Chapter 12
Cell Metabolite Analysis on Microfluidic Platform

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Abstract Cell metabolite is related to every aspect of biology and biochemistry. Cell metabolite analysis can help us to understand disease like tumor. It has been reported that abnormal metabolic states impact on cellular function and tissue dysfunction leading to many human diseases. Microfluidic platform is a powerful and promising analytical tool for cell metabolite analysis with the advantages of high-throughput, convenience, reduced reagent and time consumption, good separation ability and portability. This chapter summarizes recent progresses in microfluidic platform of cell metabolite analysis, the models of cell culture and how to control cell microenvironment, the strategy for cell metabolite analysis and the platform application. It considers the prospects of developing new metabolic approaches to disease treatment.

Keywords Microfluidic · Cell metabolite · Cell microenvironment Methods · Application

Abbreviations

2D	Two-dimensional
3D	Three-dimensional
GC	Gas
HPLC	High-pressure
LC	Liquid
μTAS	Micro
ECM	Extracellular
PDMS	Polydimethylsiloxane

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12.1 Introduction

Metabolism of living organisms is the sum of life-sustaining chemical transformations [1]. Core metabolism contains metabolic pathways involving carbohydrates, amino acids, and fatty acids, macromolecular synthesis and energy homeostasisin humans [2]. The scientific study of initial metabolism can be traced back to several centuries ago. Subsequently, animals testing are studied in early studies, followed by individual metabolic reactions has been developed. In 1614, Santorio Santorio is the first people who determined human metabolism by controlling experiments in his book. He found that most of food consumed through insensible perspiration. However, metabolism research development is accompanied with human diseases. About 2000 years ago, found Celsus knew found gout was greatly related to rich foods and drink precipitated [2]. The research has revealed that ants can be attracted by the urine of diabetic patients, but normal urine can not. The period of metabolic activities in biological research has blossomed from 1920s to 1960s, which was named "the golden age". But, newer areas of metabolic research are gradually displayed the momentum in biological research in the latter half of the 20th century. The search for the metabolic detection is benefit for clinic diagnoses and for study on the mechanisms of various diseases such as cancer, not only study on metabolic states in these diseases. More and more attention of researcher is focused on diseases in terms of inherited or somatic mutations such as signal transduction, cellular differentiation, not on bioenergetic or metabolic terms.

Before the year 2009, the definition of metabolism is not as clearly defined. In the year 2009, Kraly et al. [3] defined metabolomics related to metabolomics, metabonomics, metabolic fingerprinting, metabolic profiling, and targeted metabolic profiling. Metabolomics are similar to genomics and proteomics, which is quantitative analysis of all metabolites in an organism, study of relation between the metabolites and physiological and pathological changes. Metabonomics are aimed to monitor the time-depending concentration of metabolic markers. The goal of making up metabolomics and metabonomics together is to search biomarkers of disease. It also aim to illustrate pathways related with metabolite changes. Based on pattern recognition, the goal of metabolic fingerprinting is the determination of a whole profile of metabolites, which can be used to identify specific biomarker's profile. The field of metabolomics is targeted metabolic profiling. The history of this research is long and it often analyzes one or two targets tracked with time.

Metabolism analysis is vital consequences for disease detection and therapeutic strategy development which can provide the evidence for cancer pathophysiology. In order to predict the physiological response by applying new drug or new chemical substance in vivo, the metabolism analysis in vitro screening systems has been developed with long history [4]. Moreover, to reduce the cost and the risk of failure during clinical trials, in vivo animal studies are applied. Although the environment of study in animal testing is similar to that in human body, animal studies are expensive and labor-intensive. In vitro screening systems are widely

used to investigate drug development, screening, and toxicology [5]. Conventional in vitro cell-based assays is limited by the lack of the complexity of the physiological environment and thus leads to deviation of results. In addition, because the functions of cells can be easily lost during long-time culturing, they are limited in analysis metabolites for periods of time. With micrometer chambers and channels, microfluidics has been widely used to analyze cell metabolite [6]. Comparison to conventional in vitro cell-based assays, microfluidics technology offers various advantages. It allows simulation in vivo micro-environment, integration of functional components, high-throughput, ability to accurately control fluid flow, and automatization [7]. Importantly, with low volume and high sensitivity, microfluidics is beneficial for analyzing cellar secretions as well as suiting for single-cell analysis [3].

Looking into the past, microfluidic-based cell metabolite analysis involved various techniques, such as hydrodynamics, cell culture, separation method, and analytical method. As development of fluid propulsion in the nanolitre (nL) and picolitre (pL) range, the first microfluidic technology in biological research was developed in the early 1950s [8]. By microfluidic technology, Terry et al. [9] has realized a miniaturized gas chromatograph (GC) in the year 1979. Based on Si-Pyrex technology, the first microfluidic device was fabricated including high-pressure liquid chromatography (HPLC) column microfluidic device in 1990 [10]. During the beginning of the 1990s Manz et al. first proposed "lab-on-a-chip" or "micro total analysis systems" (μTAS), which provided controllable automation of liquid handling by microfluidic integration. Microfluidic technology for biosensor has blossomed in the early 2000s. The microdevices coupled with fluorescent imaging are feasible and highly sensitive. Meanwhile, microfluidic platform combined with highly sensitive methods can be applied to dynamic study, which is benefical to investigate the procedure of cell metabolism. Therefore, recent work of cell metabolite analysis on microfluidic platform has been focused on the following aspects: (1) study numerous mechanisms of disease and identify the regulatory pathway. (2) Investigation of dynamic mechanisms. (3) Metabolism alters cell signaling such as enzyme activity.

The microfluidic-based cell metabolites analysis is major to mimic cells in vitro. Thus, the technologies of cell culture are of significance. The original two-dimensional (2D) cell culture on microfluidic platforms is like commercial culture plates. Although simple 2D culture has been well developed, some features of the cell are eventually lost. Consequently, the cell culture on-chip are developed three-dimensional (3D) culture that are used to mimic microenvironment in vivo, finally complex 3D cultures are developed to organ on chip.

This chapter reviews the microfluidic technology are employed for cell metabolism. The incorporation of cell culture models in microfluidic systems, different microenvironment on chip, and separation and detection system is reviewed. In addition, the applications of clinical diagnostics, drug research and development, and toxicology study are discussed.

12.2 Models of Cell Culture on Microfluidic Platform

12.2.1 Cell Culture Models in Microfluidic Systems

Cell culture has played a very important role in biochemical study including clinic diagnose, drug development and screening. Different cell culture method will affect cell metabolites, and metabolites can feed back cell culture methods [11]. People have to rack their brains to develop the cells culture method to simulate the native "in vivo" microenvironments [12]. Even though some kinds of cell culture techniques are reliability and facility in amount of research, they are limited for more advanced and complex research. The interactions of cell and cell, cell and the extracellular matrix (ECM), even systemic factors are determined the differentiation and phenotype of cells in vivo [13]. The most common technologies of cell culture are the usage of dishes and flasks. These strategies cannot control the spatial distribution of the cells and others in vivo. Microfluidic has received more and more attention because it can manipulate cells such as single cell analysis [14], enhance the spatial and temporal resolution, and improve the microenvironments of cultured cells [15, 16]. As a result, a major revolution of microfluidic cell cultures is "organs-on-chips" that are highly complex models with organ-level functions. The increased spatiotemporal control makes more information achieved. Hence, the thermodynamics, kinetics as well as mechanism can be better understood.

