Microfluidics technology for manipulation and analysis of biological cells
Changqing Yi, Cheuk-Wing Li, Shenglin Ji, Mengsu Yang*
Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong, China
Received 4 October 2005; received in revised form 7 December 2005; accepted 12 December 2005
Available online 25 January 2006

Abstract
Analysis of the profiles and dynamics of molecular components and sub-cellular structures in living cells using microfluidic devices has become a major branch of bioanalytical chemistry during the past decades. Microfluidic systems have shown unique advantages in performing analytical functions such as controlled transportation, immobilization, and manipulation of biological molecules and cells, as well as separation, mixing, and dilution of chemical reagents, which enables the analysis of intracellular parameters and detection of cell metabolites, even on a single-cell level.
This article provides an in-depth review on the applications of microfluidic devices for cell-based assays in recent years (2002–2005). Various cell manipulation methods for microfluidic applications, based on magnetic, optical, mechanical, and electrical principles, are described with selected examples of microfluidic devices for cell-based analysis. Microfluidic devices for cell treatment, including cell lysis, cell culture, and cell electroporation, are surveyed and their unique features are introduced. Special attention is devoted to a number of microfluidic devices for cell-based assays, including micro cytometer, microfluidic chemical cytometry, biochemical sensing chip, and whole cell sensing chip.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Microfluidic devices; Lab-on-a-chip; Miniaturized total analysis system; Biochip; Cell analysis; Cell manipulation

Contents
1. Introduction ............................................................................................................... 2
2. Cell manipulation ......................................................................................................... 2
  2.1. Magnetic manipulation .............................................................................................. 2
  2.2. Optical manipulation ................................................................................................ 3
  2.3. Mechanical manipulation ............................................................................................ 4
  2.4. Electrical manipulation .............................................................................................. 6
  2.5. Other manipulation .................................................................................................. 7
3. Cell treatment ............................................................................................................. 7
  3.1. Cell lysis ........................................................................................................... 7
  3.2. Cell culture ......................................................................................................... 8
  3.3. Electroporation, electrosorption, and optoporation .................................................... 10
4. Cell analysis ............................................................................................................. 11
  4.1. Micro cytometer ................................................................................................... 11
  4.2. Chemical cytometry ............................................................................................... 11
  4.3. Biochemical sensing .............................................................................................. 14
  4.4. Electrical characterization and ion channel studies ..................................................... 17
  4.5. Whole cell assay .................................................................................................. 18
5. Concluding remarks ................................................................................................. 19
Acknowledgement ....................................................................................................... 20
Reference ............................................................................................................... 20
1. Introduction

Micro total analysis systems (μTAS), also called ‘lab-on-a-chip’, integrate analytical processes for sequential operations like sampling, sample pre-treatment, analytical separation, chemical reaction, analyte detection, and data analysis in a single microfluidic device. Microfluidic-based research has made significant advances over the last few years, and become very much a ‘hot topic’ recently. Because of their advantages including low reagent and power consumption, short reaction time, portability for in situ use, low cost, versatility in design, and potentials for parallel operation and for integration with other miniaturized devices, microfluidic chip-based systems for biological cell studies have attracted significant attention. Microfluidic technique has started to play an increasingly important role in discoveries in cell biology, neurobiology, pharmacology, and tissue engineering.

As cell-based assay is deemed to be essential for the functional characterization and detection of drugs, pathogens, toxicants, and odorants, several review articles concerning microfluidics for cell analysis have been published during the past few years. Berg and Andersson [1] presented a comprehensive review of microfluidics for cellomics, which covered the microfluidic devices for cell sampling, cell trapping and sorting, cell treatment, and cell analysis. Erickson and Li et al. [2] focused on integrated microfluidic devices for cell handling and cytometry, dielectrophoretic cellular manipulation and sorting, and general cellular analysis. Manz and co-workers [3–5] presented a series of comprehensive reviews concentrated on micro total analysis systems which covered development history and theory of miniaturization [3], fabrication of microfluidic systems [3,4], and microfluidic standard operations including sample preparation, sample injection, sample manipulation, reaction, separation, and detection [4,5]. Microfluidic-based biological applications such as cell culture, PCR, DNA separation, DNA sequencing, and clinical diagnostics were also reviewed [4,5]. Optical manipulation of cells in microfluidic devices, including the parallel manipulation of cells using optical tweezers, optical switching of cells in fluidic channels and optical handle had been reviewed by Orkan et al. [6]. Whitesides and co-workers [7] emphasized on the advantages of using PDMS for microfluidic bioassays, including cell sorting by flow cytometry and magnetic sorting on PDMS-based microfluidics. Recently, microfluidics for cell culture, flow cytometers, and other microscale flow-based cell analysis systems was reviewed [8,9], where cell detection and enumeration systems and microfluidic fluorescence-activated cell sorting systems were detailedly described [9]. In the review article presented by Toner and Ilrimia [10], principles for manipulating blood cells at micron scale and high-throughput approaches to blood cell separation using microdevices was introduced.

There are a large number of newly reported microfluidic devices for cell research in last 3 years. The article intends to provide an in-depth look at the applications of microfluidic devices for cell analysis in recent years (2002–2005). According to the different functions of microfluidic devices, this review is divided into three main sections, covering cell manipulation, cell treatment, and cell analysis. Based on different physical forces employed, microfluidic devices for cell manipulation can be categorized into magnetic manipulation, optical manipulation, mechanical manipulation, and electrical manipulation. Selected examples for each manipulation method are described in detail and compared to each other to reveal their advantages and disadvantages. Microfluidic devices for cell treatment, which includes cell lysis, cell culture and cell electrophoration, electrofusion, and optoporation, are also surveyed. Special attention is devoted to a number of microfluidic devices for cell-based analysis which can be further categorized into micro cytometry, chemical cytometry, biochemical sensing, and whole cell sensing, according to their different applications. Based on these reviews, an outlook of this promising and rapidly expanding area will be presented in the concluding remarks.

2. Cell manipulation

Fabrication of miniaturized cell manipulator is the first step towards microfluidics for cell-based assays. The commonly used methods for cell manipulation on chip can be categorized based on the manipulating force employed. In the following sections, a few selected microfluidic devices employing different forces for cell manipulation are described.

2.1. Magnetic manipulation

Magnetic manipulation, in which magnetic particles are selectively attached to cells, is a commonly used method for cell separation or purification in microfluidic device. The commonly used super-paramagnetic beads are 10–100 nm in diameter, whose extremely small size makes them very gentle on the cells with apparently no effect on the cellular function and cell viability. The magnetic field gradients generated by various methods can be employed to capture the bead and, consequently, cell attached to the bead, because of the magnetic anisotropy inherent in the paramagnetic beads. The high specificity and efficiency of magnetic methods make them quite useful in obtaining rare cell types, especially in handling red blood cells [10]. During the last few years, there are several research reports concentrating on this method [11–16]. Berger et al. [11] developed a magnetic sorting device which used asymmetry in obstacles and Brownian motion to affect separation of objects. Fig. 1 A shows the basic schematic of the microdevice: ferromagnetic wires are countersunk into the bottom of the central chamber at a 45° angle to the flow of cells and more than 350 channels feed into nine outlets where unlabeled cells will flow into the central outlet. In this microdevice, cells are forced to move along the magnetic wires because the high magnetic field gradient at the edge of each wire imposes a force on the superparamagnetic beads at an angle to the hydrodynamic force (illustrated in Fig. 1B) [11]. Westervelt and co-workers [12] combined microfluidic device with microelectromagnetic matrix to develop various methods for manipulating biological cells. The magnetic field generated by the matrix can stably control individual cells in fluid and can be configured dynamically to meet a variety of experimental needs. Furdui and Harrison [13] comparatively evaluated open
Fig. 1. (A) The basic schematic of the microfabricated cell fractionation chip. Inset: SEM image of magnetic wires countersunk into the floor of the central chamber. (B) The magnetic force separation idea. High magnetic field gradients provide forces at an angle to the flow of cells. Reproduced with the permission from [11], © 2001 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

tubular capture beds with magnetically trapped bead for the trapping and enrichment of relatively rare cells in blood samples. The magnetic bead bed approach provides more capture surface area and places cells and surfaces in closer proximity, and allows the cells to be readily released again after washing for further sample processing. An integrated microfluidic device consisting of a chaotic mixer, an incubation channel, and a capture channel was developed for magnetically separating \textit{E. coli} with the capture efficiency of 53 and 37\% from PBS and whole blood, respectively [14]. Inglis et al. [15] incorporated an array of microfabricated magnetic stripes into a microfluidic device to achieve cell by cell separation using super-paramagnetic nanoparticles. Frazier and co-workers [16] achieved magnetophoretic separation of red and white blood cells from whole blood based on their native magnetic properties. No additives such as magnetic tagging or inducing agents were needed for this separation. Magnetic method is a clean, versatile, and non-invasive method for cell manipulation in microfluidics. With the generation of new magnetic bead materials and modification methods for conjugating various ligands to magnetic beads, it is believed that the magnetic manipulation method will become more efficient and easily integrated with microfluidics for cell-based assay.

