



Review Paper

Development of cell metabolite analysis on microfluidic platform[☆]Luyao Lin, Jin-Ming Lin^{*}

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ARTICLE INFO

Article history:

Received 5 September 2015

Received in revised form

25 September 2015

Accepted 28 September 2015

Available online 30 September 2015

Keywords:

Microfluidic

Cell analysis

Cellular metabolism

Chip-mass spectrometry

ABSTRACT

Cell metabolite analysis is of great interest to analytical chemists and physiologists, with some metabolites having been identified as important indicators of major diseases such as cancer. A high-throughput and sensitive method for drug metabolite analysis will largely promote the drug discovery industry. The basic barrier of metabolite analysis comes from the interference of complex components in cell biological system and low abundance of target substances. As a powerful tool in biosample analysis, microfluidic chip enhances the sensitivity and throughput by integrating multiple functional units into one chip. In this review, we discussed three critical steps of establishing functional microfluidic platform for cellular metabolism study. Cell *in vitro* culture model, on chip sample pretreatment, and microchip combined detectors were described in details and demonstrated by works in five years. And a brief summary was given to discuss the advantages as well as challenges of applying microchip method in cell metabolite and biosample analysis.

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1. Introduction

Studying cellular metabolites under various environmental stimuli such as drug conditioned culture and multiple cell types interaction, can provide meaningful results for drug discovery and

a better understanding of important biological progress, especially those involved in the occurrence of major diseases [1,2] like cancer. Diagnosing methods defining metabolic species as indicators for carcinoma have been well established and applied in hospital. Also, rather than focusing on one single metabolite, the combinatorial study of several or even all metabolites in a certain time section, which is termed metabolomics, is increasingly attracting the interest of scientists [3,4]. Following the popularity of genomics and proteomics, metabolomics may provide a new

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methodology to interpret the cell biological state. However, there are several challenges remaining in metabolites profiling. Trace amount metabolites secreted from cells often exist in a complex mixture of various components such as proteins, nucleic acids and other biomolecules. The detection of a metabolite requires efficient separation and preconcentration scheme, and a highly sensitive detector. Besides, time consumption, chemical reagents and cost have to be balanced to achieve practical usability.

In the industry of drug development, analytical methods for compound metabolic pathway probing in a manner of high throughput, rapidness and great sensitivity have always been in avid demand, given the decades of time, billions of money and intensive labor working spent in getting one drug registered in USFDA. Actually, among several different screening steps concerning compound structure stability and bioactivity in drug discovery, clinical trial causes the largest expense. In the final step of clinical trial, most candidate compounds can be excluded by adverse effects or low efficacy [5,6], thus giving a dead end to all the former testings and relevant investment. And currently, clinical trial cannot be replaced by cell culture or animal experiment, because of the uniqueness and complexity of human body. One solution to reduce the time span and cost is to reconstruct a comparable human body *in vitro*, at least at the organ level, which is the basic functional unit in drug absorption, distribution, metabolism and excretion (ADME).

Owing to the development of microfabrication technique, the design and miniaturization of flow channels can be easily and cheaply completed in laboratory, which enables the wide use of microfluidics. Microfluidics offers an alternative method to conventional bench top analytical process and exhibits surprising advantages. According to scaling laws, the decrease of reaction volume adversely affects the mass/heat transfer, which means

enhancing the reactants exchanging by increasing the surface-volume ratio and shortening the diffusion distance. Therefore, chemical reactions can be more rapidly finished in a microfluidic reactor. In addition, the perfect matching between microfluidic channel dimension and cell size makes it a powerful tool for cell handling and culture, and applicable to researches such as cell positioning, sorting and circulating tumor cell (CTC) capture for cancer diagnosis. The precise control of cell culture micro-environment can be harnessed to mimic *in vivo* status. Also, the miniaturization largely reduces the reagent and sample consumption, and enables further integration of different functional modes into one chip. This integration of sample introduction or generation, pretreatment, and detection in a portable microchip is highly preferred in the analysis of analytes with low amount, to evade unwanted loss during off-line operation. The concept of lab on a chip (LOC) or micro-total analysis system (μ TAS) was proposed to realize above goals, and has been developed with considerable complexity of integration for high-throughput, parallel and fully automated screening.

Therefore, microfluidic device is an ideal candidate in profiling cellular metabolites [7–9] and drug screening [10–12] with the ability to cover cell culture, metabolite generation, separation and preconcentration as well as detection on one chip with the assistance from extra detectors like MS and fluorescence microscopy. In this review, we will go through works concerning critical aspects about application of microfluidic device in cellular metabolites analysis in recent years. As mentioned above, on-chip analysis of metabolite can be roughly divided into three steps: cell culture model establishment and metabolites generation, sample pretreatment and detection (Fig. 1)[13]. Accordingly, our review will have three main sections to demonstrate the corresponding issues.

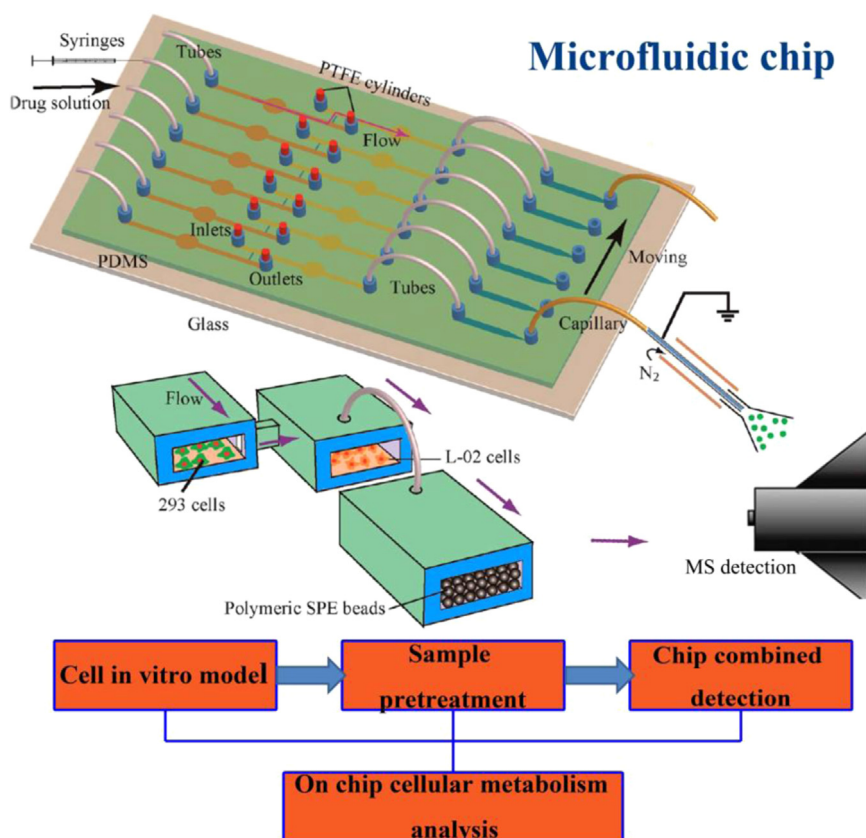


Fig. 1. Microfluidic device for cellular metabolism analysis [13].

