Chapter 7
Microfluidics-Mass Spectrometry Combination Systems for Single-Cell Analysis

Dan Gao, Chao Song and Jin-Ming Lin

Abstract Due to the existence of heterogeneities in individual cells, analysis of intercellular contents at the single-cell level has become an important direction in modern bioanalytical chemistry. The advances in miniaturized analytical systems and emerging microfluidic tools bring a new opportunity for single-cell analysis. Microfluidic systems have abilities to *the* single cell and *reagents* manipulation with minimal dilution, automatic and parallel sample preparation, and compatible with different detection techniques, which made them powerful tools for single-cell analysis. Mass spectrometry (MS) is one of the most popular analytical methods for the detection of unknown chemicals because of its unique advantages, such as label-free detection, high sensitivity, high chemical specificity, and board detection range. Recently, the coupling of microfluidics to MS for single-cell analysis has attracted substantial *interests and developments*. Nowadays, different types of ionization methods including electrospray ionization (ESI), laser desorption ionization (LDI), secondary ionization (SI), and inductively coupled plasma (ICP) have been coupled to a mass spectrometer. Owing to these ionization methods, a board range of chemicals can be detected by MS, such as proteins, metabolites, lipids, peptides, glycomics, elements, and so on. Recent progress in the fields of technologies and applications in the microfluidics-MS combination systems for single-cell analysis is described. Several analytical procedures integrated on the microfluidics such as single-cell manipulation and sample pretreatment before introduction into the mass spectrometer are reviewed. The future research opportunities by focusing on key performances of throughput, multiparametric target detection, and highly automated analysis are also discussed.

Keywords Single-cell analysis · Microfluidics · Mass spectrometry · Single-cell manipulation · Mass spectrometry interface

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7.1 Introduction

Single-cell analysis is a rapidly developing research field in recent years. Numerous researches have demonstrated that cells derived from a mother cell or from the same type of cell exhibit heterogeneity even if under the same physiological conditions or external stimuli [1, 2]. Moreover, single-cell analysis is regarded as a key step to help us comprehensively understand the cellular and subcellular endogenous substances like protein, metabolites, and nucleic acids for cell proteomics, metabolomics, genomics, and transcriptomics studies [3]. Therefore, it attracts researchers from various research fields. With respect to doctors and pharmacists, cell heterogeneity may have an impact in the understanding of diseases such as cancer, the mechanism of emerged drug-resistant cells, and the function of the immune system. With regard to biologists, cell heterogeneity may reveal insights into the fundamental biological and physiological behaviors including the size, growth rate, and morphology [4]. For analytical chemists, they are committed to develop new analytical methods to overcome the limits in single-cell analysis. Compared with conventional bulk cell assays, single-cell analysis suffers from several challenges. The biggest challenges arise from small size and volume of a cell, small concentrations of cellular components, and cellular ingredients with a wide range of concentration levels [5]. These complex and dynamic intercellular processes put forward higher requirements to scientists to develop higher sensitive, higher selective, and higher spatial-resolved methods for single-cell analysis. Earlier methods for single-cell analysis are mainly based on flow cytometry or laser scanning cytometry by rapidly screening fluorescently labeled cells in a flow [6, 7]. They are typically targeted to only one or very few molecules, but are highly specific and sensitive. Moreover, the data are collected only at a single time point, which prohibit dynamic monitoring of cell *responses*.

The recent development in microfluidic techniques has exhibited a powerful tool for single-cell analysis [8]. Compared to conventional methods, microfluidics with micro-sized channels has the advantages to handle mass-limited analytes, control the local microenvironment, integrate multiple functions into a single system and analysis in a parallel mode. The automatic analytical ability through high integration of multifunction units like sample preparation, separation, and detection can greatly reduce measurement errors generated from human operations. For single-cell analysis, miniaturized microfluidic systems are compatible to the size of a single cell (~ 10 μm in size), and they manipulate picoliter to nanoliter volumes of solution that help reduce sample loss and decrease dilution, resulting in highly sensitive assays. Moreover, microfluidics can combine with many detection techniques for online and real-time analysis, and different detection methods are allowed to be combined together for multiple types of targets detection. Sample handling is a critical procedure for single-cell analysis, and most protocols involve highly efficient manipulation of single cells. Up to now, various microfluidic-based strategies have been developed for single-cell capture, such as microwells [9, 10], microtraps [11], microvalves [12], flow cytometric methods [13], droplet-based

methods [14, 15], and optical tweezers [16]. And many detection techniques including fluorometry [17] and spectroscopy [18] can be combined with microfluidic systems for online single-cell analysis. The fluorescence microscope technique needs to pre-label the selected molecules by molecular probes or reporters, but the types of detected molecules are limited to labeling reagents. Recently developed approaches tend to label-free analytical methods, such as mass spectrometry (MS), Raman spectroscopy, and impedance measurements. These strategies do not require any tedious labeling, and cells can be observed without any intervention.

MS is becoming a powerful and well-accepted analytical approach for single-cell analysis due to its outstanding characteristics such as information-rich, high sensitivity, excellent specificity, and so on [19]. As the development of MS technology, two "soft" ionization methods, electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), are the most popular. In both methods, molecules are ionized with minimal fragmentation, so that highly accurate intact molecule weight can be provided, making identification of molecules easier. In addition, their corresponding fragment ions can be further generated through collision-induced dissociation (CID) for structure identification. ESI produces charged ions directly from a liquid, which made it convenient to online couple of chromatographic separations with mass spectrometry. However, it is tedious because sample pretreatment and chromatographic separation steps are time-consuming. For MALDI-MS analysis, the requirement of vacuum operation condition restricts its application in live single-cell analysis. In recent years, secondary ion mass spectrometry (SIMS), an advanced technology for surface analysis, is also reported for the analysis of chemicals in single cell with high sensitivity, high throughput, and spatial resolution. However, high vacuum condition is also needed during secondary ions on their way to the detector. With the invention of ambient ionization techniques, ambient MS has attracted an increasing interest since the beginning of twenty-first century [20, 21]. Samples can be directly and straightforwardly analyzed in an open-air under ambient conditions without or minimal sample pretreatment. Up until now, different kinds of ambient MS techniques have been explored for single-cell analysis, which open a new way for rapid, direct, in situ, and real-time study of the complexity and heterogeneity in single cells.

