

# Cell Analysis on Microfluidics Combined with Mass Spectrometry

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Cell analysis is of great significance for the exploration of human diseases and health. However, there are not many techniques for high-throughput cell analysis in the simulated cell microenvironment. The high designability of the microfluidic chip enables multiple kinds of cells to be co-cultured on the chip, with other functions such as sample preprocessing and cell manipulation. Mass spectrometry (MS) can detect a large number of biomolecules without labelling. Therefore, the application of the microfluidic chip coupled with MS has represented a major branch of cell analysis over the past decades. Here, we concisely introduce various microfluidic devices coupled with MS used for cell analysis. The main functions of microfluidic devices are described first, followed by introductions of different interfaces with different types of MS. Then, their various applications in cell analysis are highlighted, with an emphasis on cell metabolism, drug screening, and signal transduction. Current limitations and prospective trends of microfluidics coupled with MS are discussed at the end.

**Keywords** Microfluidic, mass spectrometer, cell analysis, cell metabolism, single-cell

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## 1 Introduction

A cell is the basic unit of survival and activity of a living body. The analysis of its metabolism, proliferation, apoptosis and other physiological activities is of great significance to the

exploration of human diseases and health. In recent years, more and more researchers have devoted themselves to cell analysis. However, cell analysis has its inherent difficulties. The first challenge comes from the complexity of the cell microenvironment. Cells are in a complex physiological environment in the body, affected by various biochemical and



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mechanic factors, including cytokines, extracellular matrix, oxygen gradients, fluid shear stress, *etc.* These factors have significant influences on cell differentiation, morphology, and other behaviors.<sup>1-3</sup> The second challenge is the small volume of the cells. The normal range for the mean cell volume is about 100 femtoliters. For deeper insights into cell biology, high throughput and high selectivity analyzing methods which can be performed in a simulated environment are in great need.

Under these circumstances, microfluidic technology has been widely used in cell analysis. Modern microfluidic chips have the advantages of micrometer-scale channels and high designability. Micron-scale channels are comparable in size to cells, which helps to effectively manipulate cells. Cells can be cultured, separated, and detected in different chambers while achieving on-line analysis and reducing reagent consumption. Compared with the traditional cell culture in a petri dish, three-dimensional (3D) co-cultivation of multiple cells and even multi-organ systems simulation in a microfluidic device are more capable of closely mimicking the cell microenvironment.<sup>4-6</sup> The high designability of the microfluidic chip makes it easier to integrate with other analytical techniques, for instance, chromatography, fluorescence, electrical detection, and MS.<sup>7-10</sup> Implementation and integration of different functional units make the microfluidic chip a powerful processing method.

It is important to note that the integration of MS with microfluidic chips has been much more attractive than other analytical tools. Due to the characteristics of high selectivity and high throughput, MS was identified as an excellent analytical tool for qualitative and quantitative analysis of biological samples.<sup>11</sup> In addition, without the requirement of sample pre-labelling, the amount of sample required for MS detection is very small. Due to these characteristics, MS has become an indispensable analysis tool in the field of life science.

Up to now, the combination of microfluidic chips and MS has played an important role in the field of drug analysis and provides a powerful tool for single-cell research. In Fig. 1, developments of microfluidic chip-mass spectrometry are summarized. In 2008, our research group developed a microfluidic chip coupled with MS technology for peptide separation and analysis.<sup>12</sup> After that, we used microparticles to simulate cells, and constructed the single-particle analysis by MS coupled with microfluidic chip analysis.<sup>13</sup> After developing on-chip cell culture analysis technology,<sup>14,15</sup> in collaboration with Purdue University, paper spray technology was designed for the interface between microfluidic mass spectrometry and the chip.<sup>16</sup> Based on the above work, we successfully developed a six-channel chip in 2010, which integrated the cell metabolite enrichment channel, and realized the cell culture and drug stimulation on the chip.<sup>17</sup> Metabolites were analyzed by MS *via* capillary tubes. Thus, a new phase of cell analysis using multichannel microfluidic chip-MS was initiated. In the following 10 years, microfluidic cell analysis was widely carried out around the world. We cooperated with Stanford University to develop cell sorting on a microfluidic chip and used membrane separation technology to realize the rapid sorting of red blood cells and white blood cells.<sup>18</sup> The application of cell analysis has also been further developed by microfluidics combined with matrix-assisted laser desorption/ionization(MALDI)-MS, and other types of MS.<sup>19-21</sup> Microfluidics-MS technology was combined with isotope labelling technology to achieve a qualitative and quantitative analysis of cellular metabolites.<sup>22</sup> With the co-cultivation of multiple cells on the chip, multi-cell-based drug metabolism and signal communication studies have also been implemented on the microfluidic chip-MS platform, which we will introduce in detail in the following paragraphs.<sup>23-25</sup>

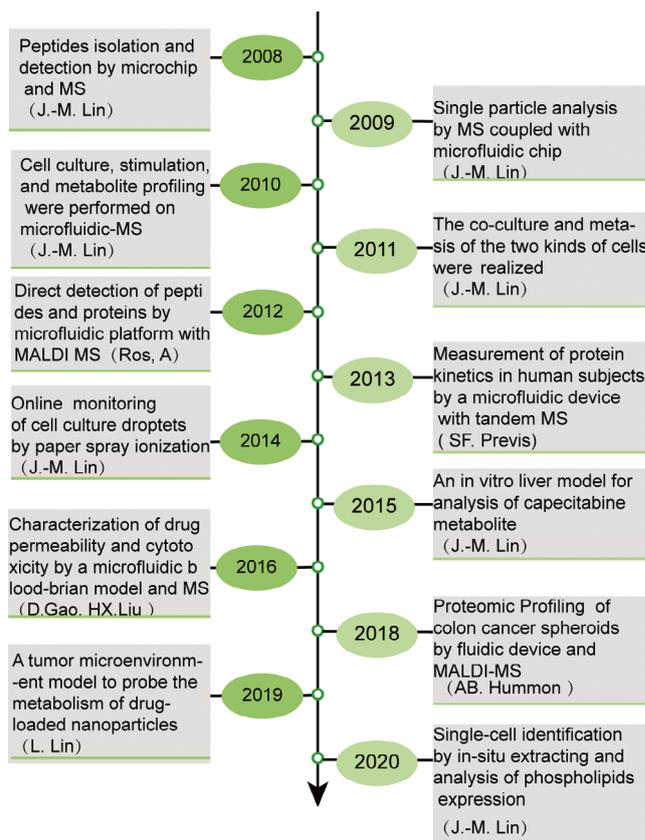


Fig. 1 The development of the microfluidic chip-MS system.