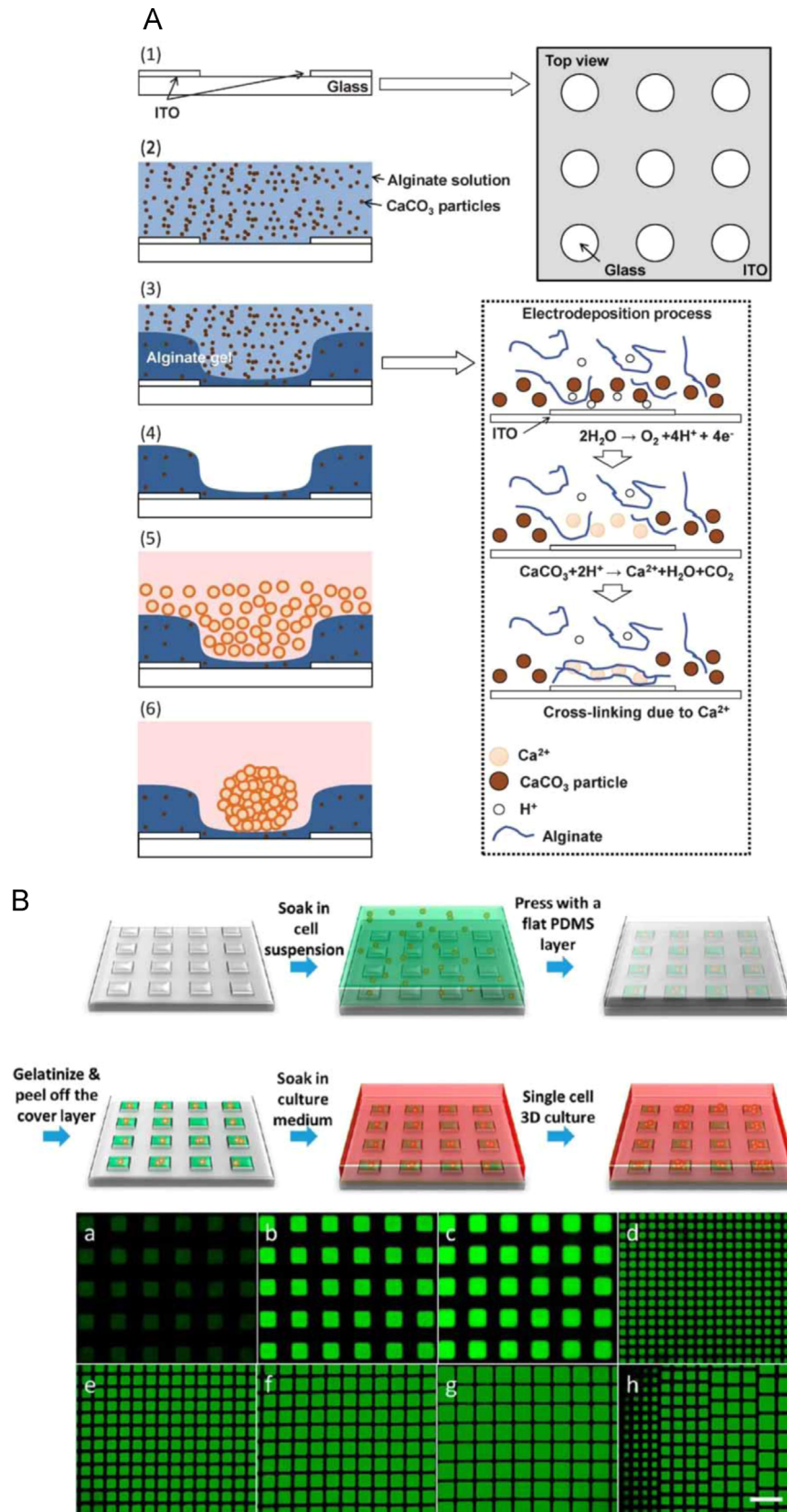


Fig. 2. Schematics of building 3D cell culture environment. (A) Fabrication of alginate microwell array by electrodeposition [25]; (B) Fabrication of microcollagen array by PDMS templating and gelation [26].

2. Cell culture on chip

The first step towards developing microfluidic platform of profiling cellular metabolites is to establish a similar cell culture environment on chip compared to *in vivo* condition [14,15]. As we all know, cells are basic building blocks constituting our body. However, in our body, the higher hierarchy structures can be much more complicated. Cells *in vivo* exist in a complex microenvironment consisting of various soluble cellular factors and insoluble extracellular matrix (ECM). These components regulate cell behaviors such as migration, mitosis and apoptosis by ligand–receptor interaction, phosphorylation, opening up the downstream signal pathway as well as affecting transcription factors. The gradient of cytokines and ECM compose the chemical microenvironment. Besides, cell–cell interaction within same or different cell types is another essential part of microenvironment. Cell–cell interaction can be regulated by secreting cellular factors or direct contact, and abnormal cell–cell interaction is an important cause of critical diseases like autoimmunity and cancer metastasis. The last component which can be easily ignored is the mechanical microenvironment. Cells in different tissues and organs may face distinct mechanical forces. Red blood cell (RBC) and leukocyte should endure the large shear forces by blood flow, and epithelium cells on the interfaces of alveolus–capillary can be stretched by the inhalation and expiration during breath. Huh et al. [16] developed a polydimethylsiloxane (PDMS) based *in vitro* lung model with porous film as alveolus interfaces to accommodate epithelial and endothelial cells on opposite site, and mimicked the breathing stretch by adding two pressure tuning side channels. When vacuum was applied to side channels, the elastic PDMS walls generated deformation and gave pulling force on the cell culture film, thus resulting in the stretch similar to an actual lung. The model was applied to investigate the cell toxicity of nanoparticles. Also, other microfluidic devices have been developed to systematically study the effects of mechanical microenvironments, such as fluidic shear stress [17] and surface strain [18,19], with tunable magnitude of forces upon monolayer of endothelial cells. Results revealed that cell orientation and formation of cell skeleton were significantly influenced by mechanical stress. Actually, 2D endothelium is especially suitable for the investigation of membrane stress due to its tendency to form integrated monolayer and easy combination with fluorescence imaging and other optical observation methods. With the assistance from pressure-mediated microvalve and precisely controlled microfluidic system, regionally selective and magnitude determined mechanical stimuli are easily achieved. Several strategies are provided to realize the construction *in vitro* culture model after the basic elements of which were reviewed.

2.1. 3D cell culture

Conventional dish culture of cell gives 2D monolayer structure, where cells adapt to show flat morphology. While *in vivo*, cells are more likely “floating” in the ECM, supported by the various structural proteins like collagen, laminin and fibronectin, thus presenting more extensive shapes. The differences of 3D and 2D culture are not limited in the morphology. Evidence shows shapes under 2D culture, cells lose functionality [20], go dedifferentiated [21] and exhibit different expression levels of several important genes [22]. In contrary, 3D culture helps cells maintain phenotypes even after weeks of *in vitro* culture [23,24]. One scheme to implement 3D culture is to accommodate cells in biocompatible hydrogen such as agarose, alginate, matrigel and collagen, supplemented with ECM components for a better cell attachment. Ozawa et al. [25] developed an alginate 3D model for embryonic stem cells and HepG2 culture based on electrodeposition of

alginate gel (Fig. 2A). Indium tin oxide (ITO) electrode is selectively patterned on substrate surface, upon which the electrolysis of water produces H^+ , dissolving the $CaCO_3$ particles in alginate solution, thus forming microwells of alginate gel. Embryonic bodies and HepG2 spheroids were formed in 3D culture, which demonstrated the validity of culture platform. Guan et al. [26] developed a facile and rapid method to generate a large microcollagen array for long-term cell culture and screening of drug resistance heterogeneity (Fig. 2B). Cell culture chamber was fabricated by fulfilling PDMS microwells with cell collagen suspension, and sequentially gelatinized to form 3D culture gel. Long-term culture was maintained as long as 30 days at single cell level, with the ability to retrieve any cell of interest for further examination. Collagen based 3D microtissue culture models for cell viability assessment [27] or immunoassay of cytotoxicity [28] under drug stimulation were also reported, which were compatible to microplate reader. This platform provides a high throughput and quantitative method for drug screening and better prediction of drug efficacy at the tissue level.

