhancement

of mixing ratio, precision on the control of the volume, and encapsulation of biomolecules for monitoring. In the early 2000s, demonstrations of fabricating complex microfluidic systems have been extensively reported. These developments provided a solid foundation for the investigations of microfluidic systems in the applications of various biomedical and chemical analyses.

1.4 Development of Application-Oriented Microfluidic Systems

By the mature development of microfluidic technology, a broad spectrum of applications has been demonstrated by the microfluidic systems. Because of the characteristics of microfluidics such as miniaturization and automation, conventional biomedical and chemical analyses could be precisely and effectively operated in a single microfluidic system. A number of advantages are obtained including less sample/reagent consumption, reduction of contamination risk, less cost per analysis, reduction of tedious operations, enhancement of sensitivity and specificity, and increase of reliability.

1.4.1 Applications of DNA Assays

Microfluidic systems have been demonstrated on DNA assays [8–15]; a pioneer work was published in 1998 [12]. Microchannels, heaters, temperature sensors, and fluorescent detectors were integrated into a single silicon-/glass-based microfluidic system. Operations such as capturing DNA, mixing reagents, and amplification, separation, and detection of DNA products were automatically

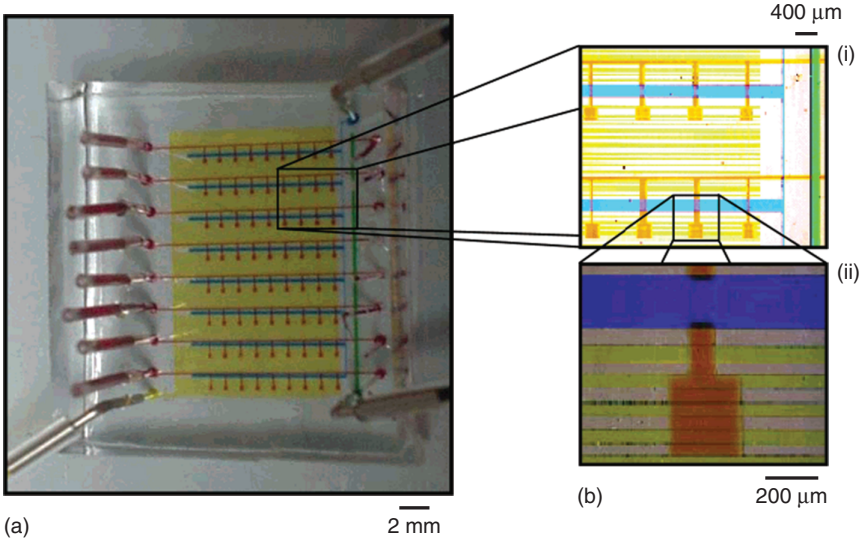

Figure 1.5 Microfluidic RT-PCR system. (a) Photograph of the system loaded with food dye. (b) Optical micrographs of eight reaction chambers (i) and one reaction chamber (ii). (Marcus *et al*. 2006 [13]. Reproduced with permission of American Chemical Society.)

manipulated by electroosmotic pumping. Detection of specific target DNA strand was successfully demonstrated, showing an integrated and automatic microfluidic device and providing a foundation of microfluidic DNA analysis. Another example was reported for performing reverse transcriptase polymerase chain reaction (RT-PCR) in microfluidic system [13]. It was shown to detect less than 50 β-actin transcripts from a total RNA template. A photograph of the microfluidic system is shown in Figure 1.5. The system was composed of three layers of PDMS bonded to a glass cover slip to construct valves and reactors. This work showed the capability of enabling highly parallel single-cell gene expression analysis. Moreover, DNA hybridization was showed to be accelerated by microfluidic technology [14]. Dynamic hybridization was achieved by local microfluidic vortexes generated by electrokinetic forces on a concentric circular microelectrode, shown in Figure 1.6a. The vortexes increased collision efficiency between target DNA strands suspended in solution and probe DNA strands