2.2. Optical manipulation

Recently, there is an increasing interest in optical manipulation of biological species on microfluidic device due to its non-contact and contamination-free manipulation process. It is well known that optical tweezers could tether biological molecules to dielectric spheres and then capture the spheres at the focal point of an electric field gradient. Due to its inherent advantages, optical tweezers can be easily combined with microfluidic systems and is the most commonly used optical manipulation method in microfluidic devices. Umehara et al. [17] employed optical tweezers to position bacteria in a single-cell microcultivation assay where single bacterial cells could be isolated and positioned in microchambers. In this way, the reproducibility of genetically identical bacteria can be studied by flowing different nutrients through the microfluidic systems. Hanstorp and co-workers [18] developed a microfluidic system which employed optical tweezers to manipulate cells without the media being dragged along with them within seconds, and evaluated it by exposing \textit{E. coli} to different fluorescent markers. Optical tweezers offer high resolution for single cells trapping, but have limited manipulation area owing to tight focusing requirements [6]. In order to improve the throughput, Flynn et al. [19,20] demonstrated the use of individually addressable vertical cavity surface emitting lasers (VCSEL), where each laser in the array was focused and acted as an individual trapping and manipulation source, as illustrated in Fig. 2, for optical trapping and active manipulation of living cells and microspheres. The high device density and compactness achievable with VCSEL arrays make them suitable for optical manipulation applications, where individual high-throughput manipulation of cells is necessary [19,20]. However, VCSELs have a relatively low output power, and hence a lower trapping strength [6]. Recently, “lab on a microscope”, which uses light intensity pattern to manipulate cells, has been presented. Wu and co-workers [21] developed optoelectronic tweezers (OET), which utilized direct optical image to create high-resolution dielectrophoresis
Fig. 2. VCSEL array optical tweezers for the parallel transport of samples on a chip. Reproduced with the permission from [6], © 2003 Kluwer Academic Publishers.

(DEP) electrodes for parallel manipulation of single particles, to achieve high resolution and high throughput at the same time. As illustrated in Fig. 3, an upper ITO-coated glass and a lower photoconductive surface are biased with an ac signal, and liquid containing cells of interest is sandwiched between them. When projected light illuminates the photoconductive layer, the virtual electrodes are created, and consequently forming non-uniform electric fields which enable particle manipulation via DEP forces. MacDonald et al. [22] exploited the optical polarizability of particles with an extended, three-dimensional optical lattice for on-chip cell sorting and achieved the sorting efficiency of \( \sim 100\% \). Though optical manipulation can provide various advantages, its applications in microfluidics for cell-based assay are still limited due to the complicated optical setup, complex operation, and expensive instrumentation.

2.3. Mechanical manipulation

Due to the complex physical properties of biological cells, manipulating cells mechanically on a microfluidic chip poses certain challenges. Separation of target cells on microfluidic structures for culture and assay purposes is the main application of on-chip mechanical manipulation. Separation of cells within the microchannels can be achieved through fabricating constriction structures such as microfilters [23–26], microwells [27–29], microgripper [30], dam structure [31,32], and sandbag structure [33], or modifying microchannel interior surface with reactive coatings [34–36], with antibodies [37,38], with selectin [39] and with enzymes [40].

Size-dependent filter-based microfluidic devices have been fabricated to concentrate microbial cells for immunofluorescent assay. Target cells with a fluorescent signal-to-noise ratio of 12 could be observed at single-cell level using a 10–100 time more dilute staining solution than conventional concentration within 2–5 min [23]. Four successively narrower regions of channels with microfilters (as shown in Fig. 4A) were constructed to separate cultured neuroblastoma cells when mixed with whole blood [24]. Moorthy and Beebe [25] developed a microfluidic device equipped with porous filters inside a microchannel to separate the blood cells from serum in real-time, without the need of an external power source. The efficiency of cell/serum separation by the porous filter was found to be comparable to separation by centrifuge. Sturm and co-workers [26] reported a microfluidic particle-separation device that made use of the asymmetric bifurcation of laminar flow around obstacles. As the gap size is reduced using nanofabrication, this device can be used to fractionate biological supramacromolecules, such as viral particles and protein complexes. Langer and co-workers [27] used a two-step process to fabricate poly(ethylene glycol) (PEG) microwells (as shown in Fig. 4B) within microchannels which could be employed to dock cells within pre-defined locations. Lee and co-workers [30] developed a microfluidic device with a SU-8-based microgripper (as shown in Fig. 4C) that could manipulate single cells in physiologic ionic solutions and hold a cell without exerting any forces in the closed position. A microfluidic device with a dam structure in parallel to the fluid flow was constructed for docking and alignment of biological cells [31]. The structure is composed of two parallel channels separated by a dam, where the height of the dam is smaller than the vertical height of the channels (as shown in Fig. 4D). The structure allows cells to move in microfluidic channels and dock in desired locations with controllable number. Cells docked on the parallel dam structure are exposed to minimal stress caused by fluidic pressure [31]. A comprehensive hydrodynamic analysis of two different types of dam structures for cell immobilization were carried out to compare the effects of dam dimensions.
and cell docking on the pressure and velocity of the flow in the microchannels as well as the hydrodynamic force and shear stress on the docked cells. The results show that the parallel dam provides a better structure for cell docking than the perpendicular dam and the hydrodynamic force and shear stress on the cells can be decreased by adjusting the pressure in the auxiliary channel of the parallel dam without significantly affecting the flow velocity and transportation efficiency [32]. Sandbag structures (as shown in Fig. 4E) were also developed to immobilize cells using single step photolithography and it was demonstrated that multi-height sandbag structure could be applied for cellular analysis using immunocytochemical staining assays [33].

Langer and co-workers [34] developed polymer-based microfluidic devices with submicrometer thin reactive coatings deposited on the interior surface to provide defined and chemically reactive interfaces for biomolecules immobilization. Modification of cell adhesion on microchannels can also be achieved by coating silica microchannel surface with polyacrylamide films [35] and octadecyltrimethoxysilane (OTMS) or N-(triethoxysilylpropyl)-O-polyethylene oxide urethane (TESP) [36]. Microfluidic cell-affinity chromatography (CAC) system uses surface-immobilized antibodies for cell separation. Toner and co-workers [37] achieved the separation of highly pure populations of T and B lymphocytes from mixtures without preprocessing incubation using microfluidic chambers coated with antibodies. Based on the effects of flow and surface conditions on the cell separation, Toner and co-workers [38] developed a cytometry platform which could create high-density leukocyte arrays for rapid optical characterization. Laser-mediated sorting of cells was also demonstrated by retrieving single selected T-lymphocytes from the cell array using laser capture microdissection (LCM) technology. Chang et al. [39] utilized selectin-based transient cell adhesions to capture, enrich, and even fractionate different types of cells in a microstructured fluidic channel. Payne and co-workers [40] reported an enzyme-based method to in situ entrap, grow, and release cells under mild conditions within a biopolymeric hydrogel matrix. Size-dependent filter-based microfluidic devices exhibit numerous advantages, such as high labeling efficiency, short detection time, high reproducibility based on simple and robust experimental procedures, high detection sensitivity at a whole cell level. However, their applications for cell-based assay are limited by their poor selectivity. Other constriction structures also face the same challenge. Microgripper can precisely manipulate single cell without any damage, but the complicated fabrication procedures make it difficult to be applicable in common biology and chemistry labs. Oppositely, dam and sandbag structures can be easily fabricated using various methods and can also efficiently immobilize cells with minimal stress on them. Dam and sandbag structures may have more and more applications in future microfluidic cell analysis. Although the coating method which use cell adhesion to capture cells suffers from relatively low immobilization efficiency, its high selectivity and
easily modifying microchannel interior surface still make it a promising method for developing microfluidic devices for cell-based assay.

2.4. Electrical manipulation

Dielectrophoresis (DEP) is the electronic analog of optical tweezers [17,18] and is defined as the lateral motion imparted on uncharged particles as a result of polarization induced by non-uniform electric fields. Scheme of two types of microfluidic DEP trap is shown in Fig. 5. The standard way to make a DEP trap is to create an electric field gradient with an arrangement of planar metallic electrodes (Fig. 5A) either directly connected to a voltage source or free-floating in presence of an ac field [41]. One advantage of DEP is that it can be scaled for parallel electrical manipulation of micro- and even nano-sized objects such as cells, DNA, proteins, nanoparticles, and possibly single molecules in aqueous solutions. Alternatively, a constriction or channel in an insulating material can also be employed to squeeze the electric field in a conducting solution. This electrodeless DEP (EDEP) (as shown in Fig. 5B and C) provides various advantages over DEP, such as a very high electric field may be applied without gas evolution as well as a huge increase in the dielectric response at low frequencies (below 1 kHz) [41]. DEP has been successfully applied on microfluidic devices to manipulate a variety of biological cells, such as bacterial, yeast, and mammalian cells [42–50].