12.2.1.1 2D Cell Culture

2D cell culture platform is the most widely used models in vitro experiments [17]. In 2D platform, cells cannot grow directly in the microchannels or on the substrate. It often requires pre-coating of ECM to facilitate cell adhesion as well as to maintain cellular function [18]. The cell cultured in microchip can simplifies the manipulation not only of single cell but also of large quantities of cells (Fig. 12.1a) because researchers can change the channel geometries and designed microchannel structures to get more functionality. Likewise, to control of spatiotemporal factors in microchip, 2D monoculture is lack of simulating the cellular microenvironment in vivo, cell coculture models are employed to simulate tissue microenvironments in vivo by co-culturing multiple relevant cell types.

Cell growth, division, survival, movement etc. have effect on the interactions of cell–cell. Cell–cell communication includes autocrine, paracrine, and juxtacrine are also altered signaling pathways. The application of microfluidic technologies can realize cell coculture, and precise control spatial and temporal by designing microchip. With these advantages, physiologically relevant distances and concentrations can be achieved, the investigation of dynamic diffusion as well as manipulation cell can be realized. Huibin Wei and co-workers designed an integrated microfluidic device that can coculture PC12 cells and GH3 cells, as a model of the nervous tissue (Fig. 12.1b) [19]. Microfluidic device combined with mass

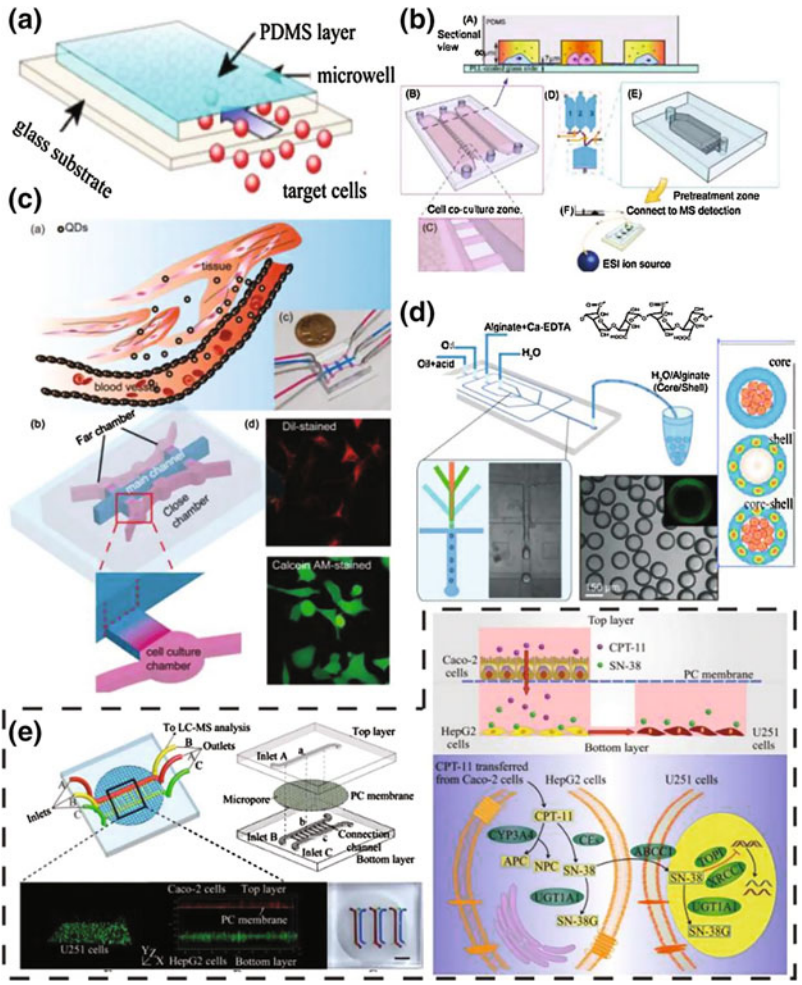


Fig. 12.1 Overview of microfluidic cell culture. **a** Monolayer 2D cell culture [30]; **b** 2D cell coculture [19]; **c** 3D cell culture [25]; **d** 3D spheroid cell culture [28]; **e** organ-on-chip system [32]. (Reprinted with permission from Refs. [19, 25, 28, 30, 32])

spectrometer make semiquantitative analysis of secreted proteins. Microfluidic cell culture is also used to study cell migration processes and their mechanism, which is different from macroscopic methods. Taking the advantages of design flexibility, real-time on-chip analysis of metabolism and low reagent consumption, Lin and co-workers developed a two-layer microfluidic cell culture device which resembles oxygen concentration gradient generator to study cell migration [20]. The microfluidic platform allowed investigation of various oxygen and distances effect on cell-to-cell communication. The cellular movement mechanisms and cell morphology are studied, and cell secretions are also on-chip detected.

Microfluidic cell culture method has ability to incorporate analytical instrumentations for the determination of living cells and cellular metabolites. One of challenges in metabolites analysis is reproduction quality. With high automation and integration, microfluidic combined with aforementioned analysis chemistry enhance the analytic precision, thereby increasing reproducibility. For instance, a two-layer microdevice was developed. This device can realize two kinds of cell coculture, and can real-time analyze the secretion proteins on the same chip. Sifeng et al. [21] developed a microfluidic system for co-culture 293 cells and L-02 cells. Cells were stimulated with different concentrations of epinephrine, and the metabolites including glucose and epinephrine were treated by on-chip solid-phase extraction and were detected directly by an electrospray ionization-quadrupole-time of-flight-mass spectrometer (ESI-Q-TOF-MS).

12.2.1.2 3D Cell Culture

Although 2D models are convenient, cells cultured by these methods often lost their functions such as differentiation due to the lack of surrounding environments. 3D culture is beneficial to understanding cellular behavior and function in their native microenvironment [22, 23]. One approach is using specific ECM [24], which can fabricate layers of extracellular matrix to achieve cell coculture [4]. Importantly, this approach reduces the lose of cell functions during the process of cell culture as well as improves tissue organization [22]. More, the secretions, nutrients and gas can be transported via the networks of 3D ECM and microchannel. In microfluidic platform, mechanical strain such as shear stress, can be controlled in the physiological range. Researchers have found cancer cells differently respond to drug when they cultured in a 3D ECM platform and in 2D platform. Jing Wu and co-workers has proposed an integrated 3D culture microdevice that can analyze quantum dot (QD) via imitating the diffusion process between blood vessels and tissues (Fig. 12.1c) [25]. The novel microdevice has been utilized with multiple constructs of 3D hydrogels and allows for a real-time monitoring cell behavior and cellular morphology exposed to both autocrine and paracrine signaling. The research showed that cell apoptosis were decreased by giving the drug 3-methyladenine (3-MA) at low concentrations of QDs, which proves that QD cytotoxicity effect on cell autophagy.