With the development of paper spray technology, microfluidics-MS has realized a high-throughput online analysis of cell metabolism.<sup>26</sup>

In this review, we focus on the recent advances in cell analysis by microfluidics combined with MS. The strategies of different designations to achieve 3D cell culture, single-cell manipulation, and sample treating, are classified and discussed in detail. Then, the application of different types of MS in microfluidic cell analysis is discussed. Finally, several typical applications of microfluidic chip-MS, such as signal transduction, metabolites, and drug screening are summarized and expected.

## 2 Different Functions of Microfluidic Devices

As we mentioned above, the high designability of the microfluidic chip allows it to be used for cell culture, sample preparation, and manipulation of cells. We have summarized these applications in Table 1. In this section, we will divide the mentioned three parts to discuss the application of microfluidic chips. We believe that the application of microfluidic chips is extensive. In the future there will be more possibilities for combining multifunctional microfluidic chips with MS, opening new avenues for cell analysis.

### 2.1 Cell culture

Cell culture is the foundation of cell research. The most traditional and the most widely used method of cell culture is 2D culture on Petri dishes, which have been used for over 50 years. This method allows for the cultivation of only a single kind of cells, and it is quite different from the actual physiological

Table 1 Application of microfluidic devices with different functions combined with MS

Functions of microfluidics	Purpose	Applications	Analytes	Ref.
Cell culture	Cell microenvironment mimicry	Cell signaling	Epinephrine	23
		Evaluation of drug permeability	Sunitinib	98
		Absorption	Paclitaxel	106
		Toxicity	Cyclophosphamide	94
		Chemoresistance analysis	6- <i>o</i> -methylguanine	99
		Combination drug therapy evaluation	Genistein and dacarbazine	97
		Vitamin E metabolism	Vitamin E	17
Sample pretreating	Sample desalting, separation and enrichment	Methotrexate metabolism	Methotrexate	95
		Cell signaling	Growth hormone	24
		Cell signaling	Dihydroxyphenylalanine	60
		Peptide detection	Gramicidin	82
		Serum glycomic profiles analysis	Glycans	107
		Single-cell lipid fingerprints, lipid heterogeneity of adherent single-cells	Phospholipids	51, 58, and 108

environment of the cells. Then, transwell was developed for the study of two-cell co-culture, cell invasion, and migration. However, it is not possible to monitor the state of cells in real-time, nor to provide cell microenvironment factors such as fluid shear stress. Microfluidic chips provide more possibilities for different types of cell culturing modes. More than one kind of cell could be cultivated in one microfluidic chip, which allows the investigation of crosstalk between them.

In the late 1990s, microfluidic devices were created for cell biology applications. The earliest application of two dimensional (2D) cell culture on microfluidic chips was cell patterning. Different shapes of microstructures can be used to arrange cells in different ways. The cells attached only to the adhesive lanes. one of the first studies in cell co-culture in microfluidic chips was established by Folch and Toner.<sup>27</sup> Hepatocyte and fibroblast were co-cultured in different zones for 24 h. In addition to cell patterning, microfluidics also offers the possibility to deliver not only chemical but also mechanical signals, providing an extra degree of control over cultured cells. 2D cultured cells can be treated with chemical signal stimulation<sup>28</sup> and fluid shear stress stimulation of different concentration gradients.<sup>29</sup>

The three dimensional (3D) models perform better than 2D models to reproduce the *in vivo*-like environment. The earliest 3D cell culture was achieved through the 3D structure of the chip. Cells were confined in micro holes and chambers.<sup>30</sup> Subsequently, the microcolumn array in the microfluidic chip enabled co-cultivation of the two cell channels.<sup>31</sup>

With the use of gel material for support function in cell culture, more 3D cell culture models on microfluidic chips were widely developed. Combining microcolumn array and polyethylene glycol material, a novel 3D mammalian cell perfusion-culture system in the microfluidic channel was established.<sup>32</sup> Until today, this model is still widely used in pharmaceutical analysis and other fields. Liu *et al.* developed a 3D tumor-microvascular structure for the study of antioxidant effects on malignant glioma cells *in vitro* by matrigel.<sup>33</sup> Among the vast kinds of 3D cell culture models, blood-brain barrier (BBB) chips are particularly popular. Because the main difficulty in brain diseases comes from the BBB, modelling the blood-brain barrier model with selective permeation function *in vitro* will greatly benefit the development of medicines. Since 2012, a vast amount of research on BBB microfluidic chip was published. These models were mostly formed by co-culture of endothelial cells, primary brain pericytes, and astrocytes.

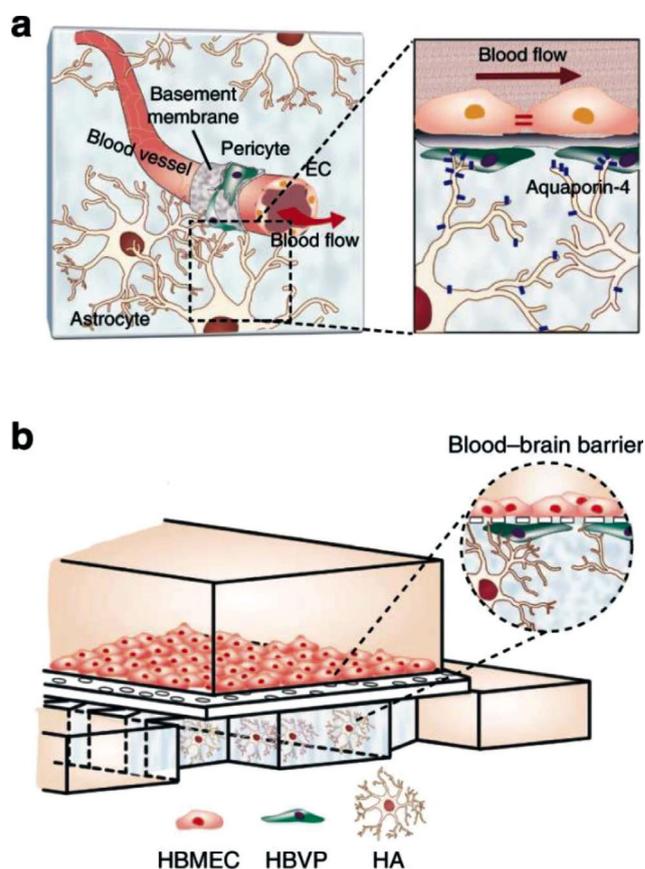


Fig. 2 Schematic description of a microengineered human BBB model. Adapted with permission from Ref. 37, Copyright 2020, Springer Nature.