Droplet technique in microfluidic has always been highlighted especially in the field of biosample analysis, due to its precise control of defined volume, minimized reagent consumption and prevention in cross contamination. Microfluidic droplet is broadly applied in constructing 3D culture models with clever art of hydrogel gelation. In a recent work from Utech et al. [29], calcium ethylene diamine tetraacetic acid (Ca-EDTA) chelate was mixed with alginate solution as the dispersed phase. After the alginate droplet was made at nozzle by flow focusing, H^+ in oil phase gradually diffused into the microdroplet and released Ca^{2+} which further solidified the alginate. Besides, the gelation process can also be conveniently controlled by sequential adjustment of the temperature for agarose or matrigel based droplet, or selective exposure to ultraviolet (UV) for PEG hydrogel. Microfluidic droplet technique realizes monodispersed, and highly uniformed gel droplet for cell encapsulation at the single cell level, providing a platform for high throughput cell culture and screening.

Combined with microarray presentation, parallel experiments can be performed in multi-parameters drug screening. Du et al. [30] reported a droplet based microfluidic system for drug combination screening (Fig. 3). In this system, droplet is confined in the array of microwells which are covered by a layer of oil to isolate each droplet. Sequential operations such as cell seeding, drug addition and stimulation, and fluorescence probing can be completed by a tapered capillary. The system was validated by exploring the effects of drug combination based on cell viability testing.

2.2. Cell co-culture

Any tissue or organ with certain function in human body is composed of large cell populations and multiple cell types. Interactions between multiple cell types constitute a stable and functional organism, while the absence of proper intercellular interaction may severely alter the behavior of cells. It has been reported that complex interaction between cancer and endothelial cells mediated by cell factors such as vascular endothelial growth factor (VEGF) and transforming growth factor- β (TGF- β) would increase the drug resistance of tumor and induce angiogenesis [31,32]. Therefore, in the aspect of drug metabolite profiling and efficiency testing, data from single cell type experiment can be rather limited. Under this circumstance, cell co-culture model is being paid intensive attention to improve the traditional screening platform. In a work of our group, a CaSki cells and human umbilical vein endothelial cells (HUVECs) co-culture model under different levels of oxygen (5% and 15%) was developed to study the cell–cell communication in cervical cancer development (Fig. 4) [33].

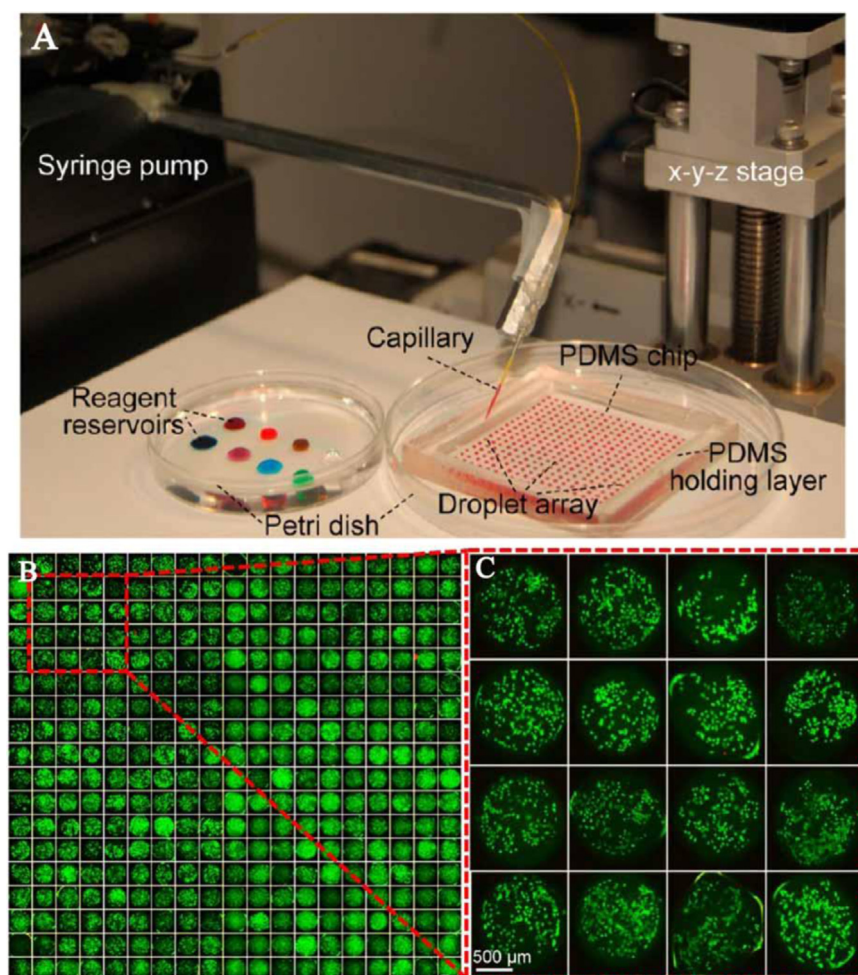


Fig. 3. Cell culture droplet array for drug combination screening. (A) Photo of device set up; (B), (C) Fluorescence images of cell culture array with A549 cells [30].

VEGF₁₆₅ was semi-quantitatively analyzed by functional nucleic acid and chromogenic system online, and other metabolism related species such as reactive oxygen species (ROS) were also studied. Another work by Mao et al. [13] suggested a cell co-culture model to emulate the epinephrine communication between 293 and L-02 cells by connecting two cell culture chambers in the dynamic on-chip culture. When acetylcholine is infused into the culture system, 293 cells on the upstream are stimulated and thus produce epinephrine, which will affect L-02 cells on the downstream chamber and enhance its glucose secretion. The final metabolite, glucose, as well as signaling molecule, epinephrine, was successfully detected by electrospray ionization-quadrupole-time of flight-mass spectrometry (ESI-Q-TOF-MS). This work provides a useful platform to study the influence of cell-cell communication upon metabolic pathway.

2.3. Organ on chip

Organ is the basic functional unit in drug metabolism, and the ADME process concerning drug efficiency is actually carried out at the organ level. To have a systematic view of drug metabolic pathway, one has to construct a model more closely emulating organ functions rather than a single or a few cell types co-culture, which is termed “organ on chip” or even “body on chip” [34–36]. Among all kinds of organs, liver is the most frequently investigated one because of its high metabolic activity in drug metabolism. Zhang et al. [37] reported an *in vitro* liver model with the functionality of metabolizing the prodrug capecitabine to produce an

anti-cancer intermediate (Fig. 5A). HepG2 was cultured to emulate the liver function, while MCF-7 cell was a representative of tumor. Prodrug and its intermediate were detected by on-line solid phase extraction (SPE) and mass spectrometry (MS) with a limit of detection (LOD) of 10 nM. Imura et al. [38] developed a more complicated biosystem to assess the drug absorption of intestine and metabolism of liver as well as its bioactivity on target cells (Fig. 5B). Caco-2, HepG2 and MCF-7 were cultured on chip to represent the modules of intestine, liver and tumor, respectively. The platform provides an efficient assessment to the oral administrated medicine.

Organ on chip or body on chip is a potential tool which may greatly promote the clinical treatment, especially point-of-care diagnosis and personal specialized therapy. However, one challenge remaining to be solved is how to accommodate dozens of cell populations and sub-cell populations into an *in vitro* model with proper proportion and position [35]. For example, there are entities of hepatic cells, vascular endothelial cells and preicytes, and other immune related lymphocytes in liver. The culture technique for some cell types is still immature and unsatisfactory. But hopefully, with the assistance of newly emerging methods such as bioprinting [39,40], one day we may construct an “organ” with more complex architectures and more power of control.