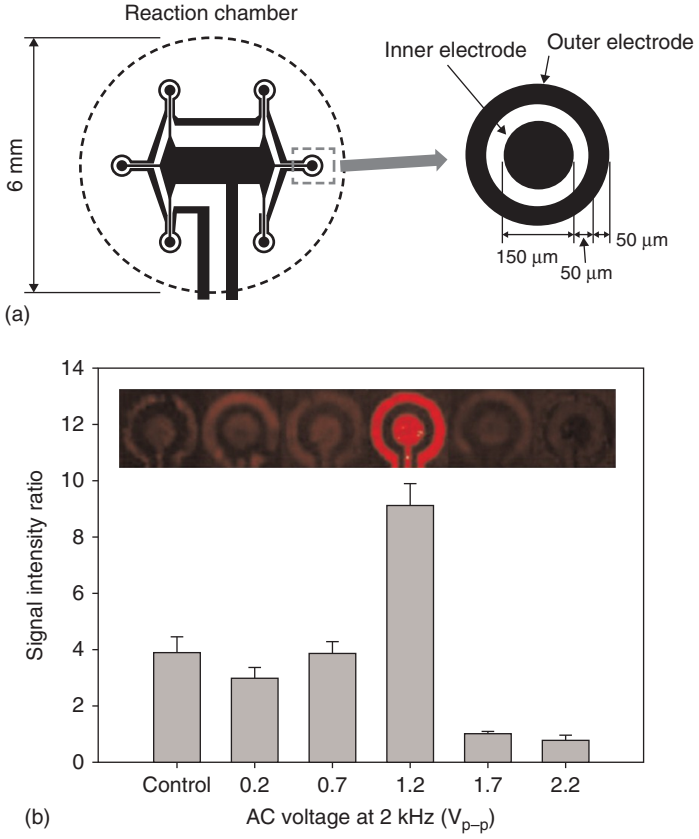

Figure 1.6 Electrokinetic acceleration of DNA hybridization. (a) Illustration of the concentric circular microelectrode for generating electrokinetic forces to achieve dynamic hybridization. (b) Results of 5-min dynamic hybridization represented by signal intensity ratio under the AC voltages at 2 kHz and different actuating voltages of 0.2, 0.7, 1.2, and 2.2 V_{p-p}. Control was 1-h static hybridization, that is, without applying electric signal. (Lei *et al*. 2015 [14]. Reproduced with permission of American Chemical Society.)

immobilized on the electrode surface. Results revealed that 5-min dynamic hybridization significantly increased the signal intensity ratio to over 1-h static hybridization, shown in Figure 1.6b. This study provided a strategy to accelerate DNA hybridization for practical rapid genetic diagnostic device.

Alternatively, digital microfluidics has been applied to the polymerase chain reaction (PCR) for potential point-of-care applications [49]. PCR in the droplets showed amplification efficiencies with no evaporation loss. The optimal hold time was found to be 9 and 30 s for denaturation and annealing/extension in thermal cycling, respectively. Droplet-based PCR can be monitored in real time and provides amplification with a cycle threshold of ~10 cycles earlier than benchtop instruments. Moreover, a digital microfluidic platform was developed for multiplexed real-time PCR [50], as shown in Figure 1.7. This system was demonstrated on the detection of DNA levels of methicillin-resistant *Staphylococcus aureus*, *Mycoplasma pneumoniae*, and *Candida albicans*. Recently, detection of Deoxyribonuclease I (DNase I) has been demonstrated by paper-based substrate using gold nanoparticle colorimetric probes [24]. In this