In these models,<sup>34-36</sup> the tight connection between cells is successfully achieved and achieve selective permeability. Drug and toxicity studies were successfully carried out. On-chip mimicry of the BBB structure and function by cellular interactions, key gene expressions, low permeability, and 3D astrocytic network with reduced reactive gliosis and polarized aquaporin-4 (AQP4) distribution was demonstrated by Kim *et al.* in 2020 (Fig. 2).<sup>37</sup>

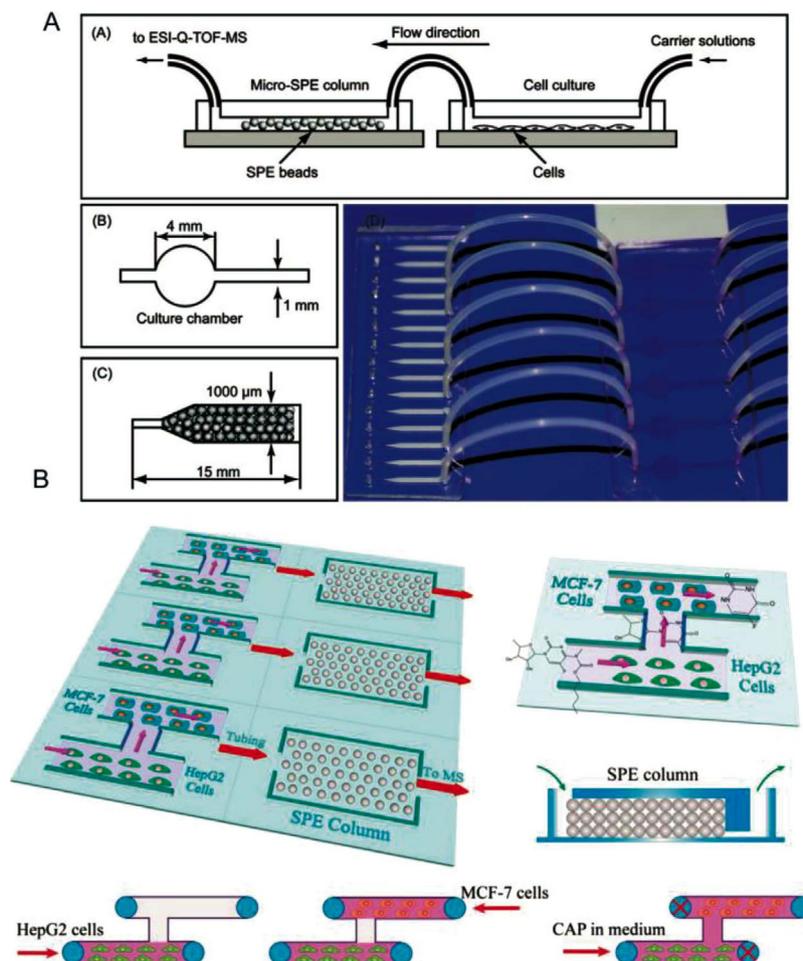


Fig. 3 Pretreating modules on a microfluidic chip. (A) Solid-phase extraction (SPE) incorporated into the microfluidic chip. Adapted with permission from Ref. 17, Copyright 2010, American Chemical Society. (B) Metabolites of co-cultured cells pretreated on a microfluidic chip. Adapted with permission from Ref. 35, Copyright 2015, Elsevier B.V.

After the establishment of mature tissue models, various organ model chips began to emerge. Microfluidic cancer organ chip models have been used to study steps during cancer progression, such as invasion, migration, and metastasis. In 2010, Huh *et al.* developed a biomimetic device for the mimesis of the lung alveolar-capillary interface in terms of structural, functional and mechanical aspects. It is worth mentioning that this three-layer PDMS-based alveolar chip is the first organ chip that successfully simulates the pulmonary breathing.<sup>38</sup> On this basis, an *in vitro* human orthotopic model of non-small-cell lung cancer (NSCLC) was developed, which helps to investigate organ microenvironment-specific cancer growth, and tumor dormancy.<sup>39</sup> Microfluidic cancer organ chip models have been used to study steps during cancer progression, such as invasion, migration, and metastasis. Breast chip, brain chip, lung chip and bone chip, were successfully fabricated and applied to pharmacologic research.<sup>40</sup>

A variety of different organ chips can be integrated to achieve *in vitro* simulation of a system of the human body. The microfabricated lung mimic device was used to fabricate an alveolar-capillary barrier on a thin, porous, flexible PDMS membrane coated with the extracellular matrix. The jejunum, liver and kidney models, along with skeletal muscle and neurovascular models, were used to test multi-organ toxicity and absorption, distribution, metabolism and excretion

processes.<sup>41</sup> These cell culture chips not only provide various factors of microenvironment for cells but also provide favorable conditions for further on-line analysis on cells.

## 2.2 Sample pretreatment

For biological samples containing a large number of salts, there would cause great damages to analytical instruments, especially for MS. So it needs to be removed before detection. In addition, since the target analytes in cells are generally low in content, an enrichment process is also required. These functions can be easily implemented on a microfluidic chip, reducing sample loss.

To remove undesired compounds, solid-phase extraction (SPE) is incorporated into the microfluidic chip. As shown in Fig. 3A, the special channel was filled with unique polymeric SPE beads, which formed a micro-SPE column. By this strategy, Gao *et al.* validated the feasibility of a vitamin E metabolism study on the developed microfluidic device and MS.<sup>17</sup> Straight microchannels with shrink ends were designed to pack the solid-phase material for sample cleanup and concentration prior to mass analysis. The total sample pretreatment time only needed about 15 min, including the desalting, preconcentration and analysis processes. Combining this with cell co-culture, Zhang *et al.* achieved online monitoring of the prodrug metabolism in the *in vitro* liver model, while the

intermediate product of the prodrug 5'-deoxy-5-fluorouridine (DFUR) was detected by MS.<sup>25</sup> (Fig. 3B) In these researches, the outlet of the SPE column was designed smaller than the diameter of SPE beads, making the beads trapped in this section. There are also some other kinds of methods for SPE column integrating into the microfluidic chip. Implementation of the laser-actuated closable valving system is demonstrated on an automated, centrifugally driven dynamic solid phase extraction (dSPE) device, by researchers at the University of Virginia.<sup>42</sup> Today, pre-processing modules such as SPE integrated on microfluidic chips have become widely used technologies.