Durr et al. [42] used a microfluidic chip with 3D arrays of microelectrodes embedded in microchannels to study the influence of channel height, particle size, buffer composition, strength, and frequency of electric field on the dielectrophoretic force, and also the effectiveness of dielectrophoretic deflection structures. Huang et al. [43] demonstrated the potential of DEP for accurate genetic analysis of specific cell subpopulations in heterogeneous samples. Voldman et al. [44] developed a microfluidic cytometer where DEP was employed to confine cells and hold them against disrupting fluid flows. The micro cytometer consisted of a cell array chip, an optical system luminescently interrogating cells, and a control system that implemented sorting function. Cell array chip was composed of a regular array of non-contact single-cell traps (as shown in Fig. 6B and C) which was designed with asymmetric extruded quadrupole geometry and electrically addressable. Schematic representation of operation is depicted in Fig. 6A. Switching the potential on the controlling electrode from +V to −V disrupts the quadrupolar potential energy by removing the electric field cage, thus ejecting the cell. Particles can be more strongly confined with maximized holding while imposed with minimized shear stress using the extruded quadrupole structure. And the trapping efficiency can be increased significantly using asymmetrical trapezoidal arrangement of the posts, because it lowers the potential energy barrier of loading the trap without affecting the trap strength. Chou et al. [45] demonstrated the trapping of E. coli and its separation from blood cells by electrodeless dielectrophoretic trapping fabricated in an insulation substrate composed of geometrical constrictions. Arai and co-workers [46] developed a system for separation of a single micro-organism from a large number of microbes in microfluidic device by using both a laser-trapping force and a dielectrophoretic force. A linear traveling wave dielectrophoretic (twDEP) microchip was developed by Cui et al. [47] for cell separation. Viable cells can be separated from heat-treated Listeria innocua cells on micro-
fabricated devices with interdigitated electrodes by making use of differences in dielectric properties of cells [48]. Recently, a specially shaped non-uniform electric field could be generated by microfabricated planar microelectrode arrays on an insulating glass, and it could be employed to develop a DEP microfluidic system which could fractionate intact biological cells in suspension into subpopulations [49]. Taking advantage of aligned top and bottom electrodes in channels, Seger et al. [50] employed deflective dielectrophoretic barriers for accurately handling of cells under controlled pressure-driven liquid flows.

DEP which uses an ac electric field to separate target cells from cell samples in a flow stream has been demonstrated to be quite effective and selective in concentrating, manipulating, and separating cells and bacteria with different sizes simultaneously. In the meantime, DEP suffers from low survival rate of biological cells in electrical field and complicated instrumentation. While, with the development of microfabrication method and appearance of newly available electrode geometries for DEP, DEP manipulation in microfluidics for cell-based assay will have more applications in microfluidics.

2.5. Other manipulation

Minerick et al. [51] delineated various fundamental driving forces in microfluidic devices with parallel and orthogonal electrode configurations by examining the motion of a suspension of erythrocytes in response to a high-frequency ac field. The results show that electrode repulsion was so far the strongest driving force in an ac field (far larger than DEP forces) and the orthogonal electrode configurations could provide stronger driving force than parallel electrode configurations. Soper and co-workers [52] studied electrokinetic cell manipulation by means of electroosmotic flow in microfluidic devices fabricated in pristine and UV-modified poly(methyl methacrylate) (PMMA) and polycarbonate (PC). Their results demonstrated the feasibility of tailoring the electroosmotic flow by changing the ionic strength of the buffer and modifying the polymer material through UV exposure [52]. Ultrasound standing wave radiation force and laminar flow have been employed for cell washing and mixing by a continuous field-flow fractionation (FFF) approach. When operating at the flow rate of 10.2 ml/min, typical transit time through the sound field was less than 0.5 s, which was significantly shorter than that required by a centrifuge [53]. Yoshida et al. [54] described a methodology for manipulation and navigation of selected single living cells to a desired location on a patterned bio-microelectromechanical system (bio-MEMS) chip that is applicable to rapid and precise formation of cell pattern.

3. Cell treatment

It is crucial to integrate cell treatment steps on chip to develop microfluidic devices for cell analysis. In order to achieve analysis of cell constituents, cell lysis is absolutely necessary. When carrying out in vitro experiments, cell culture on chip is indispensable. Besides cell lysis and cell culture, achieving on chip cell electroporation and optoporation and cell fusion is also a hot topic in microfluidics. In the following section, the basic concept of cell lysis, cell culture, cell electroporation and optoporation, and cell fusion devices are presented with selected examples.

3.1. Cell lysis

The ability of integrating the lysis of cells with analysis of their contents would greatly increase the power and portability of many microfluidic devices [1]. The choice of lysis protocol is important for the performance of subsequent assay. Several methods of cell lysis, such as thermal lysis [55], electrical lysis [56], mechanical lysis [57–60] as well as chemical lysis [61–64] have been successfully applied into microfluidic systems. Because thermal lysis may result in denaturation of proteins [59], few researchers employed this method these years. As almost all electrical lysis components in microfluidic systems are similar for cell-based assay, this lysis method will not
be discussed in this subsection. Chemical and mechanical lysis principles will be presented in detail with selected examples below.

For mechanical-based lysis, nanostructured filter-like contractions in microfluidic channels with pressure-driven cell flow are employed. Prinz et al. [57] utilized diffusive mixing to lyse E. coli cells and trapped the released chromosomal by means of DEP in an integrated microfluidic chip. Lysis of the cells was achieved by rapid diffusional mixing of water with the osmotically stressed cells. Madou and co-workers [58] developed a microfluidic compact disc (CD) platform using spherical particles for mechanical lysis of cells with a lysis efficiency of approximately 65%. The rimming flow established inside a partially solid-liquid mixture-filled annular chamber when the microfluidic CD rotated around a horizontal axis of rotation could be employed for cell lysis. Lee and co-workers [59] fabricated nanoscale-barbs in microfluidic devices for mechanical cell lysis. It is shear and frictional forces induced by subjecting cells to enter microfluidic filter region with sharp nanoscale barbs that could be utilized to lyse cells. At a flow rate of 300 ml/min within the filter region total protein and hemoglobin accessibilities of 4.8 and 7.5% were observed, respectively, as compared to 1.9 and 3.2% for a filter without nanstructured barbs. Taylor et al. [60] demonstrated cell disruptions as compared to 1.9 and 3.2% for a filter without nanostructured barbs. Taylor et al. [60] demonstrated cell disruptions and the bioavailability of 4.8 and 7.5% were observed, respectively, as compared to 1.9 and 3.2% for a filter without nanstructured barbs. Taylor et al. [60] demonstrated cell disruptions and the bioavailability of 4.8 and 7.5% were observed, respectively, as compared to 1.9 and 3.2% for a filter without nanstructured barbs.

Chemical lysis uses non-ionic, less denaturing detergents delivered from reservoirs or generated on chip for cell lysis. A microfluidic lysis device that deals with erythrocytes and up to 100% recovery of leukocytes has been achieved within 40s [64]. Single-cell capture and chemical lysis inside a 50-pl closed volume was demonstrated in a microfluidic device designed by Toner and co-workers [62]. In this device, cells and fluids were independently isolated in two microchambers of 25-pl volumes using the geometry of the microchannels and the coordinated action of four on-chip thermopneumatic actuators [62]. Beebe and co-workers [63] demonstrated the removal of zona pellucida from mammalian embryos in a PDMS microfluidic chip using chemical treatments. Zona removal was achieved by briefly washing with lysing agent (acid Tyrode’s medium) over the embryo. A microfluidic chip was developed for on-chip cell lysis based on local hydroxide electro-generation and evaluated by lysis of red blood cells (RBCs), human tumor line (HeLa), and Chinese hamster ovary (CHO) cell lines [64].

Because extensive experiences and well-established protocols for large-scale samples are available, chemical-based lysis can be easily integrated with microfluidics. However, in order to achieve efficient deliveries of lysis reagents or on-chip generation of lysis reagents, more microfluidic components should be fabricated, that will increase the complexity of the micro-devices in a way. Mechanical-based lysis also faces the same challenge. Though not discussed in this section, electrical-based lysis is most widely used in microfluidic cell lysis due to its easy integration and simple structure.

3.2. Cell culture

Microfluidic devices are especially suitable for biological applications, particularly on cellular level, because scale of channels is commensurate with that of cells [65], and scale of the devices allows important factors (e.g., growth factors) to accumulate locally forming a stable microenvironment for cell culture [66]. Compared to traditional culture tools, microfluidic platforms provide much greater control over cell microenvironment and rapid optimization of media composition using relatively small numbers of cells. Because a group of cells can more easily maintain a local microenvironment within microchannels than in macroscale culture flasks, cells grow significantly slower in microchannels than cells in traditional culture flasks [67]. Microfluidic devices have been fabricated with three different materials (silicon, PDMS, and borosilicate) and tested for embryo culture. Results showed that using microfluidic technology for in vitro production of mammalian embryos has great potentials [68]. And the results from the perfusion culture of fetal human hepatocytes (FHHs) in microfluidic bioreactors addressed the efficacy of such microstructures in future liver tissue engineering [69]. In this subsection, selected examples of microfluidic devices for cell culture will be reviewed.

PDMS has become one of the materials extensively used in microfluidic devices, due to its biocompatibility, low toxicity, high oxidative and thermal stability, optically transparent, low permeability to water, and low electrical conductivity, further more, it could be easily fabricated into microstructures using soft-lithography. The influence of PDMS composition on the attachment and growth of several different types of cells were investigated thoroughly by Whitesides and co-workers [70]. Their results showed that the ability of different PDMS surfaces for cell attachment, growth and proliferation was cell-type dependent. No significant effect on cell growth for all cell types could be observed when varying the composition of normal PDMS by excess base, and cells were able to reach confluence on these surfaces. PDMS having excess curing agent caused differences in the growth of HUAEC and HeLa cells, but did not affect the growth of 3T3 fibroblasts and MC3T3-E1 cells. And the stiffness of substrate did not influence attachment and proliferation for all cell types [70]. Patterned multiple laminar flows of etching solutions in capillaries was employed by Whitesides and co-workers [71] to achieve easy adjusting topographical features in PDMS for mammalian cell culture. Hung et al. [72] presented a high aspect ratio PDMS microfluidic device for culturing cells inside an array of microchambers with continuous perfusion of medium. The high aspect ratio (~20) between the microchamber and the perfusion channels offers advantages such as localization of cells inside the microchamber as well as creation of a uniform microenvironment for cell growth. Taylor et al. [73] reported a microdevice for direct growth of neurites and their isolation from cell bodies. This PDMS microchip consisted of two compartments separated by a physical barrier. Neurites could grow across compartments while fluidic isolation was maintained due to a number of micrometer-size grooves embedded in them. Basing on the low diffusion properties of laminar flows in microchannels, a PDMS microfluidic device consisting of an array of...
C. Yi et al. / Analytica Chimica Acta 560 (2006) 1–23

microinjectors and a base-flow channel could be employed to achieve localized drug application to cell cultures [74]. Ion and co-workers [75] developed a plasma-based dry etching method that enabled patterned cell culture inside microfluidic devices by allowing patterning, fluidic bonding, and sterilization to be carried out in one single step.