Nowadays, the most applied 3D cellular model is the spheroid [26, 27]. With cell growing, one kind of cells aggregates to form spheroids. The inner core of spheroids contains another kind of cells. The availability of oxygen and nutrients are gradually decreased from the medium to the inner core move away, which is a common tumor microenvironment. The standard in vitro model is 3D spheroid culture in the application of drug discovery and screening. Based on droplet-based microfluidics, Chen et al. [28] developed 3D core-shell scaffold by assembling hepatocytes cells and fibroblasts cells, forming an artificial liver (Fig. 12.1d). This in vitro model has potential for drug assays. Although 3D cellular models in the microfluidic technology are ECM platform and spheroid, the most applied model is

mimic vessels. Based on a blood vessel and adjacent tissue model, Wu Jing developed a microfluidic 3D-culture device that is composed of a main channel and two sets of cell culture chambers [25]. In this device, HepG2 cells were cultured in an agarose matrix on the microfluidic device. In this model, cell apoptosis, intracellular reactive oxygen species (ROS) and glutathione (GSH) were detected and QD cytotoxicity was evaluated.

12.2.1.3 Organ-on-Chip System

Building in vitro model of organ level on chip holds a promising future for drug discovery and pathological study. In vitro culture cell is an alternative for animal model. With great capacity of displaying, cell culture technique has advanced many breakthroughs in molecular biology and pathology. But surely it is retarded by not taking into consideration the complex interactions between cells and extracellular matrix and different cells. Recently, 3D cell culture incorporated with extracellular matrix has appeared as a new approach to give cell culture in a way closer to in vivo situation. Succeeding in rehabilitate cell morphology, still it fails in constructing organ-level model, and thus limits the application in pathology and drug discovery. Organ is a highly organized and functional unit consisting of different cell types and unique structures [29]. Cell co-culture methods incorporate 2 or even more cell types to study cell communication and enable partly or even fully simulation of in vivo organ. However, there are challenged about organ model building in chip, first of which lay is in the culture of primary body cell. Still, it is impossible for modern cell culture technique to launch a long term primary cell culture. Most of the existing works about organ on chip circumvented the bottleneck by using immortal cell line instead, and thus reduced fidelity to in vivo human body. A few crucial problems need to be tackled before constructing organ on chip model. Interfaces between different tissues make the barrier to separate functional parts of organ and limit flow of fluids like blood as well as myriads of signal molecular. In vitro mimicking biological interfaces can be a determining step to arrange cells or tissues into organ. One current solution is the employment of thin porous film with pores in micro scale which could stop cell but permit free exchange of cellular factors and nutrient, and herein implement cell co-culture in opposite sides [30, 31]. Jie and co-workers have developed a multi-type cell microfluidic integrator for co-cultivation and simulation of drug absorption, metabolism, and anticancer activity (Fig. 12.1e) [32]. Caco-2, HepG2, and U251 cells were co-cultured as mimics of the intestine, liver, and glioblastoma, respectively. The usage of a polycarbonate semipermeable membrane, HepG2 cell channel was separated from the Caco-2 cell channel. Then, U251 cell were cultured in anther channels through the connection with a narrow channel array. In this model, irinotecan (CPT-11) metabolism and cytotoxic analysis were performed simultaneously. These studies have revealed that 3D cell cultures have a great advantage in dynamic metabolism study, which has a potential application for high throughput drug screening and personalized cancer therapy. The appropriate tissue structure, complex biochemical

environment and dynamic mechanical microenvironment such as fluid shear are important for fabricating an organ-on-chip system [33]. Fortunately, microfluidic coculture systems can integrate to study the interaction of different cells for simulation physiology, which have the potential for fabrication human-relevant disease models. Based on 3D and organ-on chip technologies development, they have potential to study not only the absorption of drugs from the lung, metabolism in the liver and others, but also prediction clinical responses.

12.2.2 Controlling the Microenvironment In Vitro by Microfluidic Technology

Metabolites assay is aimed to quantify the level of all intermediates of metabolism. It is well known that changes of cell metabolites greatly depending on cells reside in a milieu. Chemical and mechanical parameters are important for the microenvironment (Fig. 12.2a). The surrounding of cells often consist of soluble factors, cell–matrix and its interactions, and cell–cell contacts, and so on. The cells often live in a specific physicochemical environment including pH, oxygen gradient, temperature change, and osmolality. These make up a physiological environment and effect on cell metabolism. Cell microenvironment contains biochemical, physical, and physicochemical factors. Growth factors, cytokines, amino acids and other biomolecules make up of biochemical microenvironment. Combination of these elements form complex signaling pathways, and determine the fate of the cell. Studying cell microenvironments helps examining cell metabolites for understanding the progress of disease and diagnosis. Although the construction of ideal comprehensive cell microenvironment is still difficult, multiple environmental parameters can be get using soft-lithography. The major interests of microfluidics in cell microenvironmental is: (1) controlled the scale; (2) changing chip design to control the cell microenvironment, which is more close to the physiological conditions in vivo [34].

12.2.2.1 Controlled Chemical Microenvironment

Because the richness of issues, the challenge is to develop a cellular model in vivo. Chemical environment around the cell is the compositions of cell culture medium. Therefore, researchers often firstly control the cell medium in microfluidics. Chemical gradients have been to mimic natural stimuli. These gradients in human body are mainly in the role of diffusion, and effect biological processes such as cell migration, differentiation, or development. Because concentration gradients of local region are higher 2% of its diameter, cells are able to response [35]. Thus, it is required to fine control local concentration in spatial resolution. The design of microfluidic devices have good ability with controlled spatiotemporal distribution