### 2.3 Single-cell manipulation

Cells are not heterogeneous, even if they are propagated from the same cell. The results from bulk cells only show average behaviors. Meanwhile, some important physiological process, such as cancer metastasis and invasion, are usually induced by certain cells.<sup>43</sup> The analysis method at the single-cell level hold the key.

Due to its designability on a micron-scale level, the microfluidic chip has been widely used in single-cell separation and analysis. The most common method for single-cell isolation is to fabricate mechanic structures like valves, traps, and dams, which can capture only one cell. As shown in Fig. 4, one of the earliest valve-based microfluidics was fabricated by Quake *et al.* in 2000.<sup>44</sup> This active microfluidic system contained on-off valves, switching valves, and pumps fashioned entirely out of elastomer. In each unit, the upper channel was designed to control the other channel. Then this system was applied to single-cell isolation in 2006.<sup>45,46</sup> Also in 2006, Carlo *et al.* designed micro-dam arrays in microfluidic channels.<sup>47</sup> When

suspending cells flow through these wells, cell loading could be accomplished in 30 s. Using the same strategy, a large number of micro-dam and micro-well structures of different shapes have been developed and applied to single-cell research.<sup>48,49</sup> Some improvements has been made to this strategy, such as modifying recognition molecules like antibody and aptamers on microarrays to capture specific single cells.<sup>50</sup>

Compared with the above methods, MS has the advantage of label-free for microfluidic single-cell analysis. Chen *et al.* was the first to cooperated microfluidic chip with MS for single-cell analysis.<sup>51</sup> Through inkjet sampling of a cell suspension, droplets with single-cells were generated and transferred to MS. By this strategy, lipid fingerprints of single cells were obtained. By adjusting the size of the droplet to ensure adequate nutrition, a single cell can survive in the droplet for a long time.<sup>52</sup> Thus the single-cell droplet method became a promising method for

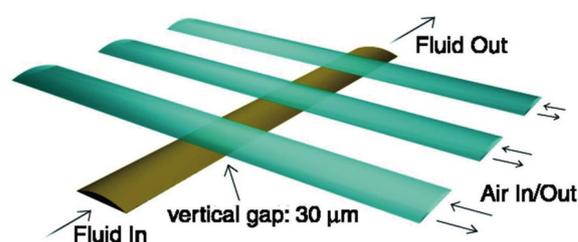


Fig. 4 Microvalves integrated on microchips. Adapted with permission from Ref. 44, Copyright 2000, The American Association for the Advancement of Science.

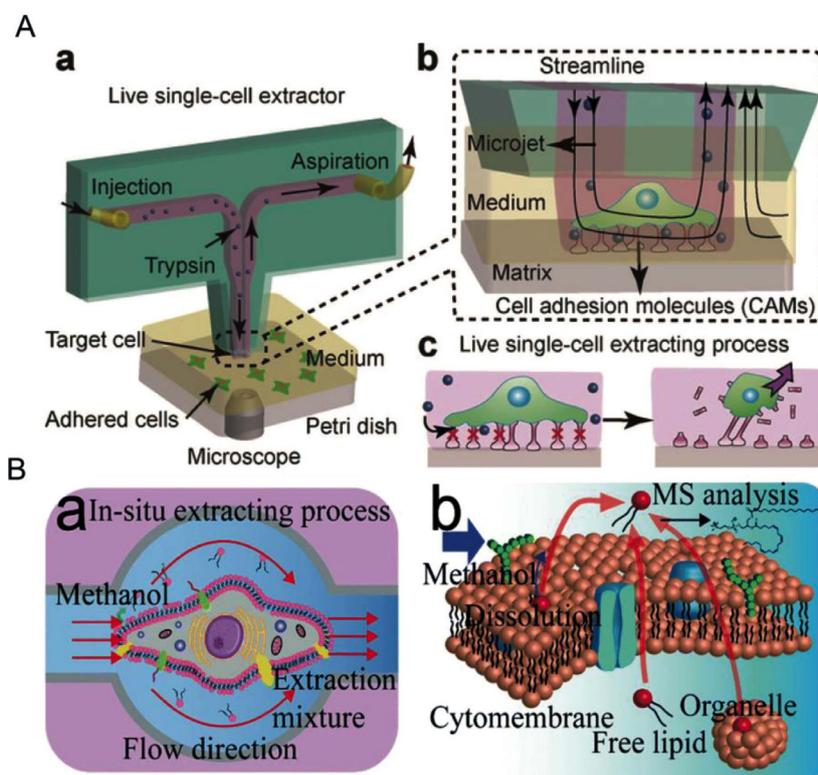


Fig. 5 Single-cell manipulation by microfluidic devices. (A) Analysis on adherent single cells. Adapted with permission from Ref. 55, Copyright 2018, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (B) Coupling of Open Microfluidic Chip and ESI MS. Adapted with permission from Ref. 58, Copyright 2020, The Royal Society of Chemistry.

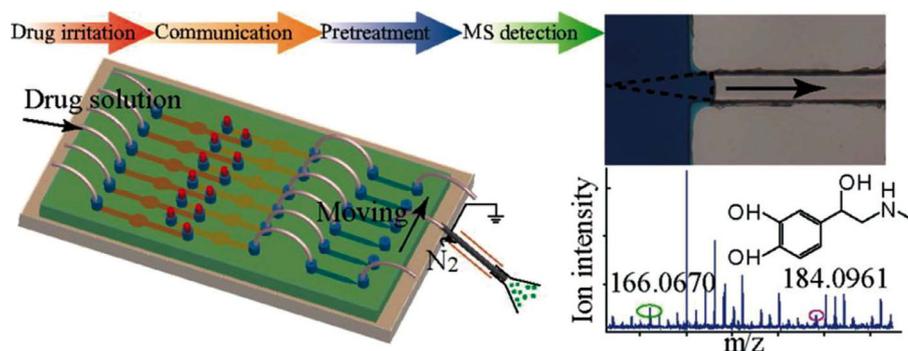


Fig. 6 Incorporation of SPE modules in microfluidic chip and ESI-MS. Adapted with permission from Ref. 23, Copyright 2013, American Chemical Society.

drug screening.<sup>53,54</sup>

Since mechanical manipulation can only fix single cells in the channel, it is difficult to perform subsequent operations on them. And manipulation and analysis of adherent single cells cannot be performed on these devices. Therefore, the control method based on hydrodynamics is more in line with the demand. For instance, Mao *et al.* developed a microfluidic chip-based live single-cell extractor (LSCE), which could analyze the adhesion strength of a single adherent cell (Fig. 5A). The cells to be analyzed were not in the microfluidic chip but were placed perpendicular to the LSCE.<sup>55</sup> Subsequent manipulation like single-cell cutting could also be achieved by the same device.<sup>56,57</sup> Subsequently, a microfluidic device for mass spectrometry analysis of adherent single cells was also developed. A single adhered cell was selected and extracted by an open space microfluidic device, then its phosphatidylcholine compositions were analyzed through MS (Fig. 5B). Subsequent studies also used a similar device to analyze phospholipids in different types of cells, further revealing the heterogeneity of different cells.<sup>58</sup> This approach opened a potential way for label-free tumor identification and auxiliary disease diagnosis. It is speculated that single-cell analysis based on hydrodynamics has great potential for further research.