Coupling of microfluidics to MS has the advantages of flexible sample manipulation, fast analysis time, high throughput, and enhanced sensitivity. The development of coupling microfluidic chips with MS in the early stages mainly focused on the MS interface. With the maturation of microfluidic fabrication techniques, microfluidics has evolved from simple infusion tools interfacing to MS to sophisticated functions that integrated with many sample pretreatment units, such as sample extraction, derivatization, and separation. A few reviews have been published about the microfluidics and MS combination systems for various applications in life science [22–24]. In this chapter, we review the advances in the field of microfluidics and MS combination systems for single-cell analysis that have been published in recent years. We firstly describe various function units integrated on

microfluidic chips, such as single-cell capture, automated sample preparation, and MS analysis. Secondly, we highlight the advances in the coupling of microfluidics to the MS interfaces with different ionization techniques. Recent applications of microfluidics-MS in nucleic acid, proteins, small molecules, and pharmaceutical analysis are reviewed and commented. Finally, we also discuss the future directions in the improvement of microfluidic techniques and ambient MS for automatic and highly sensitive single-cell analysis.

7.2 On-Chip Sample Preparation

The first step in single-cell analysis on microfluidic devices is sample preparation, including isolation of individual cells from bulk cells and docking them into a desired location for further treatment. For free cells like yeast, bacterial, or blood cells, they can be manipulated easily. But for the cells from tissue samples, they should be firstly released by chemical reagents or enzymes or by micromechanical forces [25]. With the rapid advances in micro-electro-mechanical systems (MEMS) technology, the microchannel size can be fabricated downscale to several micrometers so that the single cells can be precisely manipulated [26–28]. We highlight the most commonly used single-cell manipulation techniques and recent developments to improve efficiency and sensitivity.

7.2.1 Microwells

The microwell structures provide a convenient way to isolate and trap single cells using physical boundaries. The geometry, size, depth, and material properties of the microwells can be easily changed to capture *cells of interest* [29]. There are a few reviews reporting single-cell isolation using microwells in detail [30, 31]. One common method to achieve this goal is to design cell-sized microwells to dock them through gravity-dependent sedimentation. The excess cells outside the wells are then flushed away. The general methods to fabricate microwell arrays is based on poly(dimethylsiloxane) through soft lithography. But the process is time-consuming, and expensive chrome photomasks are needed for photolithography when the well size is less than 20 μm for single-cell capture. To overcome this limitation, Liu et al. developed a simple and cheap approach to fabricate masters for microwell generation [32]. As shown in Fig. 7.1a, a master was formed by self-assembling polystyrene microspheres on *a glass slide* and then partially melted the microspheres. The master could be used for the formation of 10- to 20-μm microwells for single-cell capture. Recently, another easy-to-use method called digital micromirror approach was developed by Yang et al. to fabricate a poly (ethylene glycol) diacrylate (PEGDA) hydrogel microwell chip [33]. As shown in Fig. 7.1b, a digital mask based on shadowed light instead of conventional physical

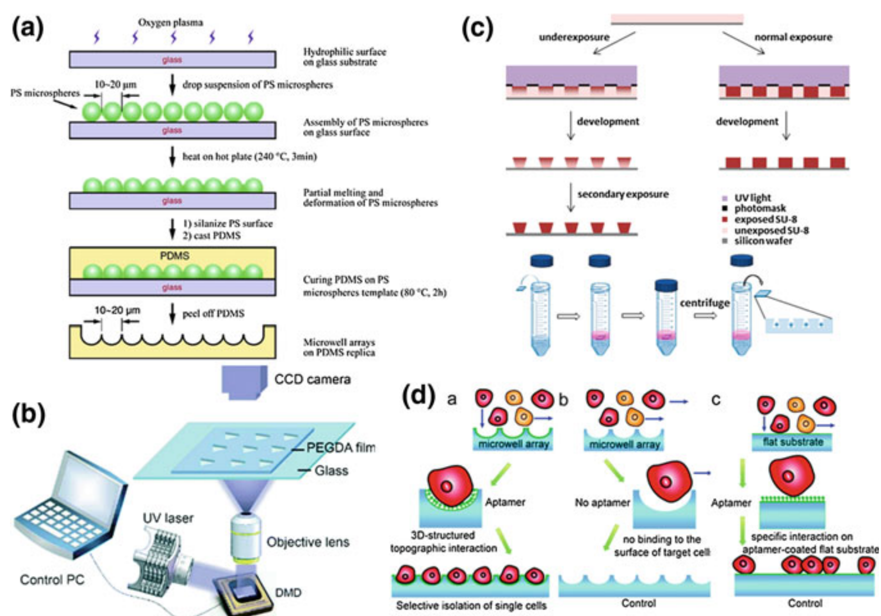


Fig. 7.1 Single-cell capture in microwell arrays. **a** Schematic illustration for the fabrication of PDMS microwell arrays and captured cell arrays on the microwells. Reprinted with permission from Ref. [32]. Copyright © 2010 American Chemical Society. **b** A schematic representation of microwell fabrication and single-cell analysis via a *digital micromirror device (DMD)*-based modulating projection printing system. Reprinted with permission from Ref. [33]. Copyright © 2017 Royal Society of Chemistry. **c** The fabrication of inverse truncated cone-shaped microposts (left) and normal columnar microposts (right) and single-cell trapping in truncated cone-shaped microwell array was realized by centrifugation assistance. Reprinted with permission from Ref. [37]. Copyright © 2010 American Chemical Society. **d** Microwells modified with DNA-aptamer for single target cell isolation. Reprinted with permission from Ref. [42]. Copyright © 2012 Royal Society of Chemistry

mask was used during the microwell fabrication process. Moreover, the constraining hydrogel film could be peeled off from glass slide for further cell analysis.