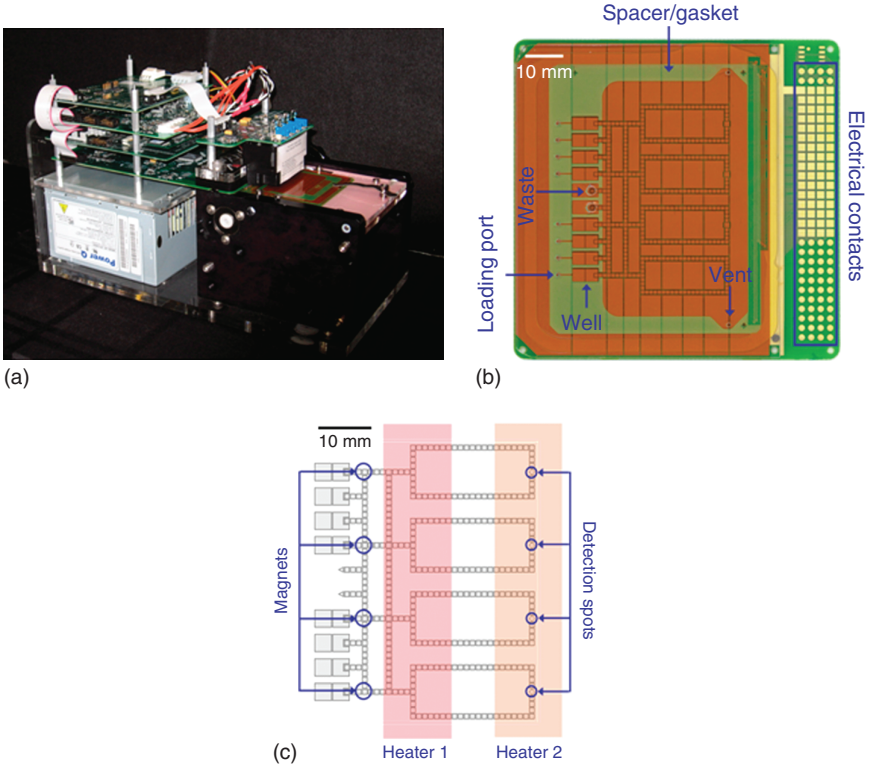

Figure 1.7 Self-contained digital microfluidic PCR system. (a) The instrument including power supply, control electronics, fluorometer module, heaters, and cartridge deck (shown with cartridge loaded). (b) Photograph of an assembled microfluidic cartridge comprising a printed circuit board chip, polymer spacer/gasket, and glass-top plate with drilled holes. (c) Schematic of the PCR chip showing electrode positions relative to heaters, magnets, and detectors. (Hua *et al*. 2010 [50]. Reproduced with permission of American Chemical Society.)

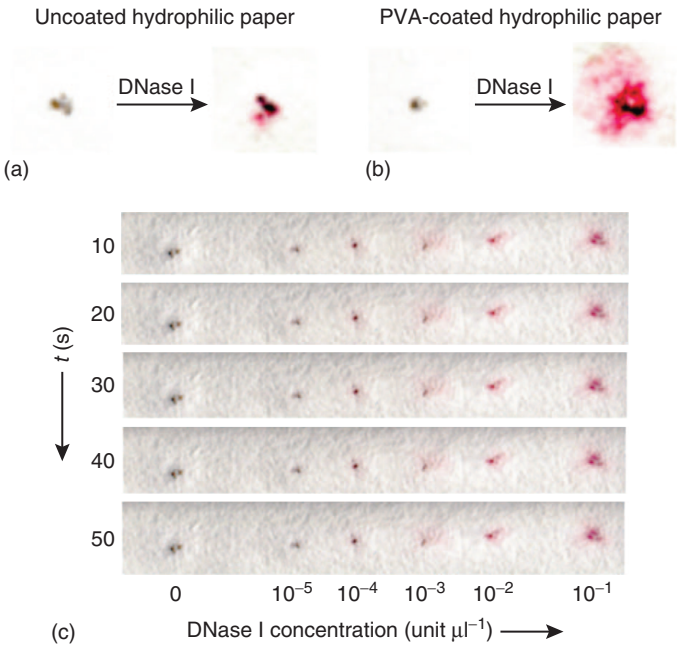

Figure 1.8 DNase I assay on (a) uncoated hydrophilic paper and (b) PVA-coated hydrophilic paper. One microliter of DNase I solution was applied in (a) and (b). Images were obtained at 20 s after adding DNase I solution. (c) DNase I assay on PVA-coated hydrophilic paper as functions of assay time and DNase I concentration. (Zhao *et al*. 2008 [24]. Reproduced with permission of American Chemical Society.)

work, colored and DNA-cross-linked gold nanoparticles aggregates were spotted on paper substrates. The addition of target DNase I solution dissociated the gold aggregates into dispersed gold nanoparticles, which generated an intense red color on paper within 1 min. Both hydrophobic and poly(vinyl alcohol) (PVA)-coated hydrophilic paper substrates were suitable for this biosensing platform and their results are shown in Figure 1.8. It was expected that it can provide a simple and practical bioassay platform for disease diagnostics, pathogen detection, and quality monitoring of food and water.