### 3 MS and Microfluidic Chip Interface

Since microfluidic chip could achieve cell co-culture, sample pretreatment, cell manipulation and other functions, when it is incorporated with MS, it can play a huge advantage in the field of cell analysis. Therefore, in recent years, much research has been devoted to the development of microfluidic chip-MS. The most challenging aspect is the design of the interface between microfluidic chips and MS. Therefore, this section mainly introduces the interface between different types of MS and microfluidic chips.

#### 3.1 ESI-MS interface

As ESI is particularly relevant for the analysis of large biological molecules, such as proteins or peptides, efforts have focused on advancing interfaces that meet the demands of nano-separation techniques that are typically used prior to MS detection.<sup>59</sup> Biological samples always contain large amounts of salts, which affect the detection results of ESI-MS, requiring a sample pretreating module in the interface design.

A common method was capillary-electrophoresis ESI-MS, where the microfluidic channel acts as a place for electrophoresis. A three-layered microfluidic chip was fabricated and evaluated

for studying the dynamics of neurotransmitters released from PC-12 cells.<sup>60</sup> Neurotransmitter including dopamine (DA), serotonin (5-HT), aspartic acid (Asp), and glutamic acid (Glu) were successfully quantified by this system. Capillary-electrophoresis ESI-MS based on microfluidics has become a very mature method with a large number of related patents and articles.<sup>61-63</sup> And further optimizations continue to emerge, such as modifying layers on the chip channels to further improve electrophoretic performance.<sup>64</sup> In addition, the combination of microfluidic and nanofluidic techniques allows samples to be processed by pressure,<sup>65</sup> and this direction still has unlimited possibilities in the future.

The most common and practical method was the incorporation of an extraction module into the microfluidic chip. As shown in Fig. 6, cell culture medium was designed to flow through a channel packed with SPE beads before entering MS.<sup>23</sup> This step can enrich the target molecules and remove a large number of interferences. Epinephrine and glucose were successfully detected using an ESI-Q-TOF-MS with short analysis time by this approach, giving a deeper insight into cell-cell communication. This kind of strategy is widely used and has been adopted by a large number of related studies, especially drug screening.<sup>66</sup>

The aforementioned method is mainly for the analysis of a large number of adherent cells. However, the analysis of a small number of cells in a droplet may yield more information. As such, droplet-ESI-MS has also become of the current hot research. Zhang *et al.* developed a high automatic platform for single-cell analysis, which consists of droplet-based inkjet printing, dielectrophoretic electrodes, and de-emulsification interface to achieve on-line single-cell encapsulation, manipulation, and MS detection.<sup>67</sup> As shown in Fig. 7, the cell culture medium is removed by droplet cutting, and the entire cell is injected to MS for detection, and information about phospholipids is obtained. Compared with the method of directly injecting single-cell droplets, this method can effectively reduce the salt concentration in the droplets and reduce the damage to the MS.<sup>51</sup> Since a single droplet is a closed and stable system, a chemical reaction can be performed within the droplet to detect the activity of biological enzymes. Due to the rapid rate of droplet generation, the detection flux is very high. Continuous infusion of droplets to a nano-ESI emitter was demonstrated for as long as 2.5 h, corresponding to the analysis of over 20000 samples.<sup>68</sup> Activity of the transaminase ATA-117 after *in vitro* expression (ivTT) in droplets was analyzed. Throughput of 0.7 samples/s was achieved with 98% accuracy using a self-correcting and adaptive sorting algorithm.<sup>69</sup> The droplet can also serve as a scaffold for three-dimensional cell

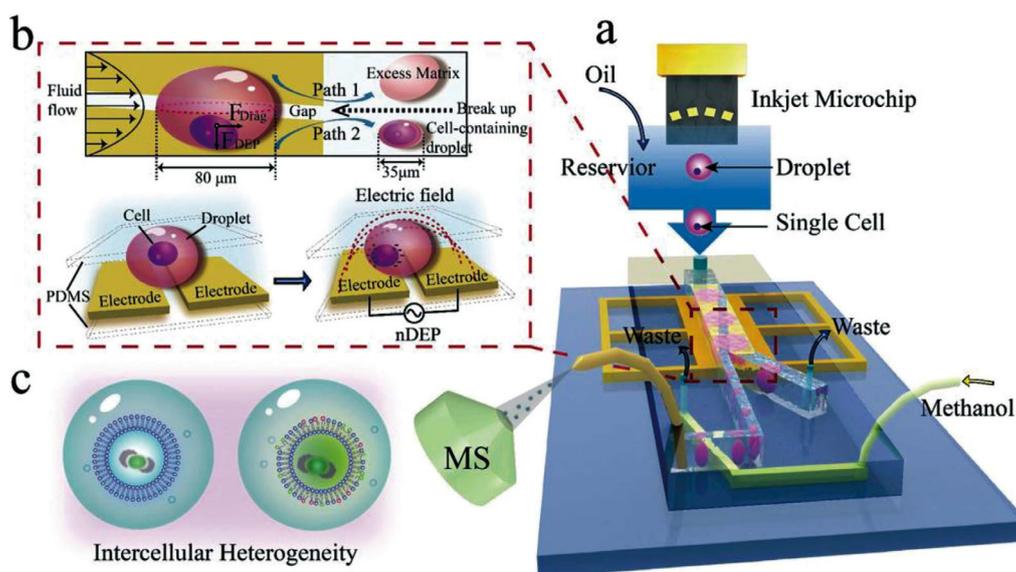


Fig. 7 The lipids of a single cell analyzed by microfluidic chip and MS. Adapted with permission from Ref. 67, Copyright 2013, American Chemical Society.

culture. Microfluidic chips can be used to synthesize droplet gel microspheres, allowing cells to grow in the gel microspheres. The metabolites diffused out of the microspheres for MS analysis.<sup>70</sup> Tumor cells and endothelial cells were co-cultured in gel microspheres, further revealing the metabolism of paclitaxel.<sup>71</sup>