One main drawback of microwell-based microfluidic devices is that single-cell occupation rate is a bit low, ranging from 2.6 to 39% [34]. To improve the capture efficiency, external operations like aided by vacuum or centrifugation [35] are applied instead of passive gravity drive. For example, Terstappen et al. designed a self-seeding microwell chip with a single 5-μm pore in the bottom of each microwell, so that single cells can be fast and easily dragged into the pore of the microwells under a negative pressure of 10 mbar generated by degassing in a vacuum chamber [36]. The single-cell capture efficiency could finally improve to 67% with this slight structural improvement. Wu's research group designed a truncated cone-shaped microwell array to trap single cells, and the single-cell capture efficiency was increased to approximately 90% with the assistance of centrifugation [37]. As shown in Fig. 7.1c, the truncated cone-shaped microwell

structure also greatly prevented significant cell loss during cell treatment. However, majority of the reported platforms have only one fluid channel on top of the microwell arrays, which allow paracrine communication between cells, making it impossible for accurate multi-parameter detection in single cells. Recently, Garcia-Cordero et al. designed a microvalve channel on top of the microwells to create an independent microenvironment for each well [38]. During the experiments, a less than 0.02 Pa of shear stress was generated inside the wells which could keep biological behaviors of cells.

The above physical structure-based single-cell capture has the challenge in selectively isolating specific single cells, such as target tumor cells in blood. To overcome this limitation, microfabricated physical structures modified with bio-recognizable molecules will be a powerful strategy and open up a new opportunity to analysis cells of interest. Moreover, surface modification without complicated cell-sized microwell fabrication is also a good way for unique cell trapping. The commonly used molecules with specific recognition function for the isolation of specific single cells are aptamer [39], antibody [40], and protein [41]. Lin's research group used DNA-aptamer to modify microwells to bio-selectively isolate target tumor cells (Fig. 7.1d) [42]. The single-cell occupancy rate was significantly enhanced from 0.5 to 88.2%.

7.2.2 Micropatterns

Micropatterning of surface is another frequently used technique for spatial arrangement of single cells by fabricating cell-adhesive spots surrounded by cell-repellent surfaces. These contact-based single-cell trapping is an easy and cheap way for high-throughput studies. Commonly used biomimetic materials and cell adhesion molecules for adhesive regions are fibronectin [43], laminin, collagen [44], vitronectin, and poly-L-lysine [45]. However, hydrophilic polymers for cell-repellent surface modification are polyethylene glycol (PEG) [46, 47], polyvinyl alcohol (PVA) [48], and alkanethiol [49], and so on. Different strategies including microcontact printing [45], ink-jet printing [50], and photopatterning [51] have been developed to produce chemical surface patterns. However, there are still some difficulties in the homogeneous distribution of cells on the patterned area because of the nonspecific absorption of cells on the cell-repellent area. Whitesides et al. have reviewed the patterning of proteins and cells using three soft lithography techniques, microcontact printing, patterning using microfluidic channels, and laminar flow patterning [52].

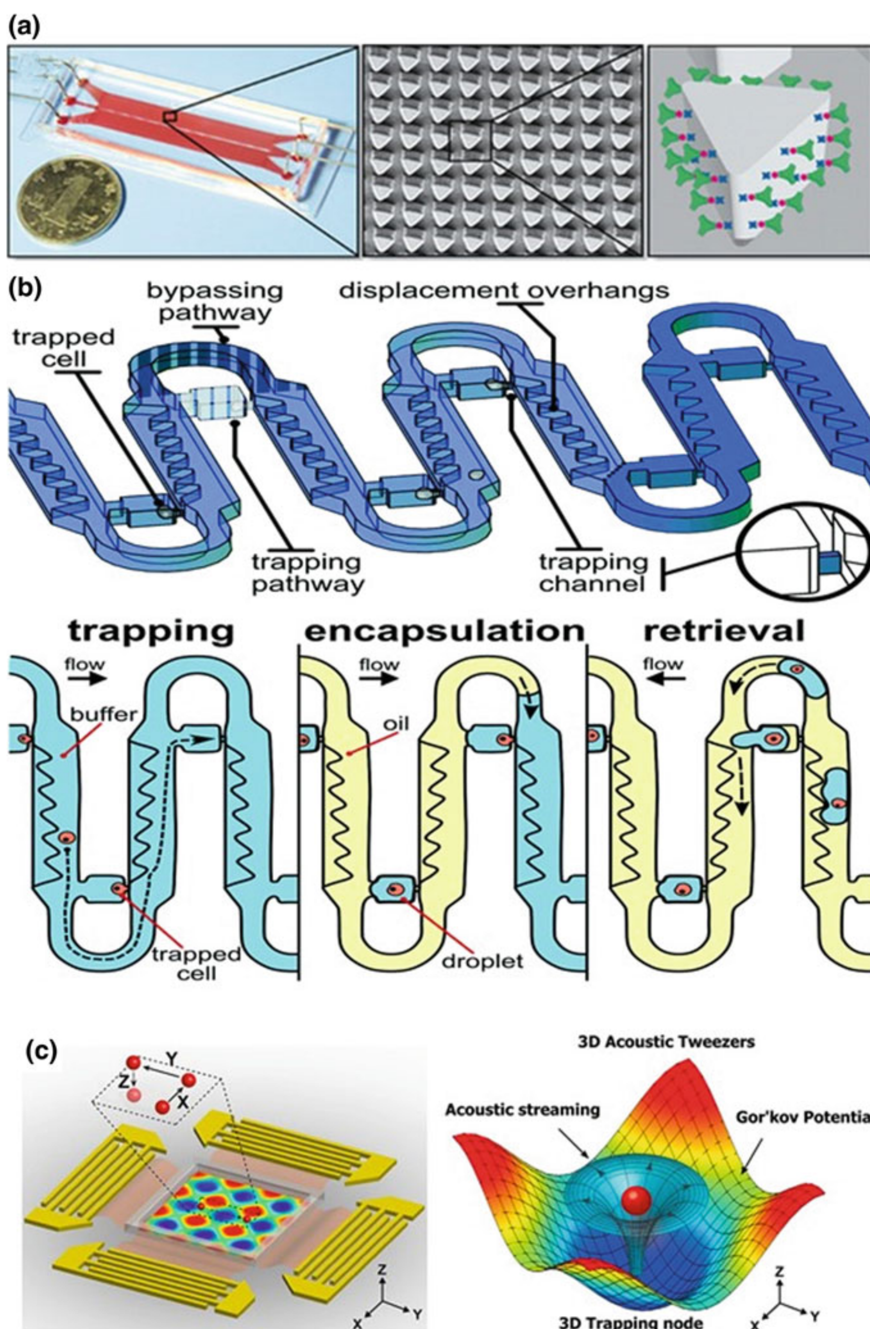
Micropatterning cells on different-shaped chemical surfaces have been widely used for cell behaviors and cell–material interaction studies, such as cell shape, division, migration, and invasion [53, 54]. Recently, Zhao et al. presented a new patterning fabrication method using tape-assisted photolithographic-free microfluidic chip for tumor migration study [55]. This technique did not need the use of microfabricated laboratory to fabricate the chip, which could be easily accessible in