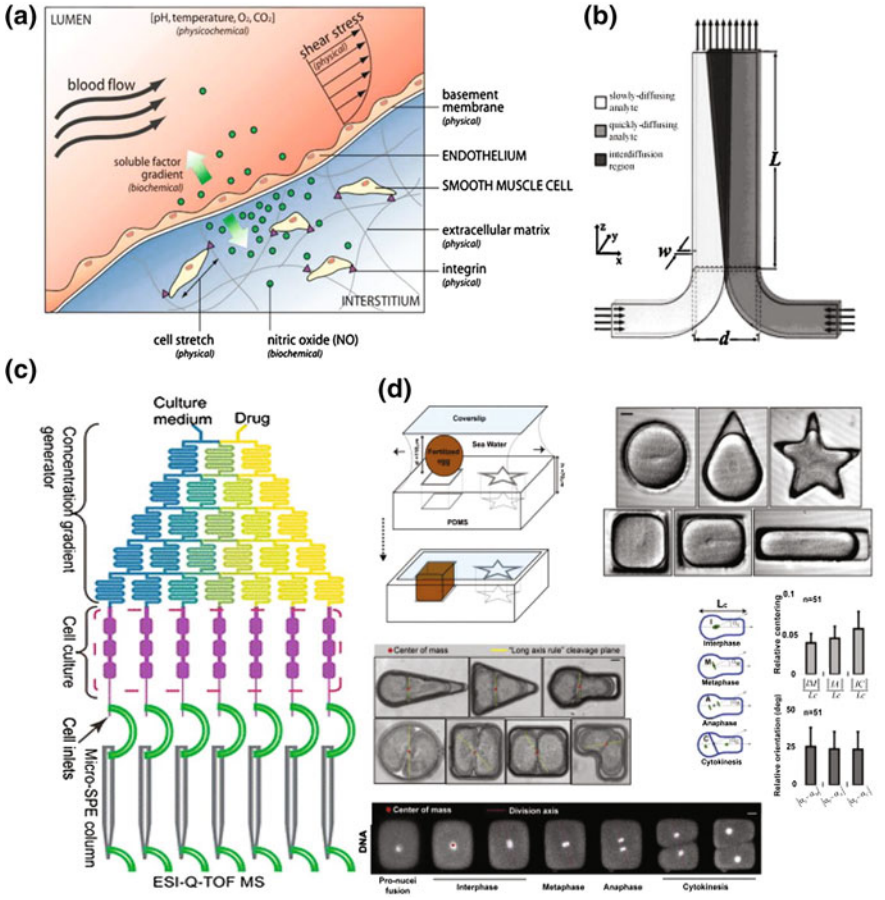


Fig. 12.2 Microfluidic plarform for fabrication cell microenvironment. **a** The cell microenvironment consists of physical, biochemical, and physicochemical factors [34]; **b** T gradient generator [36]; **c** premixer gradient generators [61]; **d** PDMS patch-clamp chip for study mechanical influence [53]. (Reprinted with permission from Refs. [34, 36, 53, 61])

and have fast response ability, which would be benefit for study the response of immune system. Researchers have successfully carried out a time invariant gradients and dynamic gradients on a microfluidic chip as well as continuous or discrete gradients based on laminar flows. Molecular gradients has been made by diffusive mixing among sever laminar streams of different composition. These gradient generators mainly depend on the flow rate and the time the streams. T-sensor is the simplest gradient generator. Time resolution of T-sensor can be achieved 10^{-2} s (Fig. 12.2b) [36], and this simple T-sensor devices are enabled to realize long-time cell culture. The simple T-sensor can also applied to study bacterial chemotaxis, endothelial cell migration. But, T-sensor device is reagent-consuming because it

often requires continuous flow. The autocrine/paracrine or cell metabolites are removed, and cells subject to shear stresses [34, 37].

One of improvement of the T-sensor is premixer gradient generators. These generators splits and recombines fluids to generate more complex gradients before enter to cell culture area (Fig. 12.2c) [15]. Dertinger et al. [38] have developed this device for generation overlapping gradients by adjusting the inlet flows. This type of device has applied to study neutrophil chemotaxis [39], neural stem cell differentiation [40]. Another kind of gradient generators including a lot of channels or chambers split the solution to generate many profiles of concentrations. A generator has been developed by Cooksey et al. [41] is composed of 16 inlets, and can combine 64 of chambers. By integrating bypass valves, it enables to format of complex biomolecules gradients at the same time with subsecond temporal resolution. Although laminar flow based on a gradient generator has been well developed, it required control of the flow rate precisely. Furthermore, Shear stresses from flow can change cells behavior and metabolism. The undesired metabolites can be found and some metabolites undergo changes. To overcome this limitation, flow resistive generators were established to eliminate shear stress around the cells. Take advantage of generation steady-state gradients, decreasing convection, and preserving metabolites by cells, the device often assembled hydrogel [42], nanopore membrane [43], or microchannel, which can promote diffusion of biomolecules to generate gradients [44].

The high permeability of PDMS has been used to control gas composition in cell cultures [30, 45]. PDMS device has been developed for generation gas gradient of the medium by controlling flowing gas. Oxygen is important for maintain growth, proliferation, and controlled cell death. Tumor cells are often incubated at 37 °C under 20% O_2 as a control, while at 37 °C under 1–5% O_2 as hypoxia because the inside tumor is found in hypoxic condition [46]. Hypoxia can increase radiation and drug resistance, and enhance metastasis of tumor cells, which is a critical factor of the tumor microenvironment [47]. Oxygen tensions on cell behavior have been commonly studied by gas gradient generator. A gas gradient generator is often formed by channels, a media chamber and a gas-permeable membrane, and then oxygen gradient microenvironment is generated by mixing carbon dioxide, nitrogen and oxygen in a predefined ratio. Furthermore, in order to obtain the desired oxygen gradients in microfluidic devices, Oppegard et al. [48] developed a microfluidic device to control oxygen tension temporally and spatially by designing multiple microchannels into a PDMS membrane to divide mixed gas. 3D cell culture in microfluidic device often need the multiple-layer microfluidic systems, and accurate control gas flow rates is a limitation. Therefore, a new microfluidic device of generating oxygen gradients was developed based on on-line chemical reaction to generate oxygen and gas diffusion. Chen et al. [49] used spatially confined chemical reactions to generate oxygen gradient in the microchip. This design applied two chemical reactions involved NaClO and H_2O_2 to generate oxygen and pyrogallol and NaOH to scavenge oxygen. Oxygen gradient was generated by the distance of

generate oxygen area and scavenging area with gas diffusion in the channel. This design simplified the structure of device and fluid flow systems, which have potential in the application of biological labs for study cellular responses although the oxygen gradients created were nonlinear.

12.2.2.2 Controlled Mechanical Microenvironment

Mechanical environment can directly affect metabolites of cells, and different metabolites can effect on mechanical environment. The common mechanical environment fabrication is to change ECM [50]. It has been reported that tumor cells show different mechanical properties to healthy cells [51]. Tumor cells are more tortuosity. This tortuosity has effect on metastasis spreading over tissues and cancer cell autophagy. Chen et al. [52] has developed a micro-device to investigate the relation between cell metabolite and mechanical environment by changing the cell culture chamber geometry. Minc et al. [53] fabricated different geometrical channels and different parameters of channels to study spatial response of cells organization (Fig. 12.2d). Chaw et al. [54] used a PDMS-matrigel device to study mechanical interactions between cells and their underlying substrates. They found that cell adhesion and mechanics were regulated by biochemical and mechanical signals. The mechanical force detection is a difficult for fabrication mechanical microenvironment. Besides of ECM, PDMS pillars or walls are applied to fabricate mechanical microenvironment of cells, which are also used to measure forces of adherent cells and substrate [55]. In most of these studies, the pillars were in the range of 2–3 μm wide, 6–10 μm high and 4–9 μm spacing apart. This method has low precision and deformable membranes have been exploited. The side force can be measured by monitoring the membrane deformation when the stress was imposed. Hohne et al. [56] developed a microfluidic device with PDMS membrane that enabled to characterize the pressure in the range of 0.1–100 kPa. Atomic force microscope, magnetic beads and so on are also applied to measure the mechanical forces, and then investigate the relativity between mechanical environment and cells.

Continuous flow microfluidic devices can control perturbation of the cellular environment in temporal and spatial resolution. Fluid flow not only transports nutrient substance, but also shear stress. Cells in a microchannel often suffer flow-stream laminar shear and splitting. Some works have showed that the migration of cells was affected fast flow of liquid caused mechanical force. Compared to fast flow, diffusion is too slow for mixing solutions, but it creates different liquid-phase environment on the changes in distance. Takayama et al. [57] applied laminar flow to study the movement of subcellular organ (mitochondria), and find that the the laminar flow can change the cytoskeletal structure of cells. In addition, magnetic field gradient are also studied by magnetic aggregates, which is used to produce cell surface stresses to investigate cell mechanobiology [58].