### 3.2 MALDI-MS interface

In addition to ESI, MALDI is also a kind of MS widely used for cell analysis, especially for the analysis of biological macromolecules. Although it cannot realize real-time online analysis like ESI, it also has the advantages of high sensitivity, *in situ* analysis, high tolerance to salt, *etc.* Coupling of MALDI-MS and microfluidic chip could bring more possibilities for cell analysis. Before the MALDI-MS analysis, a UV-absorb matrix solution mixed with samples was required to deposit onto the target plate to form matrix crystals. This step is vital for the accuracy and uniformity of the MS signals, and thus much research focused on the sample transferring steps. The primal method included the spot deposition by direct contact of a capillary tube and the sample plate,<sup>72</sup> or the direct transfer from chip surface to sample plate.<sup>73</sup> These methods were only improvements to the traditional spotting method, and they did not solve the traditional problems like the coffee ring effect. A ring-shaped piezoelectric acoustic atomizer (piezo-ring) was directly integrated into a microfluidic device to spray the sample onto the MS target substrate, which helps to form a finer crystalline matrix and present better MS signal uniformity with little sample consumption compared to the conventional pipetting method.<sup>74</sup> The use of droplet was another choice for sample deposition. Nanoliter aqueous droplets immersed in oil could be deposited on the plate, with a detection threshold reached in the nanomole or femtomole range.<sup>75-77</sup> Incorporated with an automated microfluidic system, detection of the peptide in the attomole range by fast, accurate and automatized spotting of droplets of matrix/analyte mixture was achieved, which leads to higher detection sensitivity by MALDI-TOF MS, compared to conventional droplet deposition.<sup>78</sup> These deposition methods could be utilized to test the performance of new matrix, like ion liquid, *etc.*<sup>79</sup>

In addition to the deposition device, microfluidic chips could also act as sample pretreatment module for MALDI-MS. Using a microfluidic device that integrates aptamer-based specific analyte extraction, isocratic elution, and detection by MALDI-MS, Yang *et al.* demonstrated a rapid, sensitive and label-free detection of arginine vasopressin (AVP) in human plasma ultrafiltrate.<sup>80</sup> Based on the affinity of the glycosylation pattern of proteins and a specific serum protein, a rapid glycosylation pattern analysis of transferrin was designed by Quaranta *et al.*<sup>81</sup> High integration made this assay require only 1 μL of sample per determination, and the automation allowed for the possibility of processing 54 samples in parallel in 3.5 h. Serum samples from chronic alcohol abusers were examined by this method.

Similar to interfaces of ESI-MS, electric characteristics could be utilized for sample pretreatment on the microfluidic chip and MALDI-MS. A freezing technique used for coupling microchip electrophoresis with MALDI-TOF-MS was developed by Nie *et al.*, which has been applied to the determination of peptides.<sup>82</sup> After a low-pressure evaporation and mix deposition, the analytes were transferred to a MALDI plate. Apart from electrophoresis, the isoelectric focusing (IEF) could also be accomplished on a microfluidic chip coupled with MALDI-MS. A novel method for preconcentration and purification of Alzheimer's disease-related amyloid beta (Aβ) peptides by isoelectric focusing in microchannels combined with MALDI-MS was presented by Mikkonen *et al.*<sup>83</sup> The IEF was carried out in an open microfluidic system, providing at least a 10-fold increase of the MALDI-MS-signal.

Cell manipulation and other functions were also implemented on the microfluidic chip, coupled with MALDI-MS. A quantitative mass spectrometric approach combined with microfluidic technology reached the detection sensitivity of high abundant proteins in single cells.<sup>84</sup> As shown in Fig. 8, immunoassay, digestion and isotopic labeling of apoptosis-related protein Bcl-2 was accomplished on one chip. This study indicates the possibility of single-cell sensitivity for assessing a protein copy number of 10<sup>6</sup> molecules from a single cell, by adapting the design of the microfluidic chip. Some special chips could replace the commercial MALDI-MS plate to realize *in situ* imaging. A microfluidic paper-based analytical device

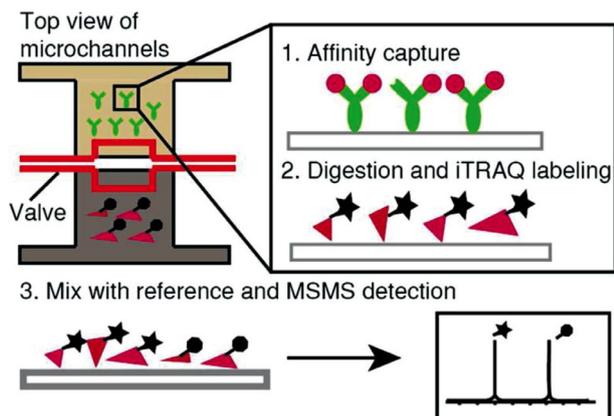


Fig. 8 Incorporation of complex sample pretreating in microfluidic chip and MALDI-MS. Adapted with permission from Ref. 84, Copyright 2016, American Chemical Society.

coupled with MALDI-MS was fabricated to understand the color gradient based on glucose detection.<sup>85</sup> It can be speculated that in the future, more types of microfluidic chip-MALDI MS devices will be widely used.

At present, ESI-MS and MALDI-MS are the main types of MS commonly incorporated with microfluidic chips. However, there are more types of MS, such as paper spray and glass spray,<sup>86,87</sup> which can be incorporated with microfluidic chips to provide more powerful tools for cell analysis.<sup>88</sup>

## 4 Applications

### 4.1 Cell metabolism

Since metabolites can have a wide range of functions in the cell and organism and reflect the overall effect of the genome, proteome and external stimuli, monitoring of metabolites could lead to a deeper study on the mechanism of life activities.<sup>89</sup>

Altered metabolism is one of the vital features of tumor cells. Online and dynamic monitoring of metabolites would contribute to the diagnosis of tumors. Liu *et al.* developed an online