routine biological lab for biological mechanism studies. Isolating specific cell subtypes of certain disease is extremely important for early disease diagnosis with no clinical symptoms. Referring to cancer disease, circulating tumor cells (CTCs) play a key role in metastatic procedure through hematopoietic dissemination [56]. The isolation of rare CTCs from peripheral blood sample can help doctor guide cancer care, but it faces some technical challenges. Micropatterning the surface of the microchannel or micropillars with chemical linkers like aptamer *or* antibody is an effective strategy to solve this problem. Yang et al. functionalized micropillars with anti-epithelial cell adhesion molecule (anti-EpCAM) antibody to capture CTCs from blood samples (Fig. 7.2a) [57]. The shape and location of the micropillars were optimized hydrodynamically to provide lower shear stress so that contact time between CTCs cells and immunodecorated micropillars would be increased to improve the capture efficiency. The retrieve of captured cells from initial capture site is also important for downstream biological analysis. A microfluidic device incorporated with a photodegradable hydrogel functionalized with leukocyte-specific antibodies was recently reported by Revzin et al. to capture and release target cells [58]. Human CD4 or CD8 T-cells from a peripheral blood sample were captured by the modified antibodies, and then the desired cells were released by UV-induced photo-degradation for the following flow cytometry analysis.

7.2.3 Traps

Similar to well- and pattern-based cell capture approaches, trapping of single cells at fixed positions by active or passive capture strategies in microfluidic systems has benefit for cell biological analysis which needs the maintenance of cells for a longer period. The related techniques *include* hydrodynamic, mechanical, magnetic, optical, electrical, and acoustic traps. Laurell and colleagues have previously reviewed both the contact and non-contact mode trapping techniques in detail [59]. Here, we will focus on the recent advances in the most commonly used microfluidic-based single-cell trapping methods.

Cells trapped by hydrodynamic flow are the most commonly used mechanism. In general, cells are stopped and trapped from the flow of a cell suspension by microscale structures (such as U-shaped *structures* [60–62]) or by bypass-channel traps [63]. Referring to bypass-channel traps, the bypass channels were usually perpendicular to the main flow channel, *and* the cells can be suctioned into the small side channel through focusing flow. Various shapes like dams, weirs, and holes can be designed for the trap structures. For example, Sauzade et al. developed a serpentine-shaped microchannel with a linear array of hydrodynamic trapping sites and filtering structures to isolate, capture, and retrieve individual cells [64]. As shown in Fig. 7.2b, incoming cells initially displaced toward the unoccupied trapping site by focusing structures. Additional cells were then diverted the flow through bypass pathway and occupied the downstream traps. The trapping scheme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3D Acoustic Tweezers
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◂**Fig. 7.2** Single-cell trapping by micropatterns and trap methods. **a** Simulated and experimental results *demonstrated* the size-dictated interaction of particles in *Size-Dictated Immunocapture Chip*. Reprinted with permission from Ref. [57]. Copyright © 2017 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. **b** Schematics of the microfluidic circuit and work flow for true single-cell encapsulation. Reprinted with permission from Ref. [64]. Copyright © 2017 Royal Society of Chemistry. **c** Illustration of 3D acoustic tweezers for particle or cell trapping. Reprinted with permission from Ref. [67]. Copyright © 2016 National Academy of Sciences

can improve the single-cell capture efficiency to a near-perfect rate. However, the hydrodynamic trapping technique usually generates mechanical stress on cells, which will have negative effects on physiological function of cells. Recently, a mechanical trap array with four optical transparent optical arms was developed by Gracias et al. to capture and encapsulate single cells [65]. The four arms consisted of SiO and SiO_2 on a quartz substrate can fold by tailoring a thin film stress to encapsulate cells without any perturbation.

Except to those passive techniques, some non-contact and active trapping techniques like dielectrophoretic (DEP) and acoustic-driven traps are also reported in recent years to manipulate individual cells with high precision. For DEP traps, cells can be moved by forces generated in a non-uniform electric field. The key point for DEP trapping is to control a scalable array individually to increase the number of cells available for analysis. To solve this problem, Zhang et al. combined an alternative pause-and-sort Raman-activated cell sorting on microfluidic device with positive DEP for single-cell trap and release [66]. This method allowed the single-cell trapping, sequence position and separation, and individual detection by Raman in a high-speed flow. Acoustic actuation, like ultrasonic standing waves, *offers* dynamic control of cell environment for short-term analysis. Cells or particles can be pushed toward pressure nodes by the acoustic radiation force generated by acoustic waves. Huang's research group has focused on the surface acoustic waves driven for many years, and they have received many excellent achievements. Recently, they integrated surface acoustic waves into the microfluidic device to generate an array of 3D trapping nodes for trapping and *manipulating single cells* and particles (Fig. 7.2c) [67]. The operating frequency of acoustic waves has significant effect on cell viability and behavior. Neild's research group systematically investigated the relationship between acoustic power and cell viability [68]. They found that the critical acoustic power for lymphocytes was less than 570 mW, and the lysis threshold power was different to different cell types. However, one main shortcoming for acoustic cell trapping is *the disability to keep* cell viable for long-term analysis.

7.2.4 Droplets

Droplet-based microfluidics has emerged as a new forerunner for massive parallelized single-cell analysis in recent years. The fact can be attributed to the

following reasons. Firstly, single cells and reagents can be isolated and encapsulated in monodisperse picoliter liquid droplets at a throughput of thousands per second. Secondly, droplets provide an isolated compartment so that the risk of cross-contamination can be largely reduced. Thirdly, small-sized droplets facilitate rapid mixing of encapsulated solution, thereby minimizing sample dilution. Joensson's research group have reviewed the technical advances on the droplet microfluidic field for single-cell analysis and the application of these technical developments to further biological understanding [69]. Later, Dittrich et al. discussed the advantages and limitations of the droplet microfluidic approach for single-cell analysis [70].