3. Sample pretreatment on chip

After the construction of biomodel on chip and drug stimulation, the following procedure is to detect drug metabolites with

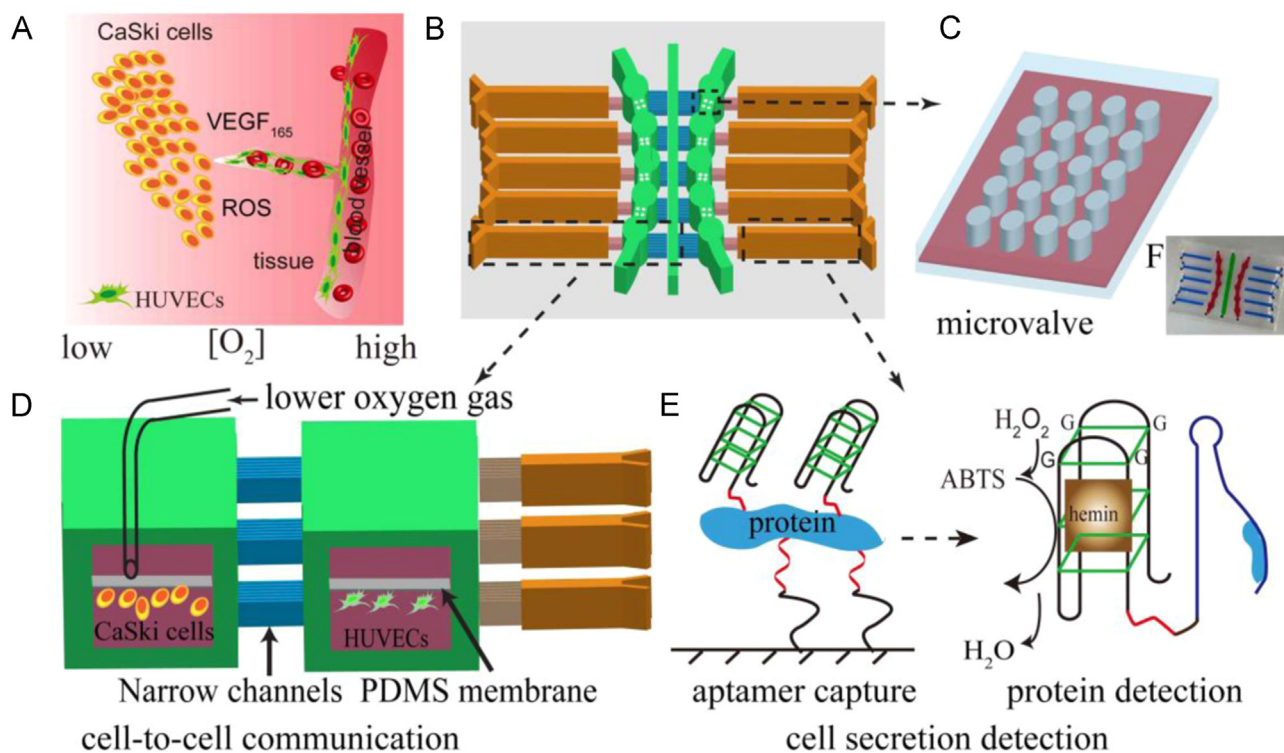


Fig. 4. Cell co-culture microfluidic system under different oxygen gradients. (A) Oxygen induced cell-cell communication; (B) Illustration of microchip structure; (C) Microvalve constituted by micropillars; (D) Two-layer cell co-culture device under low oxygen condition; (E) Schematics of cell secretion detection [33].

the assistance of an external detector. However, the sample pretreatment has to be carried out on microchip in advance, because the complex compositions among culture medium may severely inhibit the ionization efficiency and interfere the detection of a target molecule of low abundance or trace amount. Considering the limited volume of analysis system, pretreatment, roughly divided as preconcentration and separation, must have low sample consumption. And on-line pretreatment is preferred to increase the integration and automation of the platform, while avoiding unnecessary sample loss occurred in off-line method.

3.1. Preconcentration methods

Our group has published a series of works employing micro-SPE columns integrated to cell culture chip for establishment of drug cellular testing platform [41], study of cell-cell communication [13] and drug metabolism [42,43]. Commercialized SPE beads were infused and immobilized in a microchamber with shrinking ends to receive the upstream cell culture medium with drug metabolites. Analytes were captured and thus desalted by the micro-extraction process. When elution buffer was added, the dissolved metabolite was online analyzed by ESI-MS. Highly parallel experiments can be performed on the same chip by arranging multiple cell cultures and SPE channels to investigate the effects of different concentrations of a drug.

Another method developed by our group realized the extraction of cell metabolites from culture medium via microdialysis (Fig. 6A) [44]. A homemade microdialysis hollow fiber was immersed into cell culture medium, and as dialysate flowed into the fiber, glucose from culture media diffused through dialysis interface. The other end of the fiber came to paper spray ionization for MS detection. With the continuously flowing dialysate and generating droplets, on-line monitoring at glucose level in cell culture medium was achieved with the shortest time interval of 1.5 s. This work demonstrated a potential platform with label free MS detection for the monitoring of cellular culture system.

3.2. Microseparation

Chromatography is a highly efficient method for the separation and identification of analytes among complex matrix. There are standards of drug metabolite analysis based on the mature gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS) techniques. For microseparation, microchip electrophoresis (MCE) assumes the most successful miniaturization of chromatography, including operations of sample introduction, derivatization, separation and detection in a confined small area of few square centimeters. MCE is applicable to analysis of genes, amino acids, proteins and even single cells. The principle of MCE is based on the employment of electro-osmotic flow, the rate of which can be controlled by modifying inner surface or adjusting buffer composition, thus realizing the separation of substances with different motilities. Frequently used detecting methods coupled to CE separation include UV absorbance, fluorescence and electrochemical sensor. Normally, absorbance test requires an enough high concentration of analyte, or a large molar extinction coefficient, which is often inapplicable to cell metabolite analysis. Although fluorescence and electrochemical method are frequently used in metabolite detection, each of them has their own advantages. Fluorescence provides significantly better sensitivity even at the single molecule level, but specific probes are necessary to label the targets. For electroactive substances, electrochemical method is label-free without requirement of derivatization or extra bulky device which increases the portability of the device. For instance, electrochemical method is an ideal tool for saccharides analysis, because there is no functional group for the addition of fluorophore, neither do saccharides have large molar extinction coefficient. Our group reported a microchip-CE method for assay of multiplex cell metabolism related proteins (Fig. 6B) [45]. Aptamers for recognition of platelet-derived growth factor-BB (PDGF-BB), VEGF₁₆₅ and thrombin were designed to have different lengths, which modulate the electrophoretic mobility of respective proteins by specific bonding. The