To achieve the goal of long-term cell culture and culturing cells up to higher density and larger numbers, continuous nutrition and oxygen supply, and waste removal through the culture medium have to be ensured. Takayama and co-workers [76] described use of horizontally oriented mini reservoirs arrays as a gravity-driven pumping system to generate multiple fluid streams inside microfluidic cell culture channels at a constant flow rate for prolonged periods. Matharbiz et al. [77] presented a microfabricated electrolytic oxygen generator for high-density miniature cell culture arrays. Long term (over 2 weeks) cultures of muscle cells spanning the whole process of differentiation from myoblasts to myotubes has been reported by Folch and co-workers [78].

Fig. 7 shows the schematics of the microfluidic system. This microdevice could provide accurate control of perfusion rates using a gravitational flow and biochemical composition of the environment surrounding cells. Prokop et al. [79] developed NanoLiter BioReactors (NBRs) for long-term culture and maintaining up to several hundred cultured mammalian cells in volumes three orders of magnitude smaller than those in standard multi-well screening plates. Refreshable Braille display-based microfluidic bioreactors, which were more densely packed and not limited to linear and unidirectional perfusion, were developed for cell culture up to 3 weeks under perfusion [80]. Yasuda and co-workers [81] developed a type of on-chip microcultivation chamber, which could directly measure the valve opening/closing by optical microscope, for long-term cultivation of swimming cells. Fujii and co-workers [82] achieved large scale cells (up to $10^7$ cells/cm$^3$) culture in a microfluidic device with a multilayer bioreactor containing an oxygen supply system. Lee and co-workers [83] presented a microfluidic cell culture array which could assay 100 different cell-based experiments in parallel for long-term cellular monitoring. Repeated cell growth/passage cycles, reagent introduction, and real-time optical analysis could all be achieved in this microdevice.

In order to improve the efficiency of cell culture, some approaches such as fabricating three-dimensional microstructures [84–86] and attempting other biocompatible materials [87,88] are presented. Yasuda and co-workers developed a method which made use of non-contact 3D photo-thermal etch-

Fig. 7. Schematics and operation of the microfluidic device. (A) The short-term microfluidic perfusion network (ST-$\mu$FN) is shown in gray and the long-term microfluidic perfusion network (LT-$\mu$FN) is shown in black. (B) Schematics of the fragment (boxed in (A)) of the master mold showing two layers: Layer I is shown in dark gray and Layer II is shown in light gray. (C and D) Two modes of operation of the device: (C) medium is gravity-fed through the LT-$\mu$FN from inlet “1” to “2” to maintain a viable cell culture; “3–5” are inlets and “6” is the outlet for the ST-$\mu$FN; (D) hydrodynamic focusing in the device: a central stream carrying a soluble factor gets “squeezed” by two side streams; arrows indicate the direction of the flows. Reprinted with the permission from [78], © 2005 Royal Society of Chemistry.
ing with a 1480 nm [84] or 1064 nm [85] infrared focused laser beam to form shapes of agar microstructures for cultivating cells. Desai and co-workers [86] fabricated a 3D heterogeneous multilayer tissue-like structure inside microchannels for cell culture. Single- and multi-stepwise microstructures were fabricated in photosensitive biodegradable polymers poly-(ε-caprolactone (CL)-nt.-lactide (LA))-tetraacrylate for static cell culture of Hep G2 cells and fetal human hepatocyte (FHH) cells [87,88].

Other approaches for microfluidic cell culture were also studied. Cell cultivation was performed in a highly parallelized manner in fluid segments that were formed as droplets at a channel junction. Organic and cell containing aqueous phase were merged at channel junctions [89]. Multichannel microelectrode array which could influence and record electrical cellular activity was integrated into the microfluidics for cell culture [90]. A gradient-generating microfluidic platform for optimizing proliferation and differentiation of neural stem cells in culture was described by Jeon and co-workers [91].

### 3.3. Electroporation, electrofusion, and optoporation

Introduction of hydrophilic or membrane-impermeant molecules into cells is crucial for various cell-based assays. A large number of methods have been proposed, but only electroporation, electrofusion, and optoporation have been successfully applied into microfluidic systems for cell treatment until now. The ease with which arrays of microelectrodes can be patterned and integrated with networks of microchannels makes microfluidic systems an especially attractive platform for applications in cell electroporation [92–97] and electrofusion [98,99]. Optoporation offers advantage of remote operation which is quite desirable for some bio-applications [100].

Because electroporation on a microchip overcomes the potential risks of using a high voltage and generating excess heat, it is widely used for in vitro gene transfection. Electroporation microchips consisting of microchannels with gold thin film electrodes on both sides were fabricated in polymethylmethacrylate (PMMA) [92] or on glass slide [93] and evaluated by continuous gene transfection using Huh-7 and 293T cell lines. Lin et al. [94] studied the site-specific enhancement of in vitro gene delivery using electrostatic forces and electroporation microchips. An attracting electric field was employed to induce electrophoresis of negative charged DNA plasmids and to concentrate them near cell surface prior to electroporation. Compared to that without the attracting electric field, gene transfection efficiency could be enhanced up to several folds. Electroporation microchips were developed for introducing genes not only into cell lines but also into primary cells [95]. Heung and Rubinsky [96] developed a microchip for controlled electroporation of single cells in a flow-through fashion. Loaded cells were transported precisely to the electroporation site by a microfluidic channel and were electroporpermeabilized individually with virtually 100% gene transfer rate. They [97] also developed an electroporation microchip that incorporated a live biological cell in its electrical circuit and thereby induced controlled electroporation in the cell for membrane permeabilization at a single-cell level.

Despite the efficiency that can be obtained by carrying out cell fusion within networks of microchannels, so far, few experiments have been performed to exploit its advantages. Tresset and Takeuchi [98] reported a microfluidic device with high aspect ratio electrodes and low power consumption for electrofusion of liposomes and cells with the fusion yield of 75%. A schematic view of the experimental protocol is depicted in Fig. 8. After cells were introduced into the flow channel, a “pearl-chain” alignment of liposomes along the electric field lines could be obtained by applying an ac voltage. Then, a series of short and strong dc pulses disrupted membranes in the region of contact of liposomes and initiated the fusion. Orwar and co-workers [99] developed a microfluidic device for combinatorial fusion of liposomes and cells. A large number of combinatorially synthesized liposomes with complex compositions and reaction systems could be obtained from small sets of precursor liposomes.

Optoporation is a laser-based method in which the laser beam interacts with an absorptive medium. When interacting with nearby cells, a mechanical transient or stress wave, which can produce temporary alterations in the plasma membrane, can be produced because of optical breakdown, ablation, or rapid heating of the absorbing medium. Due to its complicated optical setup and complex operation, few microfluidic devices integrated with optoporation have been reported. Nevertheless, Allbritton and co-workers [100] had characterized cellular optoporation in microfluidic systems using visible laser light. The results showed that optoporation is attainable on microfluidic devices.

Electroporation and electrofusion microchips can achieve necessary electric field with a much lower applied voltage, avoiding the potential risks of using high voltage, and dissipating heat more quickly due to its large surface/volume ratio. Though they also suffer from low survival rate of biological cells in electrical fields, their advantages have not been fully exploited. A lot of work should be done to take full advantages of electroporation and electrofusion microchips to achieve on-chip gene therapy.
4. Cell analysis

Miniaturization of analytical devices promises numerous benefits, including reduced cell consumption, automated and reproducible reagent delivery, and improved performance. In following sections, microfluidic devices with specific biosanalytical applications of cell processing are reviewed.

4.1. Micro cytometer

Cytometry refers to measurement of physical and chemical characteristics of cells. Current flow cytometry is restricted to ‘benchtop’ or full-sized laboratory models which are expensive and generally dedicated to single use technology. Development of readily adaptable chip technology will allow the flow cytometer unit to be adaptable to a wide variety of potential uses with portability and low cost. Recently, micro cytometers have become a vital component in any micro total analysis system aiming to investigate biological events at the single-cell level [101]. Table 1 lists some successful attempts toward micro cytometry.

- Employing optical stretcher for deformability detection without reproducible reagent delivery, and improved performance. In following sections, microfluidic devices with specific bioanalytical applications of cell processing are reviewed.