Typically, T-junction and flow-focusing geometries' microfluidic channels were designed for the generation of highly monodisperse droplets. Water-in-oil or oil-in-water emulsions as well as complex multiple-phase emulsions can be generated (Fig. 7.3a) [71]. The size and rate of droplet formation can be regulated by a series of parameters, such as channel dimensions, flow rates, viscosities, and interfacial tension [72, 73]. Compared to other techniques, droplet microfluidics allows for high-throughput and massively parallelized studies on single cells due to the generation of droplets with high frequency from Hz to kHz. Currently, droplet microfluidics has been widely used in cell biology, clinical research, materials science, and drug discovery. Different from conventional microfluidic-based droplet generation approach, Chen et al. developed an ink-jet printing-based droplet system for single-cell encapsulation, and the single-cell lipids can be directly analyzed by probe electrospray ionization mass spectrometry [74]. As shown in Fig. 7.3b, the droplet volume can be precisely controlled by adjusting the voltage and pulse time exerted on the ink-jet head. The position of the generated droplets from the ink-jet could be adjusted by an automatic *X*-*Y* stage. In order to keep the homogeneous distribution of cells in liquid, a homemade magnetic stirring device was applied to the cell suspension reservoir. Although single cells can be encapsulated in independent aqueous microdroplets, large different sizes between cells (about 1 pL) and droplet volumes (ranged from nanoliter to microliter), and the matrix effects from cell culture medium or intercellular matrix will affect the following detection sensitivity. To address this problem, a droplet-based extraction capillary was developed by Zhang et al. to combine with ESI MS for cellular metabolites detection [75]. As shown in Fig. 7.3c, a pulled glass capillary containing extraction solvent at the tip of the capillary, manipulated by a three-dimensional manipulator, could be placed close to the surface of a single cell for cellular component extraction. Different desired metabolites could be easily extracted by using specific extraction solvent. *Although* most of the microfluidic-based droplet systems have been successfully applied in single-cell analysis, *they* still face the challenges of adding reagents into the generated droplets. Fang's group developed a solid pin-based droplet system to dip and deposit liquids on a two-dimensional and movable oil-covered hydrophilic pillars for liquid–liquid reactions and assays [76]. As shown in Fig. 7.3d, by using solid pin-based liquid "dipping-depositing-moving" manipulation strategies, they could *easily realize additional reagents adding* to the sample droplet. However, this technique still suffers from

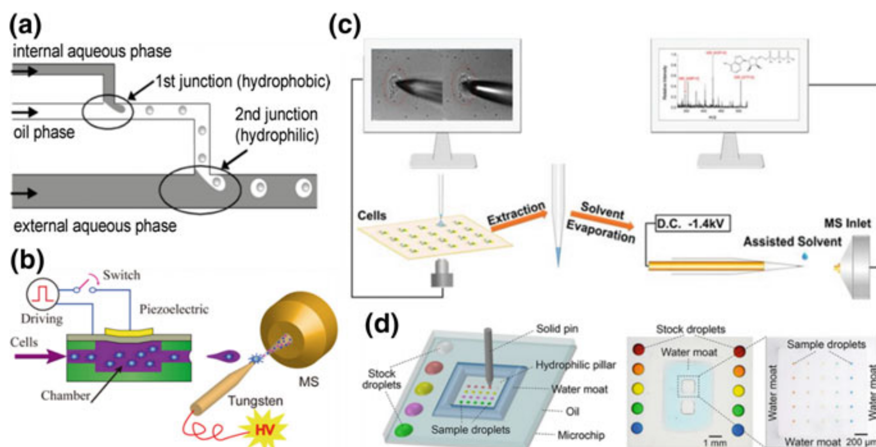

Fig. 7.3 Single-cell capture by microdroplets. **a** Basic concept for preparing double emulsions (W/O/W) using T-shaped microchannels. Reprinted with permission from Ref. [71]. Copyright © 2004 American Chemical Society. **b** The novel method for single-cell analysis and lipid profiling by combining drop-on-demand ink-jet cell printing and probe electrospray ionization mass spectrometry (PESI-MS). Reprinted with permission from Ref. [74]. Copyright © 2016 American Chemical Society. **c** A method that integrated droplet-based microextraction with single-cell mass spectrometry. Reprinted with permission from Ref. [75]. Copyright © 2016 Springer Nature Limited. **d** Setup of the solid pin-based droplet system. Reprinted with permission from Ref. [76]. Copyright © 2018 American Chemical Society

limited sample pretreatment procedures. Later, they developed a nanoliter-scale oil-air-droplet microfluidic system for single-cell proteomic analysis [77]. Multistep complex sample pretreatment and injection procedures could be realized on the established platform with minimum sample loss; thus, the analytical sensitivity was significantly increased for single-cell samples.

7.3 Chip-MS Interface Development

As we all known, a single cell contains small amount of biomolecules with a wide range distribution, which brings huge challenges for single-cell component analysis. Therefore, there is an urgent need of detection methods with high sensitivity. Fluorescence, electrochemical, PCR-based detection, and mass spectrometry are the most commonly used techniques for microfluidic-based single-cell analysis. Among these detection methods, mass spectrometry receives extensive attention due to its high sensitivity and capability to identify unknown molecules without *pre-labeling*. Different types of ablation and ionization methods can be coupled to a mass spectrometer, such as ESI, laser ablation/laser desorption ionization (LA/LDI), secondary ionization (SI), inductively coupled plasma (ICP), and ambient

ionization. The development of technologies and applications in microfluidics and mass spectrometry combination systems have been previously discussed from the year 2008 to 2013. Lin's research group has reviewed the progress made in the techniques about the *microchip-MS* and related applications in proteomics and cell analysis [22.77-79]. Over the past two years, a few reviews have been published in high-impact journals, such as *Angewandte Chemie* [78], *Journal of the American Chemical Society* [79], *and Trends in Analytical Chemistry* [80], to thoroughly overview recent advances in mass spectrometry-based single-cell analysis. Mass spectroscopy types have been developed from those requiring high pressure and a vacuum to transport the ionized molecules to the MS with ambient ionization techniques. The most important *issue* for coupling of microfluidics to MS is *to develop* stable and effective interfaces.