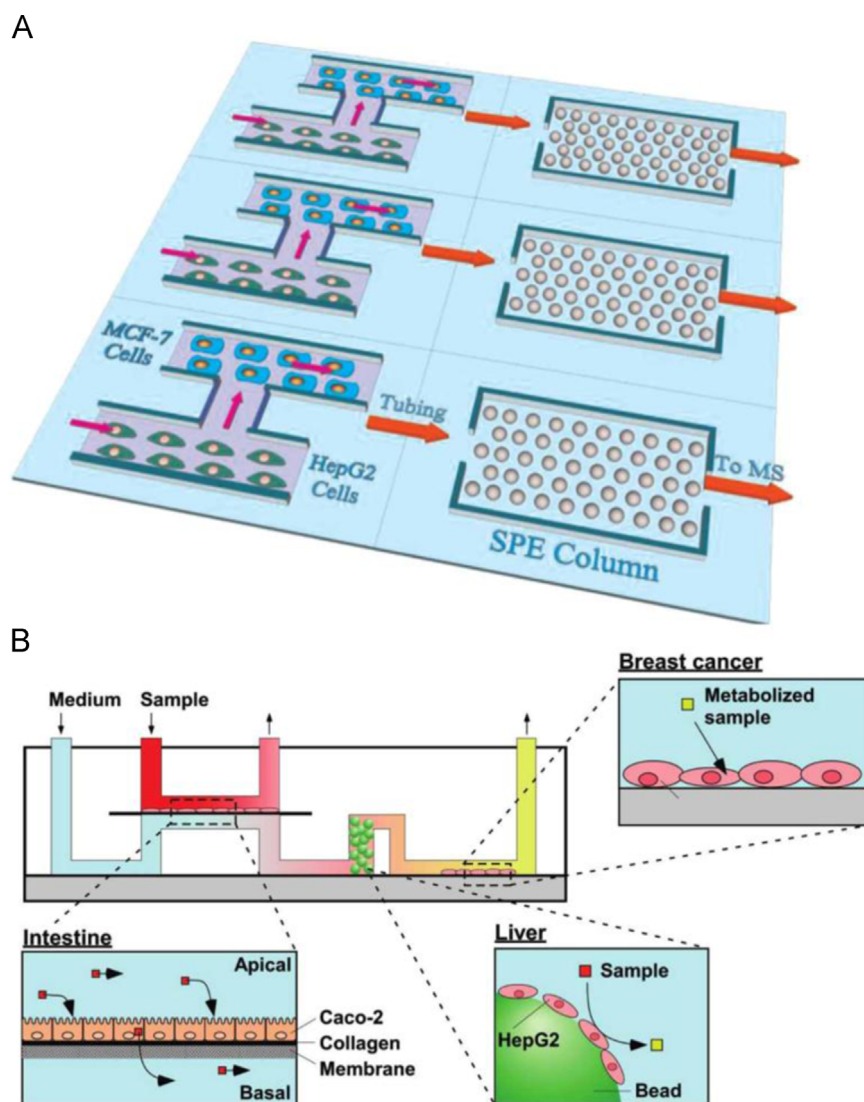


Fig. 5. Schematics of constructing on chip organ model. (A) Multiple parallel channels for cell culture, metabolism and metabolite extraction in an *in vitro* liver model [37]; (B) Assessment of drug absorption, metabolism and effect on tumor by intestine-liver-cancer model on chip [38].

employment of SYBR gold staining increases the sensitivity while reduces the matrix interference. There are other works concerning the application of MCE in metabolites probing with combination of electrochemical [46] or ESI-MS detection [47]. The sensitivity and quantitative ability of CE can be further strengthened via modification of sample introduction manner such as inkjet direct sampling. (Fig. 7) [48].

Another miniaturization by integrating LC column into a microchip combined with MS detection also demonstrates a great capacity for metabolite analysis, which can be termed microchip-LC-MS. Compared with conventional LC, microchip-LC separation is much faster and costs lower sample consumption, thus largely increasing the throughput. Sainiemi et al. [49] reported a silicon LC system on chip with micropillars functionalized with SiO₂ or C₁₈ coating as a basic separation unit. Separation can be completed in 5 min with good sensitivity of MS or fluorescence detection. Also, microchip based liquid chromatography system has been well established and commercialized by several companies [50].

4. Detection

As mentioned above, commonly used detectors for microfluidic cellular analysis platform include fluorescence microscopy,

electrochemical and MS methods. High sensitivity, good specificity, low cost and short time period are preferred characteristics for a detector, especially in microanalysis of complex system (for a detailed and comprehensive discussion about detectors suitable for biospecies detection, readers can refer to previously published review [51]). But sometimes, the size of detector may determine the usability of a platform, if portability is in high favor. For example, electrochemical method can be miniaturized to a micro-electrode, which can be selectively patterned in microfluidic channels and realize region confined detection. Several works reported a patch type metabolite monitor with an array of micro-electrodes on needle tip for non-enzymatic detection of interstitial glucose level [52] or lactate level [53]. Although in animal models, the method may be short-lived compared with *in vitro* experiments, it is still a potential complement to disease therapy like diabetes. Besides, the integration of features in nano scale may greatly increase the detection performance by exhibiting large contact area and thus gaining high probability in capturing target molecular or target cells [54]. But in general, MS is the most powerful tool in metabolite analysis, due to the abundant structural information given in a MS spectrogram. With the ability to complete simultaneous detection of multiple analytes by different charge-to-mass ratios, the analytical capacity of MS covers

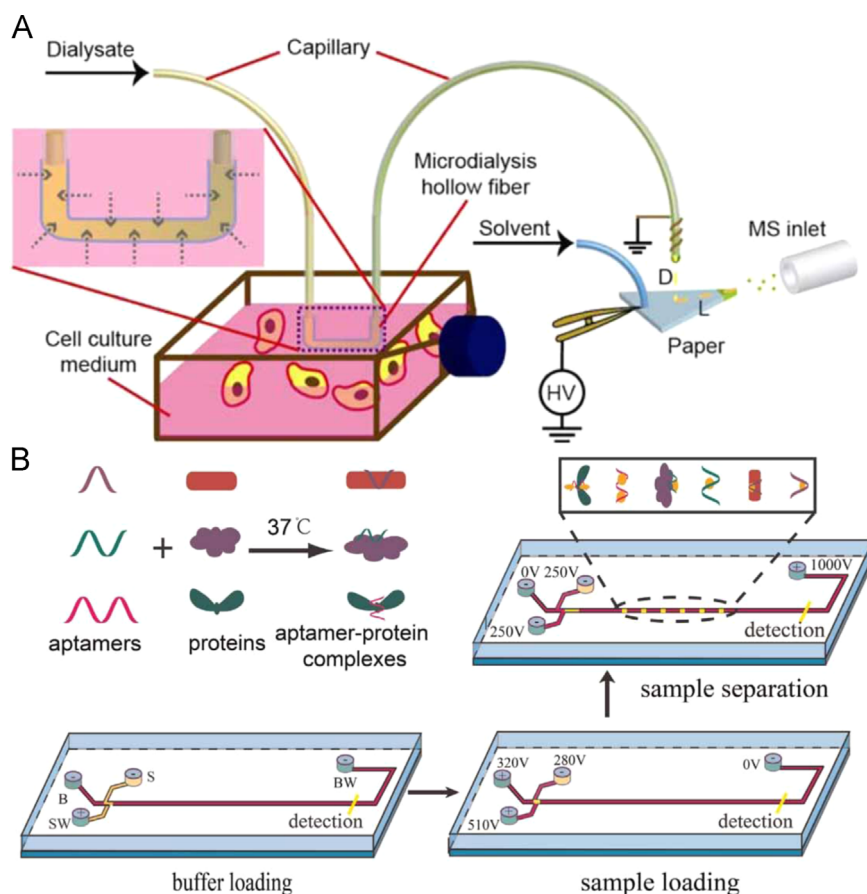


Fig. 6. Cell metabolism related sample preconcentration and microseparation methods. (A) Microdialysis to extract glucose for paper spray ionization from cell culture medium [44]; (B) Cell metabolism proteins assay based on tunable aptamer and microchip-CE [45].