Differing from conventional flow cytometers which usually employ fluorescence detection, microfluidic flow cytometry can be mounted on a variety of detection instruments. Schwille and co-workers developed a micro cytometer which supported confocal detection of fluorescent cells and particles and subsequently allowed for their sorting in the fluid phase with respect to spectroscopic properties. This micro flow cytometer comprised a branched channel system which could initiate fluid mixing, hydrodynamically focus the sample solution for confocal detection and allow direct implementation of reaction steps prior to detection and sorting. A deformability based cell sorter, which employed optical stretcher for deformability detection without the need for specific labeling, was developed by Geck and co-workers [106]. Gawad et al. [109] developed a cytological tool which was based on the micro Coulter particle counter (μCPC) principle for cell counting and separation. The device measures the spectral impedance of individual cells and allows screening rates over 100 samples/s on a single-cell basis. Influences of different cell properties, such as size, membrane capacitance, and cytoplasm conductivity, on the impedance spectrum also had been studied in detail [110]. Ramsey and co-workers [121] developed a micro flow cytometer which employed coincident light scattering and fluorescence to detect electrophoretically transported and fluorescently labeled E. coli.

An integrated micro flow cytometer should incorporate many of the necessary components and functionalities of a typical room-sized laboratory on to a small chip. Quake and co-workers [101] developed a microfabricated cell sorter which incorporated various microfluidic functionalities, including peristaltic pumps, dampers, switch valves, and input and output wells, to perform cell sorting in a coordinated and automated fashion. The whole cell sorting system is self-contained and the sorting schemes can be implemented on the device to perform time-course measurements on a single cell for kinetic studies. Kruger et al. [108] demonstrated the feasibility of integrating micro-optical components and using a flip-chip technique to bond a high gain photodiode directly over the sorting microchannel in their micro cytometer. Wolff et al. [112] developed a pressure-driven microfabricated high-throughput fluorescent activated micro cytometer which integrated the “smoking chimney” structure for hydrodynamic focusing of sample and cell sorting, a holding and culturing chamber and waveguides for optical detection of cells. The fluorescent activated cell sorting at a sample throughput as high as 12,000 cells/s at 100-fold enrichment can be achieved using this microchip. Several different optical elements such as waveguides, lens and fiber-to-waveguide couplers were incorporated into a microfluidic device designed by Wolff et al. [114] for sorting and analyzing different kinds of cells and particles. Three different signals (forward scattering, large angle scattering, and extinction) were measured simultaneously. Skerlos and co-workers [118] developed a multi-angle micro cytometer in which integrated solid-state lasers and silicon-based PIN photodiodes were employed to perform flow cytometry measurements. The observation cell is designed with integrated microgrooves that permit the physical registration of optical fibers which served as optical waveguides for laser excitation and fluorescence detection. The multi-angles design could help to increase signal-to-noise ratio and to achieve multiple simultaneous cytometric measurements at a single interrogation point. Nucleic acid-labeled fungus (S. cerevisiae) can be sorted at a fast count rate of 500 particles/s using this microchip.

There are also other some other approaches of microfluidic cell sorter. Wang and co-workers [102] developed a microfluidic system using gravity and electric force to drive cells for flow cytometry. Under driving of their own gravity force, cells flowed passing the detection region in an upright microchip, then entered into the sorting electric field and were sorted by a switch-off activation program. Marr and co-workers [113] exploited the inherent laminar nature of microscale fluid dynamics and incorporated applied fields and image cytometry to develop micro cytometer. Any visually identifiable differences between cells or particles could be employed for their sorting. Recently, Wang et al. [115] described a high-throughput microfluidic cell sorter with an all-optical control switch for live cells. As shown in Fig. 9, cells pass first through an analysis region, then through the optical switching region situated just before a Y-shaped junction, where the flow splits into two microchannels. When a cell is detected and determined to be a target cell, the optical switch will be activated and a focused laser spot deflects the cell to the target output channel. Sorting runs of cell populations ranging from as few as 1000 cells up to 280,000 cells can be completed in less than an hour. The result showed a major improvement in purity and yields.

4.2. Chemical cytometry

Understanding molecular mechanisms of many fundamental biological processes and serious health disorders requires simultaneous analysis of a large number of chemical species within single cells. Chemical cytometry refers to the use of high sensitivity analytical tools to characterize chemical composition of single cells [122]. In this section, selected examples of microfluidic chemical cytometry will be reviewed.
Table 1  
Recently published examples of micro cytometer

<table>
<thead>
<tr>
<th>Featured items</th>
<th>Driving force</th>
<th>Detection method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A cell sorter which is incorporated with various microfluidic functionalities, including peristaltic pumps, dampers, switch valves, to perform cell sorting is described</td>
<td>Pneumatically actuated pumps and valves</td>
<td>Fluorescence</td>
<td>[101]</td>
</tr>
<tr>
<td>A method based on gravity and electric force driving of cell for flow cytometry in an upright microfluidic chip system is described</td>
<td>Gravity focusing electric switching</td>
<td>Confocal fluorescence</td>
<td>[102]</td>
</tr>
<tr>
<td>A mechanism for cell sorting in a microfluidic device with combined electroosmotic and hydrodynamic pressure-driven flow is described</td>
<td>Hydrodynamic pressure and electroosmotic</td>
<td>Fluorescence</td>
<td>[103]</td>
</tr>
<tr>
<td>A valveless switch for microparticle sorting with the electrophoretic switch technique and the two phases laminar flow streams is presented</td>
<td>Hydrodynamic electrophoretic switching</td>
<td>Fluorescence</td>
<td>[104]</td>
</tr>
<tr>
<td>A microfluidic chip, which comprises a branched channel system to initiate fluid mixing and to hydrodynamically focus the sample solution down to a thin flow layer for confocal detection, is described</td>
<td>Hydrodynamic focusing electrokinetic switching</td>
<td>Confocal fluorescence</td>
<td>[105]</td>
</tr>
<tr>
<td>A deformability based cell sorter which employs the optical stretcher for deformability detection without the need for specific labeling is described</td>
<td>Hydrodynamic pressure</td>
<td>Optical stretcher</td>
<td>[106]</td>
</tr>
<tr>
<td>A confocal microfluidic particle sorter using fluorescent photon burst detection is presented</td>
<td>Electrophoretic focusing and switching</td>
<td>Confocal fluorescence</td>
<td>[107]</td>
</tr>
<tr>
<td>A microfluidic device which integrates micro-optical components and sorts single cells by means of an off-chip valve switching technique is described</td>
<td>Hydrodynamic focusing off-chip valve switching</td>
<td>Fluorescence</td>
<td>[108]</td>
</tr>
<tr>
<td>A microfluidic cytological tool which measures the spectral impedance of individual cells or particles and allows screening rates over 100 samples/s on a single-cell basis is described</td>
<td>Hydrodynamic focusing</td>
<td>Impedance spectroscopy</td>
<td>[109,110]</td>
</tr>
<tr>
<td>A microfluidic flow cytometer, which employs electrokinetic forces for flow focusing and sample switching, and incorporates buried optical fibres for the on-line detection of cells or particles, is described</td>
<td>Electrokinetic focusing and switching</td>
<td>Fluorescence</td>
<td>[111]</td>
</tr>
<tr>
<td>A microfluidic fluorescent activated cell sorter with several integrated, functional structures is described</td>
<td>Hydrodynamic focusing and switching</td>
<td>Fluorescence</td>
<td>[112]</td>
</tr>
<tr>
<td>A method, which exploits the inherent laminar nature of microscale fluid dynamics and incorporates applied fields and image cytometry to enable sorting based upon any visually identifiable difference between cells or particles, is described</td>
<td>Hydrodynamic focusing optical switching</td>
<td>Fluorescence</td>
<td>[113]</td>
</tr>
<tr>
<td>A microchip flow cytometer, in which several different optical elements (waveguides, lens and fiber-to-waveguide couplers) are integrated with microfluidic channels, is described</td>
<td>Hydrodynamic focusing</td>
<td>Scattered light</td>
<td>[114]</td>
</tr>
<tr>
<td>An all-optical control switch for live cells and its implementation in a high throughput, fluorescence-activated microfluidic cell sorter is described</td>
<td>Hydrodynamic focusing optical switching</td>
<td>Fluorescence</td>
<td>[115]</td>
</tr>
<tr>
<td>A multi-channel device for cell or particle counting and sorting utilizing a digital image detection system is described</td>
<td>Hydrodynamic focusing electrokinetic switching</td>
<td>Digital image detection</td>
<td>[116]</td>
</tr>
<tr>
<td>A microfluidic system that can separate motile sperm from small samples basing on the ability of motile sperm to cross streamlines in a laminar fluid stream is described</td>
<td>Hydrodynamic pressure</td>
<td>Fluorescence</td>
<td>[117]</td>
</tr>
</tbody>
</table>


Table 1 (Continued)

<table>
<thead>
<tr>
<th>Featured items</th>
<th>Driving force</th>
<th>Detection method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A PDMS based multi-angle microfluidic flow cytometer which used solid-state</td>
<td>Hydrodynamic focusing</td>
<td>Fluorescence</td>
<td>[118]</td>
</tr>
<tr>
<td>lasers and silicon-based PIN photodiodes to perform flow-cytometry measurement is described</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The feasibility of using auto-fluorescence (AF) for the detection of single</td>
<td>Electroosmotic focusing and</td>
<td>Confocal fluorescence</td>
<td>[119]</td>
</tr>
<tr>
<td>living cells for label-free cell sorting in microfluidic systems is</td>
<td>switching</td>
<td></td>
<td></td>
</tr>
<tr>
<td>demonstrated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A microchip flow cytometer which uses an air-liquid two-phase microfluidic</td>
<td>Aerodynamic focusing</td>
<td>Fluorescence</td>
<td>[120]</td>
</tr>
<tr>
<td>system to produce a focused high-speed liquid sample stream of particles and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cells is described</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A microfluidic device which employs coincident light scattering and fluorescence to detect electrophoretically transported fluorescein labeled E. coli is described</td>
<td>Electrophoretic focusing</td>
<td>Coincident light scattering</td>
<td>[121]</td>
</tr>
<tr>
<td></td>
<td>and Fluorescence</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Some attempts focused on detection of small molecules which are related to cell metabolism. The high-throughput chemical analysis of single cells was demonstrated by McClain et al. [123] on an integrated microfluidic device. The average analysis rate was 7–12 cells/min, which is 100–1000 times faster than standard benchtop CE analysis of single cells. Fang and co-workers [124] demonstrated the feasibility of integrating the whole process of single-cell analysis on a microfluidic. A throughput of 15 samples/h and a retention time precision of 2.4% R.S.D. (n = 14) were obtained.