12.3 Strategy for Cell Metabolite Analysis on Microfluidic Platform

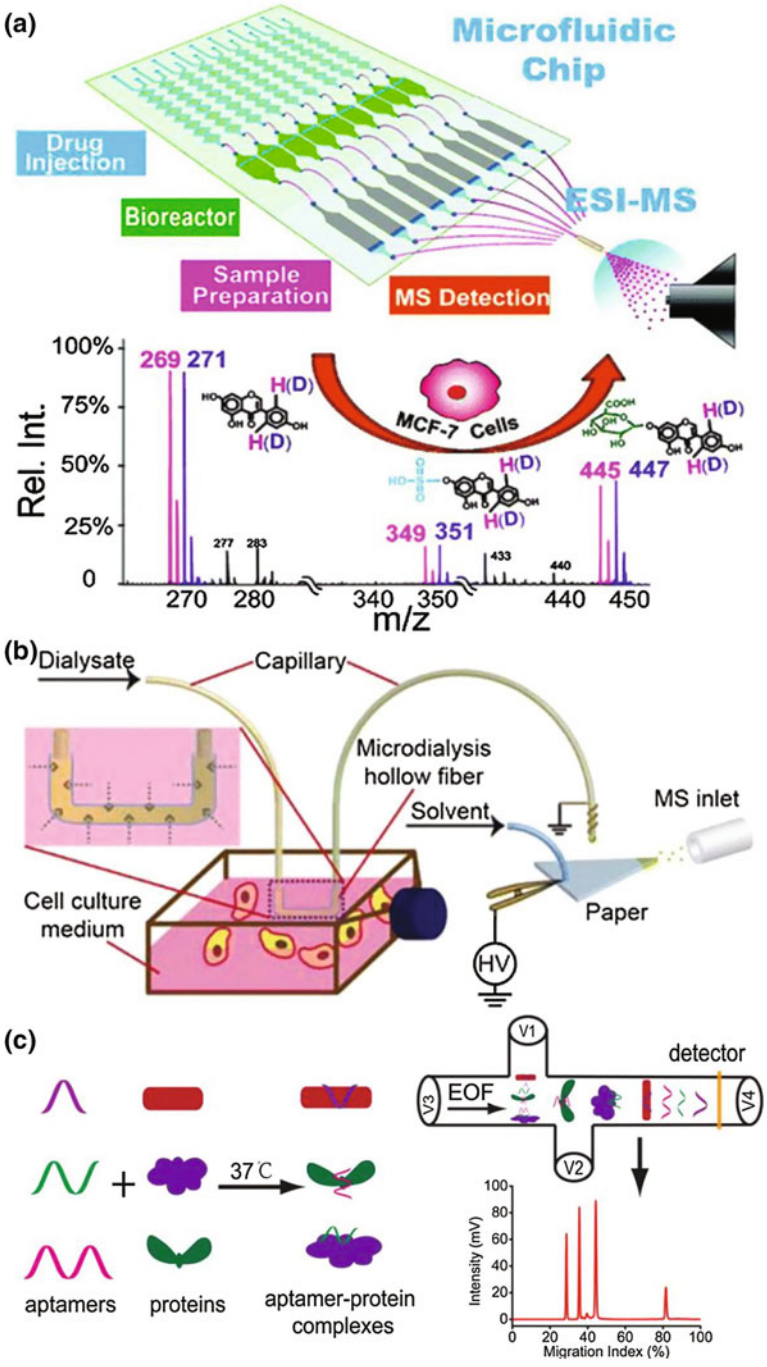
Although microfluidics are still developing, microfluidic systems are already used in many different aspects of cell metabolism and development. Microfluidic technology has the ability to integrate biosensor with separation system [59]. In combination with separation techniques possessing faster separation with smaller volumes, microfluidic chips are powerful tools for highly parallel, fast, easy and multiplexed metabolites assays. Researchers have developed sample separation technologies such as liquid chromatogram (LC), droplet technologies on microfluidic platform for cell metabolite analysis. Membrane techniques and electrokinetic separation techniques are often used for cell metabolite assay for sample pretreatment. The majoy technology of membrane technique is microdialysis sampling (MD). The frequently used electrokinetic separation techniques contained capillary electrophoresis (CE), gel electrophoresis, electrochromatography or isoelectric focusing (IEF).

12.3.1 Sample Separation on Microfluidic Platform for Cell Metabolite Analysis

12.3.1.1 LC Technology

The combination of micro separation or enrichment columns in microchip for cell metabolites treatment, and mass spectrometry or other detector can be directly detected cell metabolites. For instance, Chen et al. developed a microfluidic system for drug metabolite assay. The device consists of a microfluidic network for reagent supply, cell culture chambers and on-chip solid-phase extraction micro-columns for sample pretreatment. The quantitative analysis of drug metabolite was based on the carbonation of sample pretreatment system and stable isotopelabeling-assisted electrospray ionization mass spectrometry (Fig. 12.3a) [60]. Furthermore, this system was used to study drug-induced cell apoptosis. Gao et al. [61] also established a similar microfluidic system cell culture system to investigate on the HepG2 and Caco-2 cells absorption of methotrexate and the drug's effect. Cooksey et al. [62] applied ribosome activity inhibitor cycloheximide to investigate used green fluorescent protein decay in microfluidic cell culture system and tradition cell culture system. They found that the comparable results were obtained from different cell culture system, and microfluidic cell culture system showed higher levels of confidence.

Fig. 12.3 Strategy for cell metabolite analysis on microfluidic platform. **a** On-chip micro-SPE ▶ columns for drug metabolite analysis [60]; **b** a microdialysis-paper spray ionization-mass spectrometry (MS) system for cell glucose study [71]; **c** microchip electrophoresis for cell metabolite's analysis [73]. (Reprinted with permission from Refs. [60, 71, 73])


(a)
Microfluidic Chip
Drug Injection
Bioreactor
Sample Preparation
MS Detection
ESI-MS
100%
75%
50%
25%
0
Rel. Int.
269 271
H(D)
MCF-7 Cells
445 447
349 351
277 283
433
440
270 280 340 350 440 450
m/z
(b)
Dialysate
Capillary
Microdialysis hollow fiber
Solvent
MS inlet
Cell culture medium
Paper
HV
(c)
V1
detector
V3
EOF
V4
V2
37℃
aptamers
proteins
aptamer-protein complexes
Intensity (mV)
Migration Index (%)