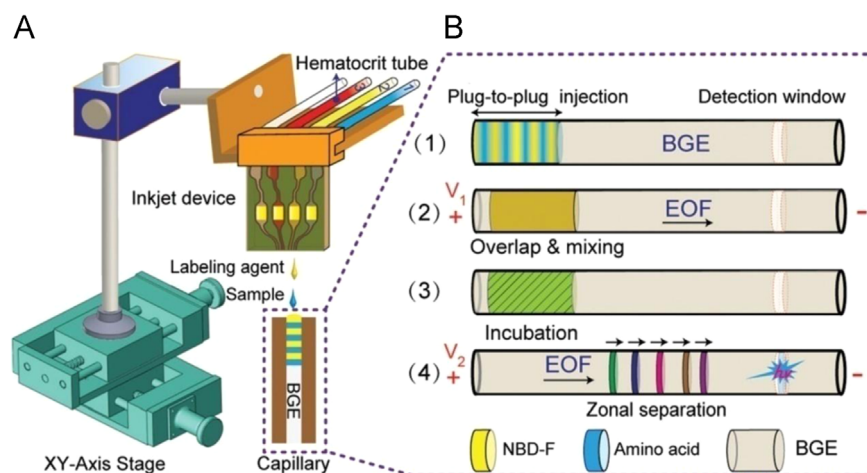


Fig. 7. Drop-by-drop sample introduction by ink jet for CE method. (A) Illustration of ink jet sample introduction to CE; (B) NBD-F derivatization of amino acids by drop-by-drop sample introduction [48].

compounds and biomolecules from small molecules to peptides, proteins and nucleic acids. Recently, the rapid development of high resolution MS pushes LOD to fmol. Besides, fast speed of over one hundred times full-spectrum scanning per second is compatible and applicable to probe biochemical reactions completed in milliseconds inside cell. As for drug metabolite identification, the composing elements and structural information of drug compound can be easily obtained by multiple determining methods [55,56]. In microchip-MS combination, one critical issue is the interfaces between microchip flow and MS ionization region. Therefore, ionization methods such as ESI, paper spray and matrix

assisted laser desorption ionization (MALDI) are carefully reviewed to address above problem.

4.1. ESI

ESI method enables direct ionization of target molecule in liquid buffer, and is often conveniently combined with LC separation. For microchip-MS method, the flow rate of microfluidic dynamic culture system is comparable to nanoflow ESI, and via external connecting silicon fused capillary, sample can be directly introduced from microchip into ESI with on-line SPE channels (Fig.

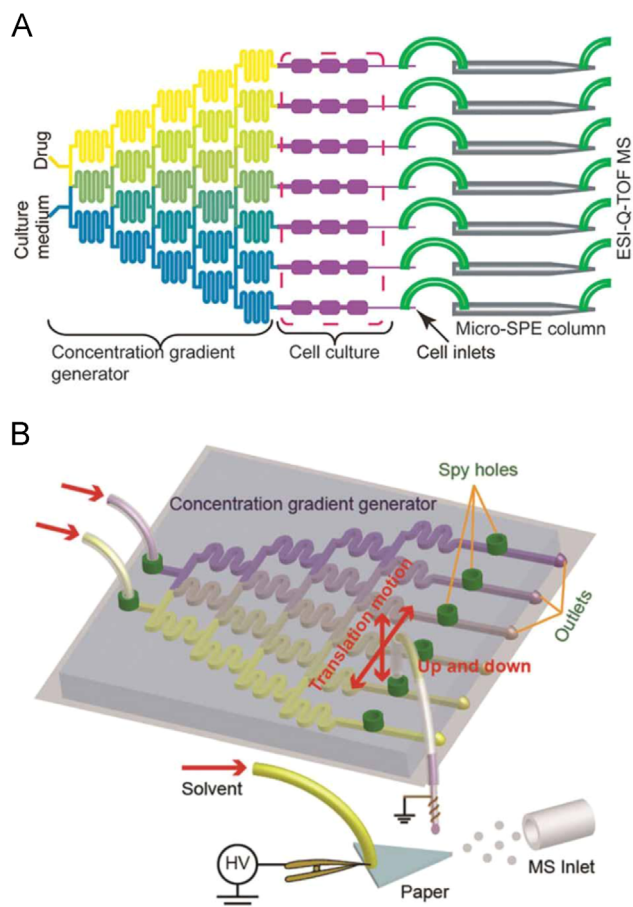


Fig. 8. Microchip-MS interfaces with multichannel set up for concentration dependent screening. (A) Combination of microchip to ESI-MS detection [41]; (B) Combination of microchip to paper spray MS detection [64].

8A) [41]. Several works also reported microchip based emitters for electrospray composed of PDMS [57] or glass material [58,59]. The integration of electrospray emitter onto microchip gives better combination to other on chip functional blocks such as MCE, and further increases the sensitivity as well as throughput by densely arrangement of multiple nozzles. Mao et al. [60] introduced a multi-nozzle emitter array consisting of 96 10-nozzle emitters in a circular array on a silicon chip. The platform provided advantages such as no cross-contamination and highly parallel experiments.

4.2. Paper spray

Paper spray is a facile, cheap and highly efficient ionization method for qualitative and quantitative MS analysis of complex samples such as dried blood [61], fruit juice [62] and body metabolites [63]. Paper spray presents great matrix tolerance with simple sample introducing fashion. Buffer with analyte is directly dripped onto paper surface, and as the paper gets wet, the analyte driven by electric field gradually moves to the tip and spray. In the whole process, electro-inert substances remain still, thus realizing a separation of target molecule from matrix. Our group has developed a multichannel microchip device with a movable capillary to sequentially aspirate different samples in different channels (Fig. 8B) [64]. The sample was transported in droplet and separated by air plug until it fell onto the paper surface and sprayed. The process was in full automation and controlled by the computer, which made the device a potential platform for high throughput screening of biomolecular interaction or cell

metabolite under drug stimulation.

4.3. Maldi

MALDI is well tolerant to salt and non-volatile components of buffer system. But in the region of molecular weight under 500 Da, fragments from matrix composition significantly raise up the background noises which are unfavorable to small molecule analysis [65]. Due to the vacuum condition required by MALDI, the on-line coupling of microchip to MALDI is rather difficult. Instead, off-line methods of depositing samples onto MALDI target plate after microchip operation are well developed. A series of works about constructing a micro-bioreactor by covalently immobilizing trypsin onto glass fiber [66] or microchannel inner surface have been reported [67,68]. Proteins such as hemoglobin and cytochrome c were digested by trypsin and further collected for deposition to MALDI target and MS detection. With the assistance of microfluidic reactor, time period for proteolysis can be reduced to less than 5 s. The platform demonstrated great capacity for identification and analysis of complex protein samples. Mikkonen et al. [69] reported an open microchannel for charged sample pre-concentration and direct usage as MALDI target plate. Peptides of cytochrome c digested by trypsin were pre-concentrated at different positions of microchannel, and determined by pH gradient of water electrolysis. MALDI-MS detection was carried out by depositing matrix and enhancing crystallization into channel. This work provides a direct combination of microchip-MS method, thus evading the possible cross contamination and sample loss.

5. Conclusions and perspectives

Construction of cell *in vitro* model, on chip sample pretreatment and detection are three critical parts concerning a successful integrated microchip method for cellular metabolism study. Since the appearance of microfluidic chip, lab on chip method has always been paid intensive attention, especially in the field of bio-sample analysis. Microfluidic platform has been proved an ideal candidate for cell metabolism analysis with at least four advantages:

- i. The micro scale of channel geometry provides precise control of cell culture microenvironment and delicate cell manipulation scheme;
- ii. On-line separation and preconcentration methods avoid the loss of sample and increase the detection sensitivity;
- iii. Microchip interfaces are compatible with various detectors such as MS, fluorescence microscopy and electrochemical sensor with little or without necessary further modification, which strengthens the capability of metabolite detection;
- iv. With integrated functions and multiple parallel channels, microfluidic platform allows operation in full automation with high throughput.