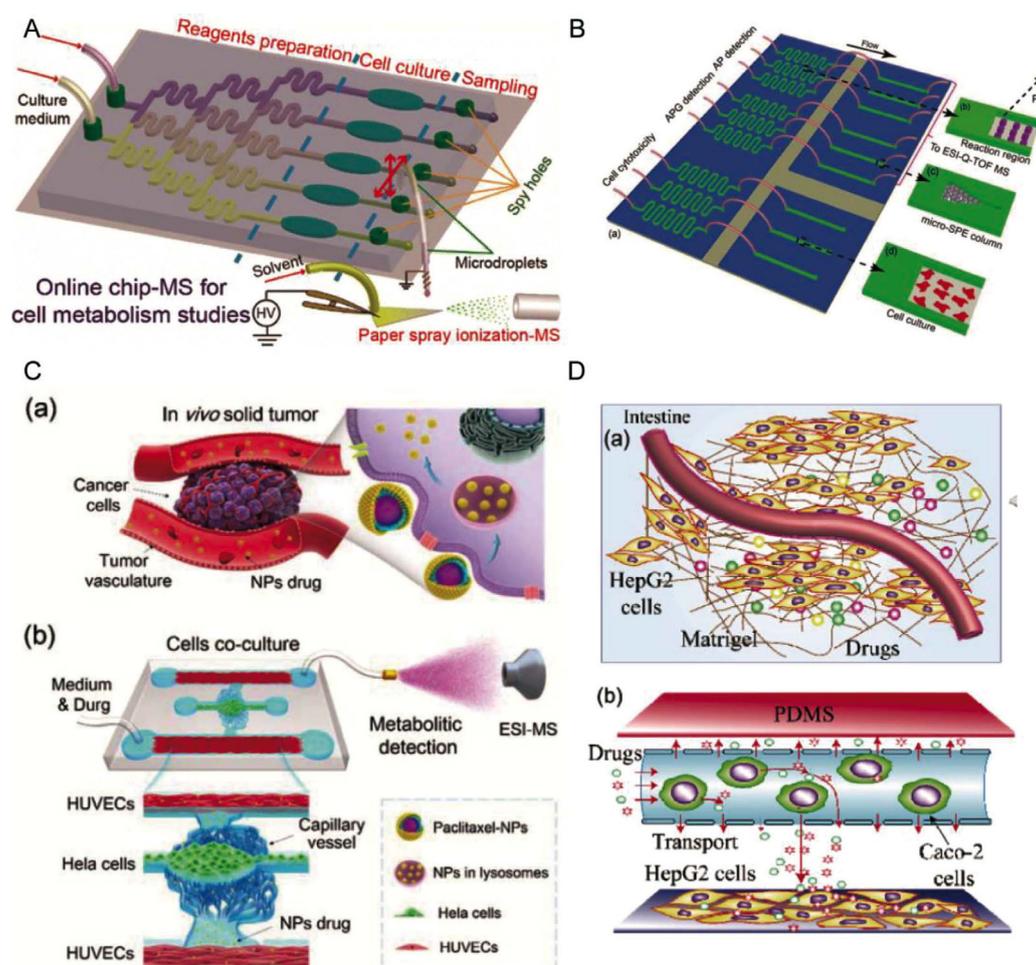


Fig. 9 Metabolites detected by microfluidic chip and MS. (A) Integrated multichannel microfluidic chip-MS for cell metabolism studies. Adapted with permission from Ref. 90, Copyright 2016, American Chemical Society. (B) Microfluidic device for cell culture, metabolite analysis and cytotoxicity assay. Adapted from Ref. 94 with permission from The Royal Society of Chemistry. (C) Schematic of the integrated microdevices for mimicking the tumor microenvironment. Adapted from Ref. 96 with permission from The Royal Society of Chemistry. (D) Schematic illustration of the microfluidic intestine-liver model. Adapted with permission from Ref. 97, Copyright 2017, Springer Nature.

multichannel microfluidic chip-MS platform for monitoring of cell metabolism studies.<sup>90</sup> After cell culture and hypoxia stimulation on the microfluidic chip, the medium was injected into the paper spray MS in droplet form for detection. As shown in Fig. 9A, lactate efflux rates of different cells under normoxia and hypoxia were demonstrated by this strategy.

The signal biomolecules AMP, ATP, and CoA could also be separated and identified by microfluidic free-flow electrophoresis *via* MS.<sup>91</sup> Metabolites of microbes could also be detected by the microfluidic chip-MS system. Online monitoring of bioactive metabolites from incubated actinobacteria in picoliter droplets was achieved.<sup>92</sup> Encapsulation of microbes and detection of fluorescence was accomplished in a microfluidic chip. *In situ* produced streptomycin was detected by ESI-MS, providing a tool for the discovery of bioactive metabolites produced by microorganisms. Facile collection and MS characterization of a secreted neuropeptide were realized by a neuron-in-capillary platform.<sup>93</sup> This platform allowed online cell culture and metabolites detection, providing a powerful tool for characterizing secreted neuropeptides.

Besides the analysis of the metabolites of the cells themselves, the metabolism of drugs in the cells can also reveal important mechanisms. The label-free advantage of MS allows simultaneous detection of multiple metabolites of drugs. Imitation of drug metabolism in human liver and cytotoxicity assay was achieved by coupling of a microfluidic chip and MS.<sup>94</sup> Bioreactors containing poly(ethylene) glycol (PEG) hydrogel encapsulated human liver microsomes were fabricated in the first channel, followed by a SPE chamber for MS detection and cell culturing chamber for cytotoxicity analysis (Fig. 9B). Metabolism of acetaminophen was monitored by this system, indicating that this system was a useful tool for drug metabolism studies and cytotoxicity assays. High-throughput analysis of drug absorption and evaluation of cytotoxicity could be simultaneously realized with a concentration generator in the microfluidic chip and an online ESI-MS.<sup>95</sup> Coupling with a dual-isotopic labeling strategy, effective qualitative analysis of multiplex metabolites was performed by microfluidic chip and MS, with high stability, sensitivity, and repeatability.<sup>22</sup>

In addition to simple cell culture and sample preparation, more sophisticated microfluidic chip-MS systems can achieve more functions. A novel integrated microfluidic platform with microvessel network channels coupled with MS was developed for real-time probing of nanoparticles (NPs) drug delivery in the vascular niche.<sup>96</sup> As shown in Fig. 9C, two kinds of cells were co-cultured on chips, while the NPs were transported through this microvessel structure to the Hela cells. The secreted NPs drug (paclitaxel) metabolites were analyzed by ESI-MS. Many oral anti-cancer drugs require gastrointestinal absorption, liver metabolism and other processes to enter the lesion, so the impact of these absorption and transportation processes must be considered for drug screening. An intestine-liver model for multiple drugs absorption and metabolism is presented in Fig. 9D. Caco-2 cells were inoculated in the HF cavity to simulate the intestinal tissue for drug absorption and transporting, while HepG2 cells were seeded in the bottom chamber to mimic the liver for metabolism-related studies.<sup>97</sup> Combination drug therapy of the anti-tumor drugs, Genistein and dacarbazine, was tested on the model. This dynamic, co-culture microchip successfully allowed long-term observation of absorption, transport, and metabolism of combination drugs. The combination of these biomimetic microfluidic chips can be more suitable to mimic the real cell microenvironment, to conduct more realistic and accurate research on drug metabolism.