12.3.1.2 Droplet Technology

Among many microfluidic techniques used for cell analysis, droplet microfluidic (including digital microfluidics) has attracted increasingly attention in biochemical assay because the low volume [14]. The volume often is pL to nL [63]. Based T-sensor devices and the developed technologies, droplets can be generated by the application of kinds of immiscible medium [64], such as a water and oil, water or gas [65]. In order to achieve uniform size, shape, and monodispersity of the droplets, hydrodynamic methods have been developed by using channel structure and properties of fluid flow because controlling droplets generation are depended on shear forces in fluids. Electrohydrodynamic technologies, electrowetting and other are also been developed to produce fine droplet in molecular biology such as gene mutation, DNA assays and molecular evolution. Chaoyong James Yang produced water-in-oil droplets for cancer biomarker EpCAM and small toxin molecule aflatoxin B1 analysis [66]. One of application is to encapsulate single-cells into individual droplets that made of hydrogels such as agarose. Then, single cell physiology and genomic content can be investigated. More, the cell-secreted molecules can be remain within the droplet, and thus can rapidly analyze. Another application is for the high-precision timing of reaction kinetics because mix pL volumes of reagents, which can be used to identifying the productivity and activity of enzymes [67]. On the other hand, with the advantages of identify such super-producing cells, droplet microfluidic technology allows for identification of cells metabolite that can be as biomarker or be used to distinguish cell type and identify genetic elements that are responsible for phenotype study. Nowadays, Chen et al. [28] used droplet-based microfluidics to reconstitute "organ in a droplet". Heterotypic cells were assembled in a 3D core-shell hydrogel to imitate liver tissue (Fig. 12.1d). These droplets can arranged multiple types of cells to create an artificial liver. Then, the time-depended albumin secretion and urea synthesis were investigated [68].

12.3.1.3 Microdialysis

Microdialysis technique is a powerful tool for analysis of cell metabolism and drug metabolism [69]. With the advantage of collective molecule of interest from the extracellular fluid, microdialysis membrane was integrated on microchip to do on-line microdialysis [70]. Furthermore, microdialysis technique can be combined with almost any detector which is very important for cell metabolites analysis with low volume requirements. The most common system is microdialysis coupled with microchip electrophoresis and laser-induced fluorescence detection. The usage of microdialysis, Scott and co-workers has developed an on-animal separation-based sensor for monitoring drug metabolism in freely roaming sheep. By the application of microdialysis, Liu et al. [71] have developed an online microdialysis-paper spray ionization-mass spectrometry (MS) platform for cell metabolism studies

(Fig. 12.3b). In this system, cell metabolite glucose rates for cells were detected, and hormone regulation of the glucose concentration was investigated.

12.3.1.4 Microchip Electrophoresis

A high-resolution separation technique is used for separation targets from complex mixtures of cell metabolites. Using microfluidics techniques, electrophoresis has been successfully integrated to microchip, and has been widely applied in various fields. Microchip electrophoresis (MCE) has the advantages of portability, simplify, rapid analysis, flexibility and low cost. The ability of this technique is to integrate sample preparation, derivatization, separation, detection and other functional units in a single device. By applying voltages to integrated electrodes, samples can be analyzed throughout channels. Analytes are separated in microchannels based on the different mobility of substances, and the length of separation channels often range from 10 to 75 mm. Electroosmotic flow is an important factor for the function of separation. Surface modification of the microchannels and changing composition of the buffer can alter electroosmotic flow, resulting in changing the separation behavior. Electrochemical methods are more portable due to it do not need derivatization, which is the most frequently analysis method coupled with MCE for the determination of cell metabolites. Holcomb et al. [3] coupled electrical conductivity detector with MCE device for monitoring metabolites such as glucose, catechins, and so on. Compared with electrochemical detection, fluorescence analysis is high sensitivity with several orders of magnitude. As an interesting development, MCE coupled with detector are employed to monitoring protein levels by aptamer-based microfluidics [72]. In addition, MCE can be used for real-time sample analysis due to its short separation time. The affinity probe for fluorescence analysis can be labeled and has been developed for multiple proteins assay containing $VEGF_{165}$, PDGF-BB, and thrombin (Fig. 12.3c) [73]. It uses non-specific DNA as an internal standard to achieve quantitative assay and reduce the interference. Finally, the established method was successfully applied to cell metabolite assay.

12.3.2 Cell Metabolite Detection Systems

Cells remain in a homeostatic state through the release of ions, small molecules, peptides, and proteins. Analysis of cellular metabolite faces analytical challenges. One is the frequent sampling collection for the dynamic nature of the release of bimolecular [74]. Otherwise, the dynamic nature would be altered average and transient events [75]. It also requires high sample collection rate, which makes low level of analysts, analytical methods with high sensitivities. In addition, cells often release a lot of substances at any given time. Thus, the assays required high selectivity for the analysts of interest. The final challenge is that cells are often in

high-salt media, which is problematic for many analytical techniques. To overcome these problems, many creative approaches have been developed. Microfluidic devices have become a powful tool for investigation of cellular secretions. Nowadays, kinds of biosensors have been designed and are applied to various fields such as diagnosing disease, food safety, and metabolites assay. A biosensor is often contained three parts: (a) a sample preparation system that ensures the capture substance is the target analysts, (b) a detection system can transform the biological phenomenon into a detectable signal, and (c) a data processing system [76]. Cell metabolites assay aims to quantify the level of all intermediates of metabolism. The mostly applied method of cellular metabolites is enzymatic assays. These assays are based on antibody. Although many antibodies are available for common peptides and proteins, it is well known antibodies are difficult to analyze small molecules or toxic compounds. To develop assays without antibodies, other methods have been established such as nucleic acid-based cell metabolite analysis, or enzymes-based assay.

12.3.2.1 Antibody-Based Immunoassays for Cell Metabolite

Antibody-based immunoassays in microsystems are the most common type in diagnostics, medical research, and disease mechanisms research and so on. Because of antibody specificity, these technologies that involved with the rapid analysis of biomolecules have been rapidly developed. In these methods, the most widely used antibody-based assay is the enzyme-linked immunosorbent assay (ELISA) [77]. A typical ELISA involves the surface subtract coated with antibodies to capture the targets, and the secondary antibodies are labeled with enzymes, fluorophores or others to detect the target [78]. Mangru and Harrison developed antibody-based chemiluminescence detection for IgG immunoassay after MCE separation. ELISA was also employed to simultaneously analyze multiple cell secretions by a fluorescence based immunosensor [79]. Recognition elements are fixed on the surface of microchannel to capture the analysts. The detection system employs a small diode laser, CCD camera, and data processing system. The signal can be correlated with image analysis software. Another method performed on chip is competitive immunoassays. This method often involved an immunoreactor with MCE separation and fluorescence detection, which has been applied in drug monitoring in human serum. Immunoaffinity chromatography assays are also established with various kinds of patterns [80]. Arash Dodge and his co-workers immobilized protein A (PA) on the surface of glass and Cy5 labled rabbit immunoglobulin G (rIgG) as sample. The behavior of immunoaffinity chromatographic in the chip was studied by using electrokinetical method [81]. Although these methods have been developed and applied, they limit sensitivity of cell metabolites assay. In the past decades, many efforts have been made to improve the