7.3.1 ESI-MS

ESI was firstly introduced by Dole et al. in the late 1960s [81] and later applied in the ionization of proteins by Fenn et al. in the late 1980s [82]. In ESI, molecules in sample solutions are ionized through an electrospray emitter, which is usually a needle-shaped structure. According to the requirement, the earliest miniaturization of emitters includes microspray and nanospray ionization formats mainly using pulled glass capillaries. Referring to microfluidic chip-ESI-MS interfaces, there are two categories of miniaturized emitters, capillaries and microchip emitters. With the advances in the microfabrication techniques, the microchip emitters have developed from one ESI emitter to multi-ESI emitters [83], which greatly increased the analytical throughput. For online ESI-MS detection, salts and buffers should be firstly removed to eliminate ion suppression. Micro-solid-phase extraction (micro-SPE) is the most commonly used approaches for sample pretreatment and cleanup interferences from analytical samples. For the analysis of complex samples by MS, the interference by the background and ion suppression between molecules are required to be considered [84]. In order to overcome these drawbacks, some on-chip separation strategies like capillary electrophoresis (CE) or liquid chromatography (LC) [85] to MS can be adopted. Recently, tremendous efforts have been focused on the integration of related sample pretreatment units, such as enzymatic digestion, extraction, desalting, and preconcentration on microfluidic devices for directly MS detection [86–88].

Our group [89–91] has engaged in the coupling of microfluidics with MS for chemical and cell biology studies for nearly ten years. Due to the powerful and integration abilities of microfluidic devices to mimic the physiological system of interest, various related functional parts, including cell culture, metabolism generation or cell secretion, sample pretreatment, and MS detection, can be integrated on one microfluidic platform. For example, Gao et al. [92] firstly coupled the microfluidics to ESI-Q-TOF-MS directly through a silica-fused capillary. As shown in Fig. 7.4a, by integrating cell culture and micro-SPE functions on one

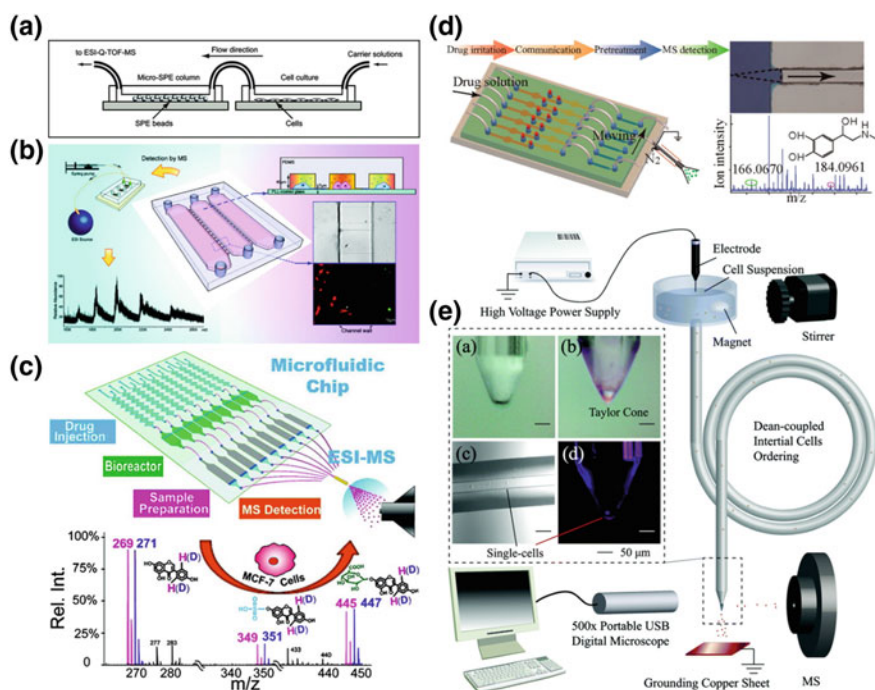

Fig. 7.4 Microfluidic-based single-cell analysis with ESI-MS detection. **a** Schematics of one unit for cell culture and sample pretreatment prior to ESI-Q-TOF-MS detection. Reprinted with permission from Ref. [92]. Copyright © 2010 American Chemical Society. **b** Schematic illustration of the microfluidic device integrated in a controlled co-culture system for detection of secreted proteins. Reprinted with permission from Ref. [89]. Copyright © 2011 American Chemical Society. **c** A stable isotope labeling-assisted microfluidic chip electrospray ionization mass spectrometry (SIL-chip–ESI-MS) platform. Reprinted with permission from Ref. [94]. Copyright © 2012 American Chemical Society. **d** A novel method for cell-to-cell communication study on an integrated microdevice. Reprinted with permission from Ref. [96]. Copyright © 2013 American Chemical Society. **e** Experimental setup of Dean flow-induced cell sorting and Taylor cone-induced electrospray for single-cell analysis. Reprinted with permission from Ref. [97]. Copyright © Royal Society of Chemistry 2018

microfluidic device, metabolism of vitamin E in human lung epithelial A549 cells can be easily studied. Compared with conventional methods, our method provides short analysis time (less than 10 min), low sample and reagent consumption (less than 100 μL). Our established platform opens up a new approach for direct, fast, and semi-automated cell-based analysis. Based on this combination technique, Lin's group later successfully detected glutamate release from neuronal PC12 cells using online ESI-Q-TOF-MS [89]. As shown in Fig. 7.4b, Wei et al. also mimicked biological bioreactions generated from *the interaction between PC12 and GH3 cells* on a microfluidic device, and the regulation of growth hormone secretion by PC12 cells was demonstrated by ESI-Q-TOF-MS detection [93]. In

order to realize more accurate quantitative analysis by the chip-MS combination system, Chen et al. introduced *a* stable isotope labeling-assisted technique for quantitation in metabolic studies (Fig. 7.4c) [94]. In the drug discovery process, prediction of drug and metabolism toxicity is extremely important to screen drug candidates during the preclinical stage. Mao et al. developed a microfluidic device to simultaneously *evaluate* drug metabolism in human liver by online ESI-Q-TOF-MS and its cytotoxicity on HepG2 cells [95]. Cell-to-cell communication plays a critical role in living tissues and *has* attracted much attention to biologists. Mao et al. later developed a “Surface Tension Plug” on a microfluidic chip for cell-to-cell communication study, and signal molecules like epinephrine and glucose secreted from 293 and L-02 cells separately were successfully detected by online ESI-Q-TOF-MS after on-chip SPE treatment (Fig. 7.4d) [96]. However, all the above systems lack automatic analytical capability, because a silica-fused capillary for the connection of microfluidics to MS should be manually moved from one channel to another for multiple cell experiments’ analysis. To overcome this drawback, Huang et al. from Lin’s group recently developed a novel Dean flow-assisted cell ordering system to generate single cells rapidly for high-throughput ESI-MS analysis (Fig. 7.4e) [97]. Based on the principle of Dean flow, the agglomeration and uneven distribution of cells in the cell suspension could be greatly reduced, which significantly improved the efficiency of single-cell MS analysis. In this platform, a spiral capillary was installed for rapid cell ordering and with the capillary tip polished and silanized for the generation of *Taylor cone* to induce ESI and *flow* in the capillary. They distinguished the cell subpopulations of several human tumor cells and confirmed a slightly different amount of phospholipids between various tumor cells.