1.4.2 Applications of Immunoassays

Microfluidic immunoassays have also been intensively demonstrated on various disease detections [51–54]. Immunoassay is a bioanalytical technique for measuring the presence and concentration of antigen in biological liquid. It is widely used in clinical, pharmaceutical, and scientific research laboratories for diagnostics. Operation of immunoassay involves repeated steps of incubation and washing. Making conventional immunoassay using multi-well microplate is time-consuming and labor intensive. By introducing microfluidic technology, immunoassay can be automatically performed by sequentially pumping samples and reagents to the reaction chamber based on various microfluidic manipulation mechanisms in the microfluidic device. For example, pneumatic

micropumps were integrated in a microfluidic device to manipulate reagents for the detection of hepatitis C virus (HCV) and syphilis from serum samples [55]. Fluid manipulation was based on peristaltic effect driven by time-phased deflection of PDMS membranes along the fluidic channel. The detection process was automatic, and it began with bonding screening antigens, that is, HCV and syphilis, to the detection chambers. Then, sample, washing buffer, horseradish peroxidase (HRP)-labeled secondary antibody, developing buffer, and stopping buffer in individual reservoirs were sequentially pumped to the detection chambers by the "spider web" pneumatic micropumps. Immunoassay results were detected by the measurement of absorbance. This work showed a highly integrated microfluidic device and the immunoassay could be performed automatically. Alternatively, centrifugal force was utilized to demonstrate sequential manipulation of reagents in a compact disk (CD)-based microfluidic device [56, 57]. The CD-based microfluidic device for ELISA is shown in Figure 1.9. Because of the mature developments of precision rotation control and optical reading in CD technology, CD-based microfluidics was expected to have great commercial potential. Immunoassay was showed to be performed by controlling the rotational speed of the disk. Different solutions involved in the immunoassay process were sequentially and automatically manipulated by centrifugal force, demonstrating the analysis of rat IgG from a hybridoma cell culture. By using the CD-based microfluidics, less reagent consumption and shorter assay time were realized over the conventional method. Alternatively, ELISA has been demonstrated using paper substrate [30, 31]. Subtyping of influenza A (H1N1) and (H3N2) viruses was reported, and the detection limits of 2.7×10^3 and 2.7×10^4 pfu/assay for H1 and H3 detection could be achieved, respectively [31]. The use of paper for the development of diagnostic devices has the advantages of being lightweight, ease-to-use, and low cost, and paper-based immunoassay is appropriate to be applied for rapid screening in point-of-care applications.

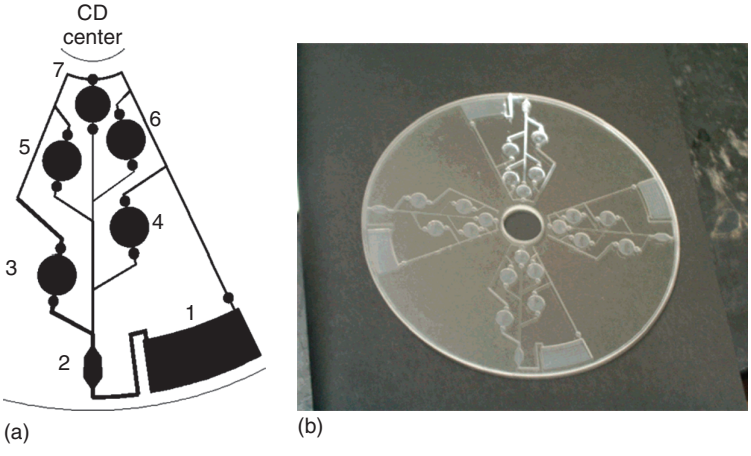

Figure 1.9 CD-based microfluidic device for the application of ELISA. (a) Schematic of five-step sequencing CD. (b) A computer numerical control-machined CD. (Lai *et al*. 2004 [56]. Reproduced with permission of American Chemical Society.)