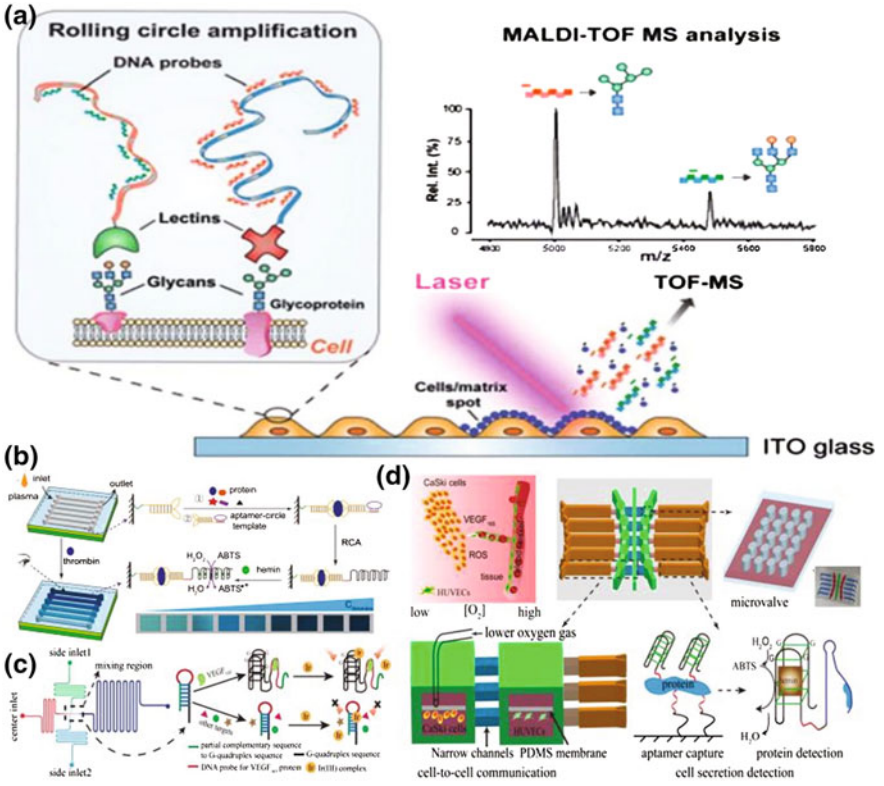


Fig. 12.4 Cell metabolite detection systems. **a** Antibody-based immunoassays for cell metabolite [82]; **b** aptamer-based biosensors coupled with RCA technology [84]; **c** real time monitoring cell metabolites with mixing device [73]; **d** microfluidic device for determination of cell metabolite with cell co-culture [20]. (Reprinted with permission from Refs. [20, 73, 82, 84])

analytic sensitivity. Heziyi et al. [82] have also developed this method for analysis of glycoproteins on cell surface (Fig. 12.4a). In this work, oligonucleotides are employed covalently linked to antibodies that recognize cell surface glycans. Upon antibody binding to lectins, the oligonucleotide probes are used as a primer, which can be utilized in the subsequent rolling circle amplification (RCA). After RCA, long single-strand DNA (ss-DNA) with repetitive sequence units is produced. After hybridizing with complementary short DNA probes, DNA duplexes are broken under laser irradiation, which makes the short DNA probes detached from the cell surface and detected by TOF-MS. This method converts the analysis of glycans to the detection of DNA probes. A benefit of it in situ proximity ligation assay is that the signal arises from specific sites in the cell, thus providing new information and improves the sensitivity and low ionization efficiency of glycans.

12.3.2.2 Nucleic Acid-Based Biosensors for Cell Metabolite Analysis

In recent years, there has been increasing interest in finding new molecules that enable to replace antibodies. Nucleic acids carry important biological information. DNA fragments store and impart genetic instructions, while RNA segments are significant for gene expression. Because nucleic acids can be rationally influenced by the base sequence, and have exciting physicochemical properties. Thus, nucleic acids have become important in cell metabolite detection because they can be used as recognition molecules, and as a material for building machine like nanodevices. The detection of DNA or RNA can be hybridized with its complementary strand with microfluidic technologies [83]. In order to improve the sensitivity, researchers often applied PCR to obtain repeated sequences of DNA fragements, and then used electrophoresis coupled with fluorescence spectrometer to separate and detect the DNA fragments. Tachibana et al. [1] have developed a self-propelled continuous-flow polymerase chain reaction in microfluidic device for amplification 232 bp of AH1pdm influenza virus and 95 bp of 16S rDNA of Escherichia coli genomic DNA. Nucleic acid-based biosensors have been widely used to detect proteins, DNA or RNA. Molecular beacon is a particular format that attracted lots of interest. Molecular beacon is often designed to various kinds of hairpin structure. This structure contains a loop domain, and a complementary sequence of analytes. A luminescence probe and a quench probe placed in start site and end site of a molecular beacon. When the target is hybrid with beacon molecules, the hairpin structure was broken, and the luminescence signal will turns on. Furthermore, as recognition factors, these probes are also applied as intracellular sensors for various molecules such as ions, proteins, DNA and RNA *in* cell *vivo*, thus enabled to do cell imaging analysis.

One of nucleic acid-based biosensors is aptamer. After the discovery of aptamers, nucleic acid-based biosensors are applied to proteins, virus, drug and even whole cells. Aptamers are single-stranded DNA or RNA molecules that are able to specific bind to targets with high affinity comparable to those of antibodies. Various aptamer-based methods have been reported in recent years, and the most common detection methods are luminescence or electrochemical methods. Lin group described immobilizing aptamer molecules on microchannel, labeling these DNA molecules with G-quadruple fragment, and replicating G-quadruple fragments, using hemin/G-quadruplex system to determinate thrombin in blood serum (Fig. 12.4b) [84]. Moreover, this method are more suitable than other methods for study the kinetics of cell metabolism because the signal can be directly obtained from analyte bind and it can decrease analytical process such as washing or labeling steps. Lin et al. have also been integrated aptasensors into microfluidic devices that could be used for on-chip aptasensor for monitoring secrets ($VEGF_{165}$, PDGF-BB, and thrombin) between cells (Fig. 12.4c, d) [20, 73]. This on-chip aptamer-based sensor is important because it extended the lifetime of the affinity biosensor, and enabled to realize the long-time monitor cell metabolism. In this case, the dynamic changes of cell metabolites are investigated.

12.3.2.3 Enzyme-Based Biosensors for Cell Metabolite Analysis

Enzymes, as catalytic biosensors, are widely applied for cell metabolite analysis. Enzyme-based biosensors are used to record analyte changes depending on time, which are the earliest biosensors in the metabolite assay. Because enzymes are not consumed, and enabled to reuse between catalyzing reaction cycles, Enzyme-based biosensors have lower detection limits over other affinity-based biosensors. Although some commercial biosensor such as glucose meter is in use, great efforts still are made. However, one of the challenges in making enzyme-based microfluidic platform is immobilized enzyme molecules and can remain functional over time. Another difficulty is to obtain the information hidden inside the cell. This requires no disruption of the cell membrane and maintain cell viability for a long time. Microfluidic technologies have their unique properties with controllable fluid transport, enzyme immobilization, and feasible manipulation, showing great potential in analysis of intracellular molecules and cell metabolites, even the whole cell. I-Jane Chen and his worker designed an interdigitated array microelectrode with enzyme-linked DNA hybridization assay into a microfluidic channel [85]. This strategy improves the limit of detection compared to conventional electrochemical biosensors. The detection of 4-aminophenol (PAP) can low to sub-nM. Batalla et al. [80] has fabricated an electrochemical microfluidic device for determination of enantiomer-biomarkers (D-methionine and D-leucine). This microfluidic strategy allowed for enantiomeric separation and covalent immobilization of the enzyme into the wall channels. In addition, hybrid polymer/grapheme based electrodes coupled with the microfluidic system enable to improve D-methionine and D-leucine analysis.