7.3.2 MALDI-MS

MALDI is another common soft ionization method, which was firstly proposed by Karas and Hillenkamp [98] and Tanaka et al. [99] in late 1980s. MALDI is generally used for large molecules analysis, such as proteins, carbohydrates, peptides, and polymers [100]. In MALDI, a laser is used to irradiate co-crystallized film of target analytes and a matrix. During the ionization process, the matrix firstly absorbs laser energy and then transfers parts of its charge to analytes to ionize them [101]. Compared with other ionization techniques, a specific characteristic of MALDI-MS is the ability to provide both chemical and spatial information. Moreover, MALDI-MS has higher tolerance to buffers, salts, and impurities in samples. However, the main factors of MALDI that limit the spatial resolution down near to the single-cell level are matrix crystal size and laser beam size. Recently, several technological and methodological advances have been made to overcome these difficulties, and some of them have been reviewed by Trouillon et al. [102]. For example, (1) the usage of the smartbeam II laser instead of standard nitrogen or solid-state laser with Gaussian beam to obtain higher spectral quality;

(2) employment of a commercial matrix application device (e.g., Imageprep) to obtain optimal extraction with minimal crystal sizes [103]; (3) stretch cell sample to compensate for the limited spatial resolution of MALDI imaging [104].

Microfluidic-based MALDI analysis is usually performed using an offline format because a MALDI target is under vacuum while the microfluidic operations are at ambient condition. Samples are usually deposited directly on a sample target by dropping, spraying, or spotting for the sequential analysis. There are a few researches about the direct analysis of cellular biomolecules at single-cell level by MALDI-MS [105, 106]. However, the cells should be manually selected which significantly reduce the throughput. To improve it, our group developed a microwell-array-based microfluidic chip to combine with MALDI-MS for automatic and high-throughput single-cell phospholipid analysis [107]. As shown in Fig. 7.5a, a high-density PDMS microwell array was fabricated to assist the formation of a cell array on an indium tin oxide (ITO)-coated glass slide. After matrix deposition, MALDI-MS imaging analysis could be automatically performed in a

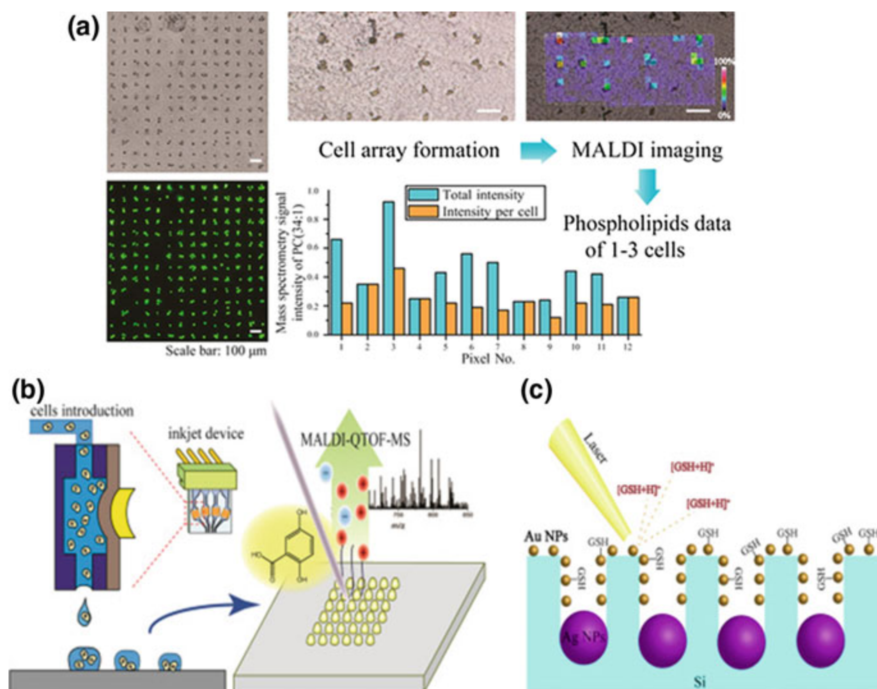

Fig. 7.5 Microfluidic-based single-cell analysis with LDI MS detection. **a** The combination of microfluidic chip and MALDI-MS for high-throughput and automatic single-cell phospholipids analysis. Reprinted with permission from Ref. [107]. Copyright © 2015 American Chemical Society. **b** Ink-jet cell introduction for MALDI-MS *analysis*. Reprinted with permission from Ref. [50]. Copyright © 2017 Elsevier B.V. **c** *Schematic* of cell analysis on *a* silicon chip with in situ synthesis of Ag and Au NPs. Reprinted with permission from Ref. [108]. Copyright © 2017 Elsevier B.V (C)

high-throughput mode by setting a matched distance between cell spacing and step size of the sample stage. However, the single-cell capture efficiency is a little low, about 30%. Korenaga et al. developed an ink-jet automatic single cells and matrices printing system to directly print sample onto a ITO glass substrate for single-cell MALDI-MS analysis (Fig. 7.5b) [50]. This technique shows controllable high-throughput analytical capabilities. Unfortunately, the most commonly used chemical matrix has strong background signals in low-mass region (< 600 Da), which makes spectral analysis more difficult. The developed surface-assisted laser desorption ionization MS (SALDI MS) offers a matrix-free way to reduce low-mass range background noise. Wang et al. in Lin's Lab developed a porous silicon chip modified with gold nanoparticles for the capture of Caco-2 cells, and intercellular glutathione was detected by SALDI MS [108]. As shown in Fig. 7.5c, the silicon chip was array-patterned for high efficient cell capture and high-throughput automatic SALDI MS detection. This method showed great potential for more efficient analysis of small thiol biomarkers in complex biological samples.