The average separation efficiency for GSH in lysed cells was $2.13 \times 10^6 \pm 0.4 \times 10^6$ plates/m. A microfluidic chemical cytometry device was developed and evaluated for electropherograms of amino acids from individual Jurkat T-cells. The total time for the analysis of one cell is less than 1 h [125]. Lilge and co-workers [126] presented a microfluidic device for single-cell CE in parallel microchannels. Calcein-labeled acute myloid leukemia (AML) cells were selected and transported by optical tweezers. After electromechanical lysis, contents of individual cells were electrophoretically separated and the labeled cellular contents were recorded by LIF detection.

Others concentrated on detection of large proteins. A microfluidic system integrating continuous lysis of bacterial cells and fractionation and detection of a large intracellular protein (β-galactosidase) has been demonstrated [127]. Wainwright et al. [128] designed microfluidic devices which were amenable to isotachophoresis (ITP)-zone electrophoresis (ZIE) separations and evaluated them by analyzing a cell surface protease (ADAM 17) in live intact THP-1 cells in physiological buffers with detection limits below 10 cells/assay. Tabuchi et al. [129] reported a pressurization technique for microchip electrophoresis that enabled 15 s separation of 12 samples of complex protein mixtures extracted from Jurkat cells.

Most attention was paid on the genetic assays. Quake and co-workers [130] developed a microfluidic chip for automated nucleic acid purification from small numbers of bacterial or mammalian cells. As shown in Fig. 10a, this chip consisted of an actuation layer and a fluidic layer. Schematic diagram of one instance of the DNA isolation process is depicted in Fig. 10a–e. Bacterial cells (red), buffer (green) for dilution of the cell sample and lysis buffer (yellow) are introduced into the microfluidic chip first (Fig. 10a), and then into the rotary mixer (Fig. 10b). The lysate is flushed over a DNA affinity column and drained (Fig. 10d). Finally, purified DNA is recovered from the chip by introducing elution buffer and can be used for further analysis or manipulation (Fig. 10e). A microchip that consists of microfluidic mixers, valves, pumps, channels, cham-
Fig. 10. DNA purification chip and schematic diagram of one instance of the DNA isolation process (open valve, rectangle; closed valve, × in rectangle). (a) Bacterial cells (red), buffer (green) for dilution of the cell sample and lysis buffer (yellow) are introduced into the microfluidic chip. (b) The cell sample, dilution buffer and lysis buffer slugs are introduced into the rotary mixer. (c) The three different liquids are mixed thoroughly and consequently bacterial cells are lysed completely. (d) The lysate is flushed over a DNA affinity column and drained to the ‘waste port’. (e) Purified DNA is recovered from the chip by introducing elution buffer and can be used for further analysis or manipulation. (f) Integrated bioprocessor chip with parallel architecture. This chip consists of the actuation layer and the fluidic layer. The width of the fluid channels is 100 μm and that of valve actuation channels is 200 μm. Reprinted with the permission from [130], © 2005 Nature Publishing Group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Bers, heaters, and DNA microarray sensors was presented by Liu et al. [131] and evaluated it by pathogenic bacteria detection from approximately milliliters of whole blood samples and single-nucleotide polymorphism analysis directly from diluted blood. The on-chip analysis started with the preparation process of a whole blood sample, which included magnetic bead-based target cell capture, cell preconcentration and purification, and cell lysis, followed by PCR amplification and electrochemical DNA microarray-based detection [131]. A microfluidic device which integrated selection of individual cells, cell lysis, and electrophoretic separation of apoptotic DNA fragments was fabricated on a CD-like plastic disk for detection of doxorubicin-induced apoptosis in individual cardiomyocytes [132]. A semiquantitative reverse transcription polymerase chain reaction (RT-PCR) procedure based on combination of competimer technology with microchip electrophoresis was developed for total RNA extraction from human colon carcinoma cell line CaCo-2. The result confirmed the role of cholesterol as a positive inducer of specific factors on ABCA1 and ApoAI gene expressions that was well estimable at low phytosterol concentrations [133].

4.3. Biochemical sensing

Because of its unique advantages such as controllable transport, immobilization, and manipulation of biological molecules and cells, as well as controllable manipulation of reagents such as separation, mixing, and dilution, microfluidic systems show great potential for analysis of intracellular parameters and to detect presence of cell metabolites, even on a single-cell level. Several examples of recently published biochemical sensing of different analytes from various cells in microfluidic devices are presented in Table 2.

A 3D microfluidic network system, in which the sensor cells were immobilized in agarose, was developed and employed for bioassay based on the expression of a bioluminescence reporter gene. In this experiment, E. coli strains having a plasmid with firefly luciferase gene (lux) under transcriptional control of
<table>
<thead>
<tr>
<th>Type of study</th>
<th>Cell type</th>
<th>Detection method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensoring mutagenicity using <em>E. coli</em> strains which have a plasmid with firefly luciferase gene (lux) under transcriptional control of mutagen-induced bacterial SOS response</td>
<td><em>E. coli</em></td>
<td>Bioluminescence</td>
<td>[28]</td>
</tr>
<tr>
<td>Monitoring the ATP-dependent calcium uptake reaction upon treatment of a concentration gradient of a test solution Studying the microfluidic bioreactor based on hydrogel-entrapped <em>E. coli</em> for cell viability, lysis, and intracellular enzyme reactions</td>
<td><em>E. coli</em></td>
<td>Confocal fluorescence</td>
<td>[31]</td>
</tr>
<tr>
<td>Studying the application of PEG hydrogel microstructures encapsulating mammalian cells in high-throughput drug screening or pathogen detection</td>
<td>Fibroblasts, hepatocytes, macrophage</td>
<td>Fluorescence</td>
<td>[134]</td>
</tr>
<tr>
<td>Monitoring extracellular acidification ratios of a large number of cells (1 x 10⁻¹⁵ - 1 x 10⁻²⁵)</td>
<td>Mouse hepatocyte cell line, TABX2S</td>
<td>Absorption spectra</td>
<td>[136]</td>
</tr>
<tr>
<td>Monitoring the kinetics of intracellular fluorescent dyeset (FDA) metabolism and calcium mobilization at single-cell level, as stimulated by glucose and pH changes</td>
<td>Yeast cell</td>
<td>Fluorescence</td>
<td>[137,138]</td>
</tr>
<tr>
<td>Monitoring allergic response, stimulated with dinitrophenylated bovine serum albumin (DNP-BSA) after incubation with anti-DNP IgE</td>
<td>Rat basophilic leukemia cell line, RBL-2H3</td>
<td>Fluorescence</td>
<td>[139]</td>
</tr>
<tr>
<td>Monitoring s,glutamate and hydrogen peroxide for studying the effects of trace levels of an endocrine disruptor, tributyltin (TBT)</td>
<td>Nerve cells</td>
<td>Amperometric</td>
<td>[140]</td>
</tr>
<tr>
<td>Quantifying amounts of adenosine triphosphate (ATP), released from erythrocytes that are mechanically deformed as these cells traverse microfluidic channels in microchips</td>
<td>Endothelial cells</td>
<td>Chemiluminescence</td>
<td>[141]</td>
</tr>
<tr>
<td>Probing naphthalene metabolism using in vitro model</td>
<td>L2 rat lung Type II epithelial cells, H4IIIE (rat hepatocytes), Hep G2C7A (human hepatocyte) cell lines</td>
<td>Fluorescence</td>
<td>[142]</td>
</tr>
<tr>
<td>Measuring the bradykinin stimulation of individual cells by observing changes in intracellular Ca²⁺ levels using Ca²⁺-sensitive fluorescent dye</td>
<td>PC12 cells</td>
<td>Fluorescence</td>
<td>[143]</td>
</tr>
<tr>
<td>Monitoring real-time production and release of glucose and ethanol from immobilized cells using chemiluminescent (CL) enzyme-based flow-through microchip</td>
<td>Yeast cells (Saccharomyces cerevisiae)</td>
<td>Chemiluminescence</td>
<td>[144,145]</td>
</tr>
<tr>
<td>Monitoring insulin secretion using on-line electrophoresis immunoassay</td>
<td>Islets of Langerhans</td>
<td>Fluorescence</td>
<td>[146,147]</td>
</tr>
<tr>
<td>Assaying cell viability, measuring ionophore-mediated intracellular Ca²⁺ flux, and measuring multistep receptor-mediated Ca²⁺ responses under the stimulation of glucose</td>
<td>Jurkat T-cells, U93719 cells</td>
<td>Fluorescence</td>
<td>[148]</td>
</tr>
<tr>
<td>Monitoring the NAD(P)H and (Ca²⁺) responses under the stimulation of glucose</td>
<td>β-Cells of Langerhans</td>
<td>Fluorescence</td>
<td>[149]</td>
</tr>
<tr>
<td>Real-time monitoring cellular glucose consumption and lactate production by NAD-linked enzymatic analysis</td>
<td>Mouse hepatocyte cell lines, TABX2S and TABX1A</td>
<td>Absorption spectra</td>
<td>[150]</td>
</tr>
<tr>
<td>Monitoring of cytochrome c distribution in a neuroblastoma-glioma hybrid cell</td>
<td>NG 108-15 neuroblastoma glioma hybrid cell</td>
<td>Scanning thermal lens microscope</td>
<td>[151]</td>
</tr>
<tr>
<td>Monitoring the quantum release of dopamine from the cell under the stimulation of acetylcholine</td>
<td>PC12 cells</td>
<td>Amperometric</td>
<td>[152]</td>
</tr>
</tbody>
</table>
Monitoring cellular ATP of E. coli