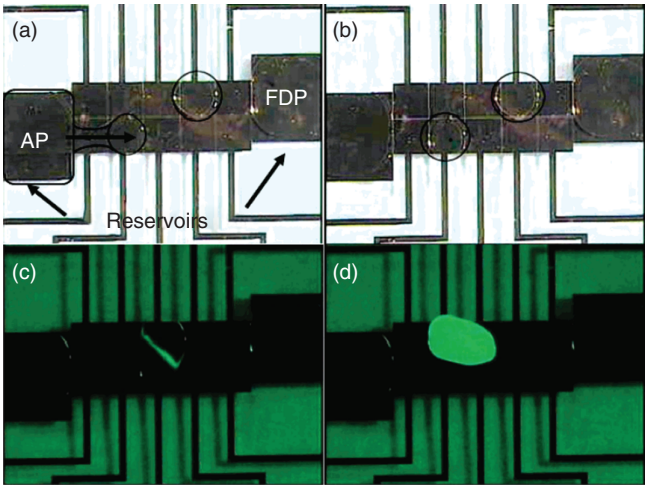

Figure 1.10 Fluorescent enzymatic assay on a digital microfluidic device. (a) A droplet containing fluorescein diphosphate (FDP) was dispensed from the reservoir on the right, while (b) a droplet of alkaline phosphatase (AP) was dispensed from the reservoir on the left. (c) When the droplets were merged under fluorescent illumination, the product was observed at the interface of the droplets. (d) After active mixing, the reaction proceeded to completion. (Miller and Wheeler 2008 [58]. Reproduced with permission of American Chemical Society.)

Furthermore, a microfluidic device based on electrowetting manipulation has been developed to perform multiplexed enzyme analysis [58]. Samples and reagents in the form of discrete droplets were manipulated on the device, as shown in Figure 1.10. Droplets of alkaline phosphatase and fluorescein diphosphate were merged and mixed on the device, and then the fluorescent product was detected by fluorescence plate reader. The detection limit achieved was $\sim 7.0 \times 10^{-20}$ M. Also, heterogeneous immunoassays have been demonstrated by efficient handling of magnetic microbeads using electrowetting manipulation [59]. A sample droplet and a reagent droplet containing magnetic beads conjugated to primary antibodies, blocking proteins, and secondary antibodies were dispensed on the system. These two droplets were then merged, mixed, and incubated by electrowetting manipulation. A permanent magnet was applied to immobilize the sandwiched microbead complexes, followed by the washing of the unbound components. Finally, a reagent droplet was applied for the chemiluminescent detection. Sandwich heterogeneous immunoassays on human insulin and interleukin-6 (IL-6) were demonstrated with a total time of 7 min to result for each assay.

1.4.3 Applications of Cell-Based Assays

Cell culture is a fundamental biological technique for various investigations such as study of physiology and chemistry of cells [60, 61] and cellular response under the exploration of tested substances [62, 63]. In conventional cell culture practice, cells are cultured in culture vessels, that is, Petri dish or multi-well microplate. During the culture course, culture medium is supplied manually and replaced

regularly. Although this operation is standardized and widely used today, it limits the throughput and possibility of automation. Because of the development of microfluidic technology, microfluidic cell culture devices have been developed and constructed a miniaturized cell culture environment [64–66]. An example of a microfluidic cell culture device composed of a 10×10 culture chamber array to demonstrate a high-throughput cell-based screening was shown [66]. Photographs of the microfluidic device are shown in Figure 1.11a. Mammalian HeLa cells were cultured in the well and grown nearly to confluency after 7.5 days, as shown in Figure 1.11b. A miniaturized perfusion cell culture environment was demonstrated, showing a promising evidence of microfluidic cell culture model. By using microfluidic technology for cell culture, there are several advantages such as providing a closed environment without the need of an incubator, monitoring cellular responses in a real-time manner, minimizing reagent consumption, and reducing the number of seeding cells.