12.4 Application

12.4.1 Clinical Diagnostics

Cell metabolite assay have considerable potential in the field of clinical diagnostics. Numerous sensor technologies in the industry have potentially been used for clinical applications. The usage of microfluidic systems in metabolite assay have attracted more and more attention in the investigation of disease mechanisms and enhance understanding of pathology. The results would be potential for the monitoring disease processes, but these techniques are not mature and reliable for routine clinical practice. The challenges are the detection of low-abundance proteins or low concentration of other molecular. Great efforts have been made over the past decade and fruitful results were obtained. The most common class of diagnostic tests for metabolites analysis is the lateral flow test based on microfluidic platforms. Biomarkers are immobilized on a membrane or paper strip to analyze the targets. The most advantage is the induction of sample by capillary action without

using intervention. When samples flow across the membrane, targets are captured in the membrane with the labeling reagents embedded. Then the targets are detected by eye to form a visible band or by specific instrument. The most application is pregnancy test. The streptococcus infections are also well tested in developed countries. In developing countries, this technology is widely used for diagnose human immunodeficiency virus. Although it is simple, the test is significant in a clinic diagnostic market. During the last few decades, many people tried to improve the method, but so far there is no significant progress [86]. Another critical application of membrane and lateral flow immunoassays is blood glucose test. The glucose test has the key effect on the entire diagnostics industry, which improve diabetic patients healthy. With the development of diagnostic technologies, microfluidic device are required much more functionalization. Indeed, iSTAT system is the first commercially successful microfluidic device in the current concept based on the combination microfluidic system and electrochemical detection [86]. The clinical medicine is greatly required new microfluidic devices, especially for high sensitivity assay with an ability to quantify.

12.4.2 Drug Research and Development

The drug development is challenging high costs and decreasing efficiency of drug. The poor advanced research of preclinical models brings the unsatisfactory clinical outcomes, resulting in drug failures in clinical trial [87]. Moreover, drugs often weakly selectivity but distribute to the whole of body, causing side effects. Therefore, great efforts have been made for drug development including drug discovery and delivery, drug sensitivity and toxicity, and drug preparation. Furthermore, metabolites are the key indicators for drug research. Microfluidic device is extensively applied to drug discovery, drug delivery, drug toxicity and so on. In order to decrease the cost and time, drug discovery and development greatly requires the rapid and high-throughput drug screening platform and metabolite analysis platform. Lin group developed a microfluidic device performed high-throughput drug screening based on integrated drug gradient generator, cell culture, and sample pretreatment system. This microchip can also rapidly analyze metabolites coupled with ESI-Q-TOF MS [61]. Although traditional culture models in microfluidic device can provide lots of relatively inexpensive data, they are lack of complex physiological conditions to predict drug responses in vivo. Microengineered cell culture models provide a solution to this requirement. Xu et al. [88] have developed 3D co-culture microchip for coculturing lung cancer cells and stromal cells, which enables to imitate lung tissue microenvironment in vivo. Then, different anticancer drugs (gefitinib, paclitaxel, and gemcitabine) with different concentration were given by a gradient concentration generator inside the chips. The cell apopotosis was used to access the drug sensitivity and toxicity. Battig et al. [89] fabricated drug delivery system based on microfluidic technologies, and evaluated the system by the metabolites (VEGF and PDGF-BB). Au and

co-workers fabricated a three-dimensional hydrogel-based microfluidic system to perform drug screening. On this plarfom, HepG2 and NIH-3T3 cells were co-cultured to simulate live microtissues and as a tissue model for drug metabolism [90]. The albumin secretion profiles and cytochrome P450 3A4 are evaluated for the delivery of acetaminophen. Recently, microfluidics is applied to synthesize drug carriers. Microfluidic based on droplet and photolithography technologies has been successfully applied to make colloids of drug. Combination with aptamer and microfluidic technologies, the selectivity of the drug carriers was improved by the application of aptamer and the release time would be controlled to the desired place. Oh et al. [91] employed an efficient "click chemistry" technique to synthesize aptamer-polymer hybrids. On recognizing the targeted cell-surface marker, tumor cells were targeted and killed by doxorubicin. Furthermore, Tan group proved that aptamer-functionalized micelles were able to go through the surface of immobilized tumor cells, modeled drug delivery in the blood system, and demonstrated the dynamic specificity of aptamer–micelles [92].

12.4.3 Toxicology Study

Cell metabolite analysis has been increasingly used in toxicological studies mostly because the analytical results have the ability to provide more detailed information to elucidate mechanism of toxicity. During a toxicological study, it can be monitored time-depending on metabolites changes by drug dose or new chemicals treatment. In vitro models and analytical methods are desirable for predicting drug or other substance effects in humans. These technologies would also provide the mechanism and manifestation of toxicity based on patterns of metabolite changes. Metabolites have different kinds of properties with polarities, volatilities, chemical reactivities, and concentrations. These characteristics of metabolites and variations are greatly challenging tasks. Therefore, the selectivity, sensitivity, and continuous monitoring are the most important factors for metabolite analysis. An ideal analytical platform for metabolite analysis should have an ability to separate metabolites as much as possible and to quantify their dynamic concentration ranges. The advantages of microfluidic systems provide opportunities to realize these requirements by the combination with various detectors. Microfluidic cell culture technology provides opportunities to fabricate bioartificial organs. Organ-on-chip can perform not only cellular microenvironment but also extracellular variety with high spatiotemporal resolution. Furthermore, the metabolite can be analyzed at any time with the desired location. These properties are critical for toxicology study especially in drug discovery process, environmental pollutants and food toxicity screening. Bergström and his co-workers cultured clusters of human induced pluripotent stem cell derived cardiomyocytes in the integrated microfluidic device to simulate cardiomyocyte clusters beating. The application of this platform, the effects of drugs doxorubicin, verapamil and quinidine were assessed by the combination of video imaging system [94]. Shintu et al. [93] designed an organ-on-chip

system to imitate liver and kidney tissue. This design system is used to evaluate the toxicity of environmental pollutant such as *N*-acetyl-para-aminophenol and ammonia based NMR technology.

12.5 Conclusions and Perspectives

Microfluidic platforms with simplify, portability, miniaturization and integration have become basic research tools for cell metabolite analysis. Great progresses have been made in analysis of metabolites over the past decades especially in the drug discovery and development, clinical diagnose and environmental contaminant studies. Although many strategies have been greatly used to analyze metabolites, challenges remain and the application facilitate to more development. If these challenges are resolved and scientific concept are reliable, cell metabolite analysis on microfluidic platform will benefit an amount of domains on drug screening, contamination assessment, disease diagnosis, and personalized healthcare, altering many fields of our life. Much remains to be done, and so there are a lot of opportunities to take part in what we hope innovations and the potential of cell metabolite analysis on microfluidic platform holds.

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