However, there are still challenges before a wider application of microfluidic platform come to drug screening and cellular events study. How can we establish a proper cell culture model with perfect simulation of tissue or organ functions meanwhile allowing transparent and thorough investigation of the system? How will the separation and detection method be developed to probe components inside cells of less content but with important biological significance? Addressing these questions may require hard work and wisdom of generations. But actually, microfluidic platform has already revolutionized the way of bioanalysis and life analysis.

Acknowledgments

This work was financially supported by National Natural Science Foundation of China (Nos. 81373373, 91213305, 21227006) and CERS – China Equipment and Education Resources System (No. CERS-1-75).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpha.2015.09.003.

References

- [1] R. Zenobi, Single-cell metabolomics: analytical and biological perspectives, *Science* 342 (2013) 1243259.
- [2] A.R. Fernie, R.N. Trethewey, A.J. Krotzky, et al., Metabolite profiling: from diagnostics to systems biology, *Nat. Rev. Mol. Cell Biol.* 5 (2004) 763–769.
- [3] G.J. Patti, O. Yanes, G. Siuzdak, Innovation: metabolomics: the apogee of the omics trilogy, *Nat. Rev. Mol. Cell Biol.* 13 (2012) 263–269.
- [4] M. Baker, Metabolomics: from small molecules to big ideas, *Nat. Methods* 8 (2011) 117–121.
- [5] J. Arrowsmith, Trial watch: phase II failures: 2008–2010, *Nat. Rev. Drug Discov.* 10 (2011) 328–329.
- [6] J. Arrowsmith, Trial watch: phase III and submission failures: 2007–2010, *Nat. Rev. Drug Discov.* 10 (2011) 87.
- [7] J.H. Sung, M.L. Shuler, In vitro microscale systems for systematic drug toxicity study, *Bioprocess Biosyst. Eng.* 33 (2010) 5–19.
- [8] J.R. Kraly, R.E. Holcomb, Q. Guan, et al., Review: microfluidic applications in metabolomics and metabolic profiling, *Anal. Chim. Acta* 653 (2009) 23–35.
- [9] Q. Zhuang, S. Wang, J. Zhang, et al., Nephrocyte–neurocyte interaction and cellular metabolic analysis on membrane-integrated microfluidic device, *Sci. China Chem.* (2015) 1–8, in press.
- [10] M.H. Wu, S.B. Huang, G.B. Lee, Microfluidic cell culture systems for drug research, *Lab Chip* 10 (2010) 939–956.
- [11] P. Neuzi, S. Giselbrecht, K. Länge, et al., Revisiting lab-on-a-chip technology for drug discovery, *Nat. Rev. Drug Discov.* 11 (2012) 620–632.
- [12] P.S. Dittich, A. Manz, Lab-on-a-chip: microfluidics in drug discovery, *Nat. Rev. Drug Discov.* 5 (2006) 210–218.
- [13] S. Mao, J. Zhang, H. Li, et al., Strategy for signaling molecule detection by using an integrated microfluidic device coupled with mass spectrometry to study cell-to-cell communication, *Anal. Chem.* 85 (2013) 868–876.
- [14] K. Ziólkowska, R. Kwapiszewski, Z. Brzózka, Microfluidic devices as tools for mimicking the in vivo environment, *New J. Chem.* 35 (2011) 979.
- [15] J.H. Sung, M.L. Shuler, Microtechnology for mimicking in vivo tissue environment, *Ann. Biomed. Eng.* 40 (2012) 1289–1300.
- [16] D. Huh, B.D. Matthews, A. Mammoto, et al., Reconstituting organ-level lung functions on a chip, *Science* 328 (2010) 1662–1668.
- [17] X. Zhang, D.J. Huk, Q. Wang, et al., A microfluidic shear device that accommodates parallel high and low stress zones within the same culturing chamber, *Biomicrofluidics* 8 (2014) 054106.
- [18] Q. Wang, X. Zhang, Y. Zhao, A multiplexed micromechanical cell stimulator for studying magnitude-dependent cell responses, *Microfluid. Nanofluid.* 18 (2014) 415–425.
- [19] Q. Wang, X. Zhang, Y. Zhao, Micromechanical stimulator for localized cell loading: fabrication and strain analysis, *J. Micromech. Microeng.* 23 (2013) 015002.
- [20] F. Pampaloni, E.G. Reynaud, E.H.K. Stelzer, The third dimension bridges the gap between cell culture and live tissue, *Nat. Rev. Mol. Cell Biol.* 8 (2007) 839–845.
- [21] K. Bhadriraju, C.S. Chen, Engineering cellular microenvironments to improve cell-based drug testing, *Drug Discov. Today* 7 (2002) 612–620.
- [22] A. Birgersdotter, R. Sandberg, I. Ernberg, Gene expression perturbation in vitro – a growing case for three-dimensional (3D) culture systems, *Semin. Cancer Biol.* 15 (2005) 405–412.
- [23] C.E. Semino, J.R. Merok, G.G. Crane, et al., Functional differentiation of hepatocyte-like spheroid structures from putative liver progenitor cells in three-dimensional peptide scaffolds, *Differentiation* 71 (2003) 262–270.
- [24] E. Cukierman, R. Pankov, D.R. Stevens, et al., Taking cell-matrix adhesions to the third dimension, *Science* 294 (2001) 1708–1712.
- [25] F. Ozawa, K. Ino, T. Arai, et al., Alginate gel microwell arrays using electrodeposition for three-dimensional cell culture, *Lab Chip* 13 (2013) 3128–3135.
- [26] Z. Guan, S. Jia, Z. Zhu, et al., Facile and rapid generation of large-scale microcollagen gel array for long-term single-cell 3D culture and cell proliferation heterogeneity analysis, *Anal. Chem.* 86 (2014) 2789–2797.
- [27] H. Zhao, L. Zhou, Q. Zhang, et al., Bi-content micro-collagen chip provides contractility-based biomechanical readout for phenotypic drug screening with expanded and profiled targets, *Lab Chip* 15 (2015) 3481–3494.
- [28] X. Yan, J. Wang, L. Zhu, et al., A ready-to-use, versatile, multiplex-able three-dimensional scaffold-based immunoassay chip for high throughput hepatotoxicity evaluation, *Lab Chip* 15 (2015) 2634–2646.
- [29] S. Utech, R. Prodanovic, A.S. Mao, et al., Microfluidic generation of mono-disperse, structurally homogeneous alginate microgels for cell encapsulation and 3D cell culture, *Adv. Healthc. Mater.* 4 (2015) 1628–1633.
- [30] G.S. Du, J.Z. Pan, S.P. Zhao, et al., Cell-based drug combination screening with a microfluidic droplet array system, *Anal. Chem.* 85 (2013) 6740–6747.
- [31] P. Carmeliet, Angiogenesis in life, disease and medicine, *Nature* 438 (2005) 932–936.
- [32] T.L. Whiteside, The tumor microenvironment and its role in promoting tumor growth, *Oncogene* 27 (2008) 5904–5912.
- [33] X. Lin, Q. Chen, W. Liu, et al., Oxygen-induced cell migration and on-line monitoring biomarkers modulation of cervical cancers on a microfluidic system, *Sci. Rep.* 5 (2015) 9643.
- [34] N.K. Inamdar, J.T. Borenstein, Microfluidic cell culture models for tissue engineering, *Curr. Opin. Biotechnol.* 22 (2011) 681–689.
- [35] E.W. Esch, A. Bahinski, D. Huh, Organs-on-chips at the frontiers of drug discovery, *Nat. Rev. Drug Discov.* 14 (2015) 248–260.
- [36] M. Baker, Tissue models: a living system on a chip, *Nature* 471 (2011) 661–665.
- [37] J. Zhang, J. Wu, H. Li, et al., An in vitro liver model on microfluidic device for analysis of capecitabine metabolite using mass spectrometer as detector, *Biosens. Bioelectron.* 68 (2015) 322–328.
- [38] Y. Imura, K. Sato, E. Yoshimura, Micro total bioassay system for ingested substances: assessment of intestinal absorption, hepatic metabolism, and bioactivity, *Anal. Chem.* 82 (2010) 9983–9988.
- [39] D.B. Weibel, W.R. Diluzio, G.M. Whitesides, Microfabrication meets microbiology, *Nat. Rev. Microbiol.* 5 (2007) 209–218.
- [40] B. Derby, Printing and prototyping of tissues and scaffolds, *Science* 338 (2012) 921–926.
- [41] D. Gao, H. Li, N. Wang, et al., Evaluation of the absorption of methotrexate on cells and its cytotoxicity assay by using an integrated microfluidic device coupled to a mass spectrometer, *Anal. Chem.* 84 (2012) 9230–9237.
- [42] Q. Chen, J. Wu, Y. Zhang, et al., Qualitative and quantitative analysis of tumor cell metabolism via stable isotope labeling assisted microfluidic chip electrospray ionization mass spectrometry, *Anal. Chem.* 84 (2012) 1695–1701.
- [43] S. Mao, D. Gao, W. Liu, et al., Imitation of drug metabolism in human liver and cytotoxicity assay using a microfluidic device coupled to mass spectrometric detection, *Lab Chip* 12 (2012) 219–226.
- [44] W. Liu, N. Wang, X. Lin, et al., Interfacing microsampling droplets and mass spectrometry by paper spray ionization for online chemical monitoring of cell culture, *Anal. Chem.* 86 (2014) 7128–7134.
- [45] X. Lin, Q. Chen, W. Liu, et al., Assay of multiplex proteins from cell metabolism based on tunable aptamer and microchip electrophoresis, *Biosens. Bioelectron.* 63 (2015) 105–111.
- [46] R.A. Saylor, E.A. Reid, S.M. Lunte, Microchip electrophoresis with electrochemical detection for the determination of analytes in the dopamine metabolic pathway, *Electrophoresis* 36 (2015) 1912–1919.
- [47] N. Nordman, T. Sikanen, M.E. Moilanen, et al., Rapid and sensitive drug metabolism studies by SU-8 microchip capillary electrophoresis-electrospray ionization mass spectrometry, *J. Chromatogr. A* 1218 (2011) 739–745.
- [48] F. Chen, Y. Rang, Y. Weng, et al., Drop-by-drop chemical reaction and sample introduction for capillary electrophoresis, *Analyst* 140 (2015) 3953–3959.
- [49] L. Sainiemi, T. Nissilä, R. Kostianen, et al., A microfabricated micropillar liquid chromatographic chip monolithically integrated with an electrospray ionization tip, *Lab Chip* 12 (2012) 325–332.
- [50] S.L. Lin, H.Y. Bai, T.Y. Lin, et al., Microfluidic chip-based liquid chromatography coupled to mass spectrometry for determination of small molecules in bioanalytical applications, *Electrophoresis* 33 (2012) 635–643.
- [51] Y. Liu, S. Wang, Y. Song, et al., Biospecies Capture and detection at low concentration, *Micro. Nanosyst.* 4 (2012) 254–272.
- [52] S.J. Lee, H.S. Yoon, X. Xuan, et al., A patch type non-enzymatic biosensor based on 3D SUS micro-needle electrode array for minimally invasive continuous glucose monitoring, *Sens. Actuator B: Chem.* (2015) 10.1016/j.snb.2015.08.013.
- [53] J.R. Windmiller, N. Zhou, M.C. Chuang, et al., Microneedle array-based carbon paste amperometric sensors and biosensors, *Analyst* 136 (2011) 1846–1851.
- [54] S. Wang, Y. Wan, Y. Liu, Effects of nanopillar array diameter and spacing on cancer cell capture and cell behaviors, *Nanoscale* 6 (2014) 12482–12489.
- [55] H. Li, Y. Zhang, J. Lin, Recent advances in coupling techniques of microfluidic device-mass spectrometry for cell analysis, *Sci. Sin. Chim.* 44 (2014) 777.
- [56] C.-H. Lin, X. Lin, L. Lin, et al., Development of LC-MS method for analysis of paclitaxel-inhibited growth and enhanced therapeutic response in human glioblastoma cells, *Chin. Chem. Lett.* (2015) 10.1016/j.ccl.2015.03.007.
- [57] X. Sun, R.T. Kelly, K. Tang, et al., Membrane-based emitter for coupling microfluidics with ultrasensitive nanoelectrospray ionization-mass spectrometry, *Anal. Chem.* 83 (2011) 5797–5803.
- [58] P. Hoffmann, U. Häusig, P. Schulze, et al., Microfluidic glass chips with an integrated nanospray emitter for coupling to a mass spectrometer, *Angew. Chem. Int. Ed.* 46 (2007) 4913–4916.
- [59] J. Wu, S. Wang, Q. Chen, et al., Cell-patterned glass spray for direct drug assay using mass spectrometry, *Anal. Chim. Acta* 892 (2015) 132–139.
- [60] P. Mao, H.T. Wang, P. Yang, et al., Multinozzle emitter arrays for nanoelectrospray mass spectrometry, *Anal. Chem.* 83 (2011) 6082–6089.
- [61] H. Wang, J. Liu, R.G. Cooks, et al., Paper spray for direct analysis of complex mixtures using mass spectrometry, *Angew. Chem. Int. Ed.* 49 (2010) 877–880.
- [62] W. Liu, S. Mao, J. Wu, et al., Development and applications of paper-based electrospray ionization-mass spectrometry for monitoring of sequentially