Monitoring the oxygenation cycle of the cell

Monitoring of expression events in single

Long-term monitoring the intracellular

Studying the intracellular conversion of

Monitoring extracellular acidification rates

Dynamic measurements of lactate during

cell permeabilization

Monitoring extracellular acidification rates

Monitoring extracellular potential caused by

Dynamic measurements of lactate during

cell permeabilization

Studying the intracellular conversion of

Monitoring extracellular acidification rates

Monitoring extracellular acidification rates

Monitoring extracellular acidification rates

Monitoring extracellular acidification rates

Monitoring extracellular acidification rates

Monitoring extracellular acidification rates

Table 2 (Continued)

<table>
<thead>
<tr>
<th>Type of study</th>
<th>Cell type</th>
<th>Detection method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynamic measurements of lactate during cell permeabilization</td>
<td>Cardiac myocytes (healthy and anoxic cells)</td>
<td>Amperometric</td>
<td>[133]</td>
</tr>
<tr>
<td>Monitoring extracellular acidification rates (with pH-sensitive field effect transistors, ISFETs), cellular oxygen consumption rates (with amperometric electrode structures) and cell morphological alterations (with impedimetric electrode structures, IDES) on single chips</td>
<td>LS174T adenocarcinoma colorectal cell line</td>
<td>Potentiometric, amperometric, impedimetric</td>
<td>[144]</td>
</tr>
<tr>
<td>Monitoring extracellular potential caused by transmembrane currents associated with spontaneously initiated intracellular calcium waves</td>
<td>Jurkat T-cells yeast cells</td>
<td>Fluorescence</td>
<td>[156]</td>
</tr>
<tr>
<td>Studying the intracellular conversion of fluorescein diacetate (FDA) to fluorescein and the degradation of an inhibitory protein, IκB, as involved in the NF-κB signaling pathway</td>
<td>HFF11 cell line</td>
<td>Chemiluminescence</td>
<td>[157]</td>
</tr>
<tr>
<td>Monitoring of expression events in single cells by profiling the activation of the transcription factor NF-κB in cells in response to varying doses of the inflammatory cytokine TNF-α</td>
<td>HeLa S3 cells</td>
<td>Fluorescence</td>
<td>[158]</td>
</tr>
<tr>
<td>Monitoring the oxygenation cycle of the cell and to investigate effects like photo-induced chemistry caused by the illumination</td>
<td>Red blood cell</td>
<td>Raman spectroscopy</td>
<td>[159]</td>
</tr>
<tr>
<td>Monitoring cellular ATP of E. coli</td>
<td>E. coli</td>
<td>Bioluminescence</td>
<td>[160]</td>
</tr>
</tbody>
</table>

mutagen-induced bacterial SOS response was used as sensor bacteria for mutagenicity [28]. A microfluidic device that integrated cell docking and concentration gradient functions was developed for monitoring calcium uptake reaction of HL-60 cells by Yang et al. [31]. In this microfluidic chip, an analyte solution could be diluted to different gradients as a function of distance along the dam structure. Real-time monitoring of ATP dependent calcium uptake reaction of HL-60 cells upon treatment of a concentration gradient of a test solution was achieved [31]. Li and Peng [137,138] developed a microfluidic system for detecting cells’ fluorescein diacetate (FDA) metabolism and calcium mobilization at single-cell level, as stimulated by glucose and co-workers [142] described a microscale cell culture analog (μCCA), which was a physical replica of the physiologically based pharmacokinetics (PBPK) model, for realistically and inexpensively studying the adsorption, distribution, metabolism, elimination, and potential toxicity (ADMET) of chemicals. Such a microfabricated device consists of a fluidic network of channels to mimic the circulatory system and chambers containing cultured mammalian cells representing key functions of animal “organ” systems. This paper described the application of a two-cell system, four-chamber μCCA (“lung”–“liver”–“other tissue”–“fat”) device for proof-of-concept study using naphthalene as a model toxicant. Naphthalene was converted into reactive metabolites in the “liver” compartment, which then circulated to the “lung” depleting glutathione (GSH) in lung cells. Peterman et al. [143] presented a microfluidic device which could repeatedly deliver chemical compounds to multiple locations and evaluated it by measuring the bradykinin stimulation of individual PC12 cells with a Ca2+-sensitive fluorescent dye. Basic computational results with experimental verification of both fluid ejection and fluid withdrawal by imaging pH changes using a fluorescent dye also were reported in their work. Wheeler et al. [148] developed a microfluidic device which can passively and gently separate a single cell from the bulk cell suspension, and precisely deliver nanoliter volumes of reagents to that cell. The cell viability assays, ionophore-mediated intracellular Ca2+ flux measurements, and multistep receptor-mediated Ca2+ measurements were demonstrated in this microfluidic device. A microfluidic device which is capable of partially stimulating an islet and allows observation of the NAD(P)H and [Ca2+]i.
responses was developed to test degree of electrical coupling within the islet. Several results were obtained in this research: the β-cells of an islet were sufficiently coupled to synchronize the [Ca$^{2+}$], response within glucose-stimulated regions, but were not coupled to the extent that allowed a glucose-stimulated [Ca$^{2+}$], response to be transmitted into neighboring non-stimulated cells; Tolbutamide, an antagonist of the ATP-sensitive K$^{+}$ channel, allowed β-cells [Ca$^{2+}$], oscillations to travel further into the non-stimulated regions of the islet. The extent of Ca$^{2+}$ propagation across the islet depended on a delicate interaction between the degree of coupling and the extent of ATP-sensitive K$^{+}$-channel activation [149]. Kitamori and co-workers [151] combined a scanning thermal lens microscope detector with a cell culture microchip to realize on-chip cellular biochemical analysis. Cytochrome c (cyt) release from mitochondria to cytosol during the apoptosis process was successfully monitored and directly imaged with extremely high spatial resolution of ~1 μm, without any labeling materials. Brischwein et al. [154] reported a multi-parametric silicon sensor chip device with potentiometric, amperometric, and impedimetric microsensors to enable investigations of cellular microphysiological patterns. The chip layout is shown in Fig. 11: five pH-sensitive field effect transistors (pH-ISFETs) for measurements of extracellular acidification; one interdigitated electrode structure (IDES) for detection of cell morphological changes; one amperometric sensor structure for assessment of cellular oxygen consumption (pO$_2$ sensor). Extracellular acidification rates, cellular oxygen consumption rates, and cell morphological alterations were monitored on single chips simultaneously for up to several days [154]. Li et al. [156] developed a microfluidic device, which consisted of special U-shaped microstructures with openings parallel to the liquid flow and weirs perpendicular to the flow. The versatility of this microfluidic system was demonstrated by studying two cellular processes at single-cell level: the intracellular conversion of FDA to fluorescein; and degradation of an inhibitory protein, IxB, as involved in the NF-κB signaling pathway. A Jurkat cell expressed with IxB-EGFP was also employed to probe any possible action of an herbal compound, isosafrole (IQ), on the degradation of IxB-EGFP. Emmerus and co-workers [157] developed a microfluidic device which could perform on-chip cell lysis and sample extraction to monitor cellular ATP of E. coli using bioluminescence (BL) detection. BL detection of ATP and ATP-conjugated metabolites was realized by using firefly luciferin-luciferase BL system. Under optimized conditions, ATP analysis could be realized within 30 s with a detection limit down to 0.20 μM and a dynamic linear range over two orders of magnitude. No interferences from ADP, AMP, and cell lysis detergent Triton X-100 were found within analysis standard deviation.

### 4.4. Electrical characterization and ion channel studies

Cells control their electrical activity by opening and closing molecular-sized pores in cellular membranes called ion channels. The ability to measure ion channel function in outer space is thus very important but difficult. Patch-clamping cells have proven to be a powerful technique for elucidation of fundamental ion channel protein biophysics and for drug discovery. Although conventional patch-clamp methods provide high information contents, they are laborious and suffer from low throughput and high overall cost. Several approaches for achieving high-throughput electrophysiology are under development, among which microchip-based patch-clamp systems uniquely achieve a higher degree of miniaturization, faster perfusion and mixing, and lower reagent cost without losing information contents [161–166].