The above discussions are based on a two-dimensional (2D) culture model where cells spread on a flat surface on a monolayer format. However, recent

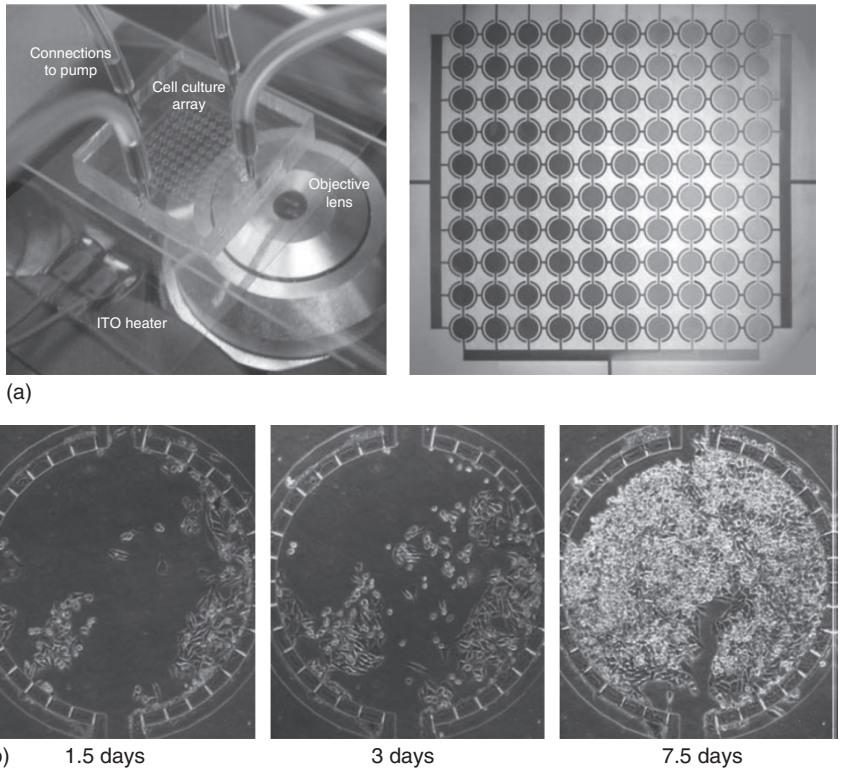

Figure 1.11 Microfluidic cell culture system. (a) A 10×10 culture chamber array on a microfluidic chip mounted on an optical microscope. (b) Cell growth inside a microfluidic cell culture chamber. Mammalian HeLa cells were cultured in the well and grown nearly to confluency at day 7.5. (Reprinted with permission from Ref. [66]. Copyright (2004) Royal Society of Chemistry.)

studies reported 2D culture models cannot well mimic the native cellular microenvironment because animal cells inhabit three-dimensional (3D) environment [67, 68]. Hence, 3D cell culture model in which cells are encapsulated and cultured in a 3D polymeric scaffold material was proposed [67, 68]. It is regarded as realizing a better approximation of *in vivo* conditions than 2D surfaces and providing a more physiologically meaningful culture condition for cell-based assays. Recently, impedimetric measurement of 3D cell culture was demonstrated by gelling a spot of cells–hydrogel mixture (1 μl) on planar electrodes [69]. On the other hand, a perfusion 3D cell culture microfluidic chip was developed to construct a precise, stable, and well-defined culture environment for 3D cell-based assays [70]. The microfluidic chip consisted of six 3D culture chambers, as shown in Figure 1.12a. A pair of vertical parallel electrodes located at the opposite sidewalls of the culture chamber was embedded for the on-site