## 4-2 Drug screening

Since the coupling of microfluidic chips and MS can be applied to monitor drug metabolism in a simulated tumor niche, it has been widely used in the drug screening process. Compared to drug screening based on cells cultured in a petri dish, cells cultured in microfluidics could achieve more physiological functions, helping to achieve a deeper understanding of the drug delivery and absorption process. The restrictive nature of the BBB is an obstacle for drug delivery to the central nervous system. Therefore, screening drugs for the central nervous system must consider the delivery of therapeutics on BBB. A BBB model in a membrane-based microfluidic chip for characterization of drug permeability and cytotoxicity was established by Shao *et al.*<sup>98</sup> The permeated amount of drug was directly quantified by an ESI-MS after on-chip SPE pretreatment. Then the permeated drug was incubated with glioma cells for cytotoxicity test. Chemoresistance of glioma was studied by analyzing the metabolism of temozolomide in an artificial glioma perivascular niche.<sup>99</sup> Chemoresistance of temozolomide on glioma stem cells and the molecular metabolism was investigated by an ESI-MS. Microfluidic chips provide a simulated microenvironment for cells, while MS provides the possibility for high-throughput detection. Metabolism and its kinetic of eight drugs are evaluated, by coupling of a liver chip and MS. Hepatic clearances were extrapolated on this strategy.<sup>100</sup> These studies have shown that microfluidic chip-MS has great advantages in drug screening.

## 4-3 Signal transduction

Intercellular signaling has been identified as a highly complex process, responsible for orchestrating many physiological functions. Precise control of the microenvironment by microfluidic chip and high throughput label-free analysis by MS allow complex mechanisms to be studied in detail.<sup>101</sup> Firstly, the microfluidic chip can reduce the loss of signal molecules during sample preparation. This strategy can effectively detect two kinds of neuropeptides secreted by nerve cells under KCl stimulation by MALDI-MS. This quantitation label-free approach is robust and is well suited for miniaturized off-line characterization from microfluidic devices.<sup>102</sup> The high-throughput advantage of MS can enable highly sensitive detection of dopamine, adenosine monophosphate, cytidine and other signal molecules in biofluids such as urine and blood.<sup>103</sup>

A more notable advantage is that different kinds of cells could be co-cultured in one microfluidic chip, making it possible to analyze cellular signal transduction on the chip. A model of the regulation of the organism by the nervous system was created by PC12 cells and GH3 cells co-cultured under various conditions.<sup>24</sup> Cells were cultured in a three-channel microfluidic chip, and the growth hormone secreted by GH3 cells during the regulation from PC12 cells was monitored by ESI-MS. Another nephrocyte-neurocyte co-culture model on a membrane-integrated microfluidic was created by Zhuang *et al.*<sup>104</sup> PC12 cells served as sympathetic nerve and cultured in the bottom channel, while 293 cells served as the kidney and cultured in the top channel, separated by a porous polycarbonate membrane. After two days' of co-culture, the acetylcholine secreted by differentiated PC12 cells would transfer to stimulate 293 cells to secrete epinephrine, which was identified by a nano-ESI-MS. This microfluidic chip-MS system was capable of mimicking the cell-cell interaction, while allowing for the collection and qualitative investigation of the secreted signal molecules. In recent years, some microfluidic chips with more complex structures have been developed for the study of cell-cell interactions. An integrated microfluidic device with three

individual components (cell co-culture component, protein detection component, and pretreatment component for drug metabolites) to probe the interaction between tumor and endothelial cells.<sup>105</sup> In the future, more and more kinds of microfluidic chips coupled with MS may help us to understand this signaling process more deeply.

## 5 Conclusion and Future Perspectives

In this review, we summarized cell analysis based on microfluidics combined with MS. First, we introduced the application of microfluidic chips in cell culture, sample preparation and cell manipulation. The microfluidic chip can realize three-dimensional co-cultivation of a variety of cells, simulating the real physiological microenvironment of the cells. Secondly, modules such as SPE can be integrated to achieve online sample pre-processing on the chip. Droplets, single-cell arrays, open microfluidics and other technologies can achieve single-cell manipulation and detection. In the following section, the interface design of the microfluidic chip and two commonly used types of MS, ESI-MS and MALDI-MS, are introduced. The microfluidic chips, integrating cell culture, sample enrichment, capillary electrophoresis, and sample purification, are connected to the ESI-MS inlet through pipeline connections to achieve online cell analysis. When the microfluidic chip is incorporated with MALDI-MS, in addition to the above functions, the spotting function can also be achieved. Finally, the applications of microfluidic chips and MS in cell metabolism detection, drug screening, and signal transduction are illustrated in detail. Undoubtedly, due to the high designability and biocompatibility of microfluidic chips and the high throughput and label-free nature of MS, the combination of the two has incomparable advantages in the field of cell analysis.

However, it must be admitted that there are still some deficiencies in the current research. The first is that the manufacturing process of the microfluidic chip that can realize three-dimensional cell co-cultivation is still relatively complicated and has high requirements for operators. For chips with advanced functions, a large number of pipelines are often required to connect modules, such as pumps, valves, and mass spectrometers, which further increases system instability and errors. These existing problems have hindered the large-scale promotion of microfluidic MS in the field of cell analysis.

But these problems can be solved with the progress of technology. The commercialization of microfluidic chips has also promoted their use. At present, microfluidic chip-MS devices that integrate automated cell flow culture, sampling, purification and injection have appeared. The standardized chip and built-in piping system reduce manpower consumption and system instability, achieving automated long-term cultivation of cells, drug stimulation and detection. With the further commercialization of technologies such as organ chips, we look forward to the day when chips can replace experimental animals. While promoting the progress of bioethics, it also promotes the process of drug research and development.

Microfluidic chip-MS also has great potential in the more advanced field of cell analysis. As one of the most cutting-edge topics in cell research, single cells still have many mechanisms waiting to be explored. With the increased sensitivity of MS and more optimized chip design, the metabolism of a single cell may also be monitored by this system in the future. With the advent of nanofluidic technology, smaller and more precise structures may help to achieve more precise manipulation of cells and study more detailed life activity mechanisms. In

general, the combination of microfluidic chips and MS is still a promising technology for the future, and is expected to make a great contribution to human health.

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