Comparison of different patch-clamp setups is summarized in Fig. 12 [161]. Traditionally, patch-clamp recording is accomplished with the help of a micromanipulator positioning glass pipette under a microscope [162,163]. As illustrated in Fig. 12A, a cell membrane patch is sucked into the glass pipette and forms a high electrical resistance seal. Current that passes through the ion channels in either the membrane patch or the whole cell membrane is then recorded at different bias voltages. In the meantime, most chip-based devices use the planar geometry shown in Fig. 12B, where the patch pore is etched in a horizontal membrane that separates the top cell compartment from the recording electrode compartment [164–166]. Lee and co-workers [161] presented a multiple patch-clamp array chip by utilizing lateral
cell trapping junctions which provided multiple cell addressing and manipulation sites for efficient electrophysiological measurements at a number of patch sites. As shown in Fig. 12C, this device geometry not only minimizes capacitive coupling between the cell reservoir and the patch channel, but also allows simultaneous optical and electrical measurements for studying roles of ion channels with respect to their cellular functions. Orwar and co-workers [162] presented a microfluidics–patch clamp platform for performing high-throughput screening and rapid characterization of weak-affinity ion channel–ligand interactions. Different agents and buffer solutions could be presented in programmable sequence to the biosensors, which could yield high screening rates. This microfluidic platform may have potential applications in competitive assays, and dose–response characterizations from which agonist–antagonist affinity and efficacy as well as receptor blockade mechanisms. They [163] also studied the electrical properties and mechanical stability of the seal using a microfluidic chip generating laminar flow in open volumes. The results showed that by applying an external force in the form of a fluid flow to a patch-clamped cell acting parallel to the cell-pipet axis, it was possible to increase patch-clamp recording times by over 100% and decrease the electrical noise level by 40%. Folch and co-workers [164] designed and fabricated microfluidic devices which incorporated arrays of nanoholes for patch-clamp electrophysiological recordings of ion channel activities and could realize the focal delivery of biochemical factors to cell surface with submicron-scale precision. 3D silicon oxide micronozzles (Fig. 12D) integrated into a microfluidic device for patch clamping has been developed by Lehnert et al. [165]. A cell could be positioned on the nozzle by suction through the hollow nozzle that extended to the backside opening of the chip. A microanalysis system for multi-purpose electrophysiological analyses has been presented by Han et al. [166]. This system has the capability to perform whole cell patch clamping, impedance spectroscopy, and general extracellular stimulation recording using integrated multi-electrode configurations.

There are also some other approaches toward electrical characterization of cells. Microfluidic devices for the culture and electrical characterization of epithelial cell layers have been developed by Hediger et al. [167,168]. The main goal was to achieve both cell culture and impedimetric and potentiometric characterization on a single device: Huang et al. [169] developed a method that employed a microfabricated device for evaluation of membrane electrical properties of single cells. Electrical resistances of dead (membrane impaired) cells and live cells were found to be significantly different. This suggested that evaluating membrane resistances of individual cells could provide an instant and quantitative measurement for determining cell membrane integrity and cell viability at single-cell level.

4.5. Whole cell assay

Recent advances in microfluidic techniques have increased the potential of high-throughput biochemical assays on individual mammalian cells. Of particular interest is the ability to parallelize up-front assay protocols and still be able to examine and treat every individual cell in the assay separately retrieving single-cell event information [170]. Several examples of recently published whole cell assay for different cells in microfluidic chip are presented in Table 3.

Shelby et al. [171] employed PDMS microfluidic channels to characterize the complex behaviors of P. falciparum-infected erythrocytes under capillary-like conditions. The results showed that mature forms of infected erythrocytes concentrate to very high parasitemia at the mouth of a blocked capillary and fresh, pliable erythrocytes can squeeze through a blockage. A microfluidic assay for bacterial chemotaxis was developed, in which a gradient of chemoeffectors was established inside a microchannel via diffusion between parallel streams of liquid in laminar flow. The random motility and chemotactic responses to l-aspartate, l-serine, l-leucine, and Ni²⁺ of WT cells and chemotactic-mutant strains of E. coli were measured. The results were amazing: WT cells dispersed better than cells lacking one or both of major receptors Tar and Tsc; low concentration of
l-aspartate (3.2 mM at the inlet) that generated a significant migration of RP437 WT cells. t-leucine acted as an attractant sensed by Tar and as a repellent sensed by Tsr [172]. Li et al. [173] developed a microfluidic chip, which was integrated with a thickness-shear mode (TSM) acoustic wave sensor, for muscle cell contraction analysis upon chemical stimuli. Jea and co-workers [175] developed a microfluidic device to study chemotaxis of metastatic breast cancer cells, MDA-MB-231, in EGF gradients of well-defined profiles. The result suggested that MDA-MB-231 cancer cell chemotaxis depended on the shape of gradient profile as well as on the range of EGF concentrations. Dertinger et al. [176] fabricated substrate-bound gradients of proteins with complex shapes using laminar flows in microchannels to systematically investigate influences of protein gradients on neuronal development. The results showed that axon specification was oriented in the direction of increasing surface density of laminin. Taupin and co-workers [179] developed a microfluidic platform that enabled parallel, quantitative analysis of stem cells at single cells level in arbitrary conditions. Using this platform, one could further interrogate the response of distinct stem cell subpopulations to micro-environmental cues (mitogens, cell–cell interactions, and cell–extracellular matrix interactions) that governed their behavior. A PDMS-based microfluidic device, which could dilute drugs with a buffer solution with serially increasing concentrations, was fabricated to perform cytotoxicity tests [181]. Zhang et al. [182] developed an integrated time of flight (TOF) optophoresis system to consistently detect significant differences between normal skin and melanoma cell lines (CCD-1037 and A375, respectively), and measure consistent difference in a cell differentiation model (HL-60 cell line with DMSO treatment).

5. Concluding remarks

After several years’ development, microfluidic devices have testified themselves to be powerful tools for cell-based analysis. Various cell manipulation methods can be incorporated into microfluidic devices for cell-based analysis, such as magnetic, optical, mechanical, and electrical manipulation methods. Magnetic method is a clean, versatile, and non-invasive method will become more efficient. Due to the non-contact and contamination-free manipulation process, optical manipulation method can be easily integrated with microfluidics for cell-based assay. With the generation of new magnetic bead materials and modification methods for conjugating various ligands to magnetic beads, it is believed that the magnetic manipulation method can be easily integrated with microfluidics for cell-based assay. With the generation of new magnetic bead materials and modification methods for conjugating various ligands to magnetic beads, it is believed that the magnetic manipulation method can be easily integrated with microfluidics for cell-based assay. With the generation of new magnetic bead materials and modification methods for conjugating various ligands to magnetic beads, it is believed that the magnetic manipulation method can be easily integrated with microfluidics for cell-based assay. With the generation of new magnetic bead materials and modification methods for conjugating various ligands to magnetic beads, it is believed that the magnetic manipulation method can be easily integrated with microfluidics for cell-based assay. With the generation of new magnetic bead materials and modification methods for conjugating various ligands to magnetic beads, it is believed that the magnetic manipulation method can be easily integrated with microfluidics for cell-based assay. With the generation of new magnetic bead materials and modification methods for conjugating various ligands to magnetic beads, it is believed that the magnetic manipulation method can be easily integrated with microfluidics for cell-based assay.
their further applications in microfluidics. Size-dependent filter-based microfluidic devices exhibit numerous advantages, such as high labeling efficiency, short detection time, and high reproducibility based on simple and robust experimental procedures. However, their poor selectivity limits their further applications in microfluidics for cell-based assays. Other convection structures also face the same challenge. Micropumps can precisely manipulate single cell without any damage, but the complicated fabrication procedures make it difficult to be applicable in common biology and chemistry labs. Oppositely, dam and sandbag structures can be easily fabricated using various methods and can also efficiently immobilize cells with minimal stress on them. Dam and sandbag structures hold promises in microfluidic cell analysis. Although the coating method which use cell adhesion to capture cells suffers from relatively low immobilization efficiency, its high selectivity and easily modifying microchannel interior surface still make it a promising method for developing microfluidic devices for cell-based assay. DEP, which used an ac electric field to separate target cells from cell samples in a flow stream, has been demonstrated to be quite effective and selective in concentrating, manipulating, and separating cells and bacteria with different sizes simultaneously. In the meantime, DEP suffers from low survival rate of biological cells in electrical field and complicated instrumentation. However, with the development of microfabrication method and appearance of newly available electrode geometries for DEP, DEP manipulation will have more applications in microfluidics for cell-based assay. Cell manipulation using magnetic and electrical methods in microfluidics is most promising due to their simple and cheap peripheral equipments and easy integration with microfluidics. A lot of work should be done to take full advantages of these two methods.

The successful applications of microfluidics for cell-based assay include cell lysis chip, cell culture chip, electroporation chip, electrofusion chip, optoporation chip, microcytometer, chemical cytometry, biochemical sensing chip, and whole cell sensing chip. On-chip cell lysis can be realized using chemical, mechanical, and electrical methods. All these lysis methods can be easily integrated with microfluidics. In order to achieve efficient deliveries of lysis reagents or on-chip generation of lysis reagents, more microfluidic components should be fabricated, that will increase the complexity of the micro-devices in a way. Mechanical-based lysis also faces the same challenge. Electrical-based lysis is the most widely used in microfluidic cell lysis due to its easy integration and simplified instrumentation and will obtain more applications. On-chip cell culture can be successfully achieved in microfluidic platform. A variety of small molecules, large proteins and DNA can be simultaneously analyzed in chemical cytometer chip within a single run. Especially arresting is the possibility to treat and analyze single living cells using microfluidic devices. A variety of intracellular parameters and cell metabolites have been analyzed and detected using microfluidics, which also demonstrates the versatility of microfluidics. With the employment of nanotechnology, single cell-based microfluidic devices for various sophisticated experiments will be the future direction of this research area.

Acknowledgement
This work is supported by the Research Grants Council, Hong Kong (CityU Project No. 9040983, CityU 102904).

References