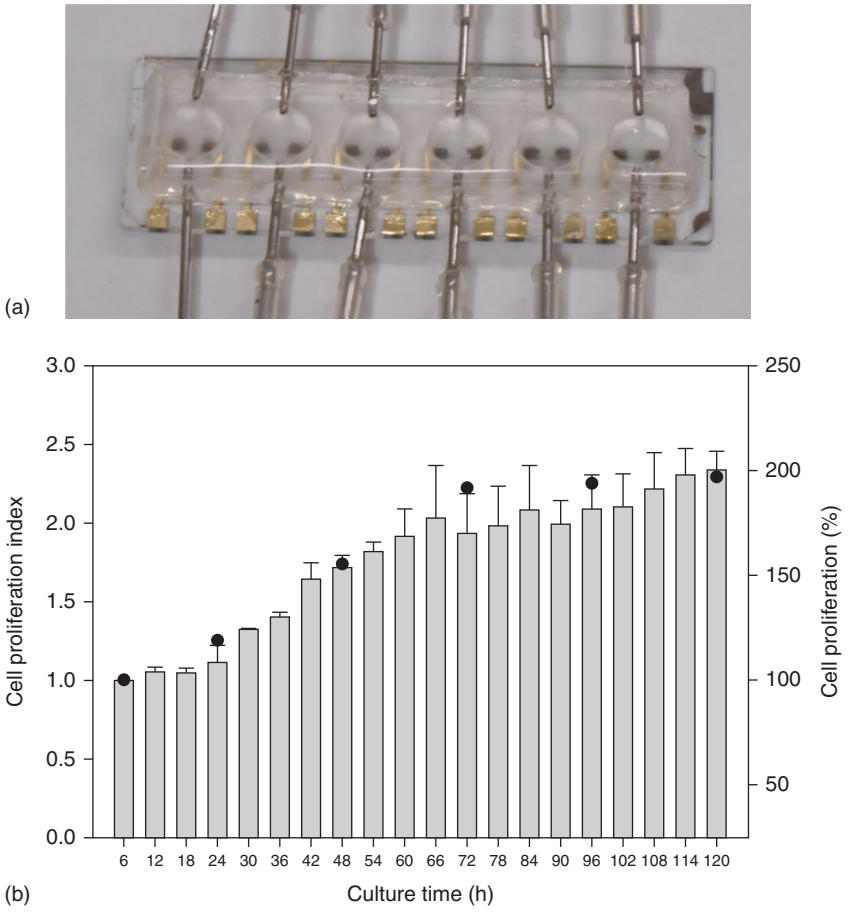

Figure 1.12 Real-time impedimetric monitoring of cell proliferation in a perfusion 3D cell culture microfluidic chip. (a) Photograph of the microfluidic chip. (b) Quantification of cell proliferation in 3D culture environment under medium perfusion for up to 5 days. (Lei *et al*. 2014 [70]. Reproduced with permission of Elsevier.)

impedance measurement. Cells encapsulated in the hydrogel were loaded into the chamber and could receive uniform electric field during the measurement. Real-time and noninvasive impedimetric monitoring of cell proliferation were demonstrated and is shown in Figure 1.12b. Quantification of cell proliferation could be realized in 3D culture environment. This microfluidic device has a high potential to develop an automatic and high-throughput platform for drug screening applications.

1.5 Perspective

The current development of microfluidic systems has been discussed and these systems have been demonstrated on various biomedical and chemical applications. These excellent works showed the mature development of microfluidic technology in research laboratories. Moreover, some of the research projects have been turned into commercial products. For example, a portable and user-friendly blood diagnostic equipment called Abbott i-STAT analyzer has been launched for clinical diagnostics. Only a few drops of blood are required for the blood analysis, and results are automatically uploaded to the patient's chart within minutes. Moreover, a commercial platform, that is, Advanced Liquid Logic, based on the technology of digital microfluidics was developed for gene and protein analysis. It is designed for life science research and provides cost-effective automation solutions for complex bioassay workflows. For microfluidic cell-based assays, benchtop equipment for real-time quantitative monitoring of cellular response has been commercialized for high-throughput drug screening applications. The equipment is named xCELLigence system, and its major advantage is to provide quantitative indexes to describe cellular responses during the culture course. However, these excellent products have not made great impact on the market. Most of the assays in clinical and research laboratories still rely on conventional equipment. It may be because these newly developed microfluidic products need to take time to compete with the existing equipment that have been perfected over the decades. But it is expected that more commercial microfluidic products will be launched in the near future.

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