- generated droplets, *Analyst* 138 (2013) 2163–2170.
- [63] J. Liu, H. Wang, N.E. Manicke, et al., Development, characterization, and application of paper spray ionization, *Anal. Chem.* 82 (2010) 2463–2471.
- [64] W. Liu, Q. Chen, X. Lin, et al., Online multi-channel microfluidic chip-mass spectrometry and its application for quantifying noncovalent protein-protein interactions, *Analyst* 140 (2015) 1551–1554.
- [65] D. Gao, H. Liu, Y. Jiang, et al., Recent advances in microfluidics combined with mass spectrometry: technologies and applications, *Lab Chip* 13 (2013) 3309–3322.
- [66] H. Bao, S. Liu, L. Zhang, et al., Efficient sample proteolysis based on a microchip containing a glass fiber core with immobilized trypsin, *Microchim. Acta* 179 (2012) 291–297.
- [67] H. Bao, L. Zhang, G. Chen, Immobilization of trypsin via graphene oxide-silica composite for efficient microchip proteolysis, *J. Chromatogr. A* 1310 (2013) 74–81.
- [68] H. Fan, F. Yao, S. Xu, et al., Microchip bioreactors based on trypsin-immobilized graphene oxide-poly(urea-formaldehyde) composite coating for efficient peptide mapping, *Talanta* 117 (2013) 119–126.
- [69] S. Mikkonen, M.K. Rokhas, J. Jacksén, et al., Sample preconcentration in open microchannels combined with MALDI-MS, *Electrophoresis* 33 (2012) 3343–3350.