7.3.3 Secondary Ion Mass Spectroscopy (SIMS)

A large number of single-cell-based works have been carried out with SIMS due to its high spatial resolution. For single-cell analysis, time-of-flight SIMS (TOF-SIMS) uses a pulsed ion beam desorb secondary ions from the very outermost surface of a single cell. The technique has the ability to detect biological molecules with molecular weight lower than 1000 Da, such as lipids, metabolites, and the resolution can downscale to subcellular level. Sample preparation is a key step in biological mass spectrometric analysis, especially for those methods requiring a vacuum environment. Probably due to this reason, microfluidic-based single-cell analysis scarcely uses the SIMS as the detection technique. Recently, inspired by our previous work [107], Wu's group developed a micropatterning PDMS stencil film to capture and form single-cell microarray with the assistance of centrifugation, and they studied drug-induced cellular phenotypic alterations by TOF-SIMS for the first time [109]. The facile single-cell patterning method exhibited higher than 90% of site occupancy and more than 97% of single-cell resolution. Most work in this field can be done to help better understand the molecular biology for many diseases and discover potential biomarkers for early diagnosis of disease.

7.3.4 Chromatographic Techniques Coupled to MS

Due to the tiny concentrations of biomolecules with a wide range of distribution in single cells, the coupling of chromatographic techniques to MS will significantly enhance separation and identification of intercellular compounds. Capillary

electrophoresis (CE) represents the most popularly used separation techniques for this purpose because of its strong abilities for separation of cellular metabolites in miniaturized sample volume. Since the development of CE coupling to MS has been reviewed recently by Fritzsch et al. [110], Kleparnik et al. [111], Zhong et al. [112], and Tycova et al. [101], we only focus on the major progress in this field for single-cell analysis. Ramsey's group integrated cell lysis, electrophoresis separation, and an integrated electrospray emitter on a crossed microfluidic device for online separation of intracellular molecules and direct analysis using ESI-MS [113]. Onijko et al. presented a CE-ESI-MS approach for metabolites identification in single embryonic cells from the South African clawed frog [114]. By *microextraction* of their metabolomes, they could identify 40 metabolites that have relationship with central metabolic networks. The differences in activities between different cell types in the wild-type, unperturbed embryos could be revealed by relative quantitation analysis.

7.3.5 ICP MS

The application of ICP MS for trace elemental analysis in single cells has attracted an increasing interest in recent years [115]. The ion source of ICP MS uses high-temperature plasma to transform the atomic or molecular ion of a sample into a charged ion [116, 117]. ICP MS has several distinct advantages as follows: (1) ICP MS can be injected at ambient pressure, enabling combining with other injection technique easily; (2) ICP MS has low detection limit, fast analysis speed, and simple spectrum; (3) the low initial ion energy made it compatible with many simple mass analyzers. Many researches have reported about the combination of droplet microfluidics with ICP MS for ultra-trace elements analysis in single cells. For example, Hu et al. presented a cross-channel droplet chip to directly sampling to time-resolved ICP MS via a miniaturized nebulization system for the quantification of Zn in single HepG2 cells (Fig. 7.6a) [118]. To match each ICP MS spike with one cell, the cells should be spatially and temporally separated. By optimizing the dimensions of the droplet generation channels and sampling flow rate, an average diameter of 25 μm droplets was formed with the droplet generation frequency of $3–6 \times 10^6$ droplets per minute, which could be applied for *high-throughput* single-cell analysis. However, the direct quantification of trace elements in cells by ICP MS still faces a large bottleneck. One reason is caused by the serious matrix effect from complex intracellular components. To alleviate this disadvantage, an appropriate sample pretreatment technique can be taken. Yu et al. in Hu's Lab integrated a magnetic solid-phase microextraction (MSMPE) column on a chip to extract the released Cd and Se from single cells after treated with CdSe QDs and directly detected by ICP MS (Fig. 7.6b) [119]. Under the optimized extraction conditions, the limits of detection (LOD) of the developed platform are 2.2 and 21 ng L^{-1} for Cd and Se, separately.

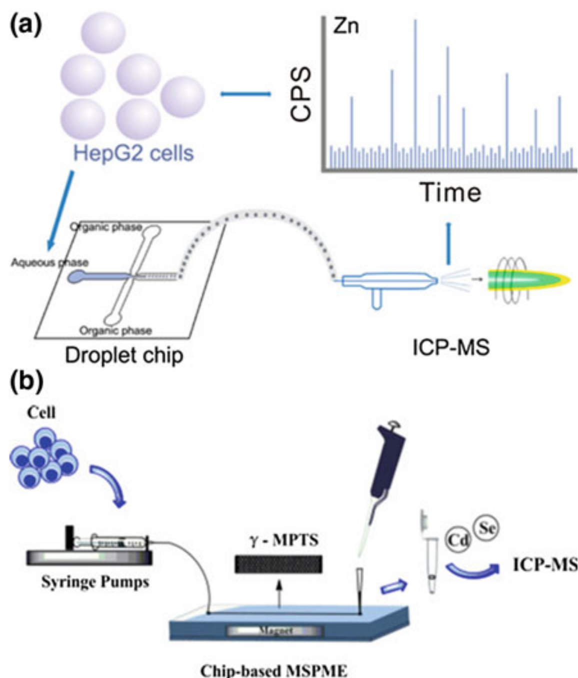

Fig. 7.6 Microfluidic-based single-cell analysis with ICP MS detection. **a** Schematic diagrams of single-cell analysis on the combination of facile droplet chip and ICP MS. Reprinted with permission from Ref. [118]. Copyright © 2017 American Chemical Society. **b** Schematic illustration *of chip-based* magnetic solid-phase microextraction coupled with ICP MS for the determination of Cd and Se in single cells. Reprinted with permission from Ref. [119]. Copyright © 2018 Elsevier B.V

7.3.6 Paper Spray Ionization MS

As discussed above, the vacuum operating conditions of some ionization techniques (e.g., MALDI and SIMS) limit their applications in living cell analysis. The recently developed ambient ionization techniques, which allow the direct analysis of complex samples under ambient condition, are good selective for living cell analysis. Since the invention of ambient ionization techniques, many efforts have been made to apply ambient MS for single-cell analysis. A series of ambient MS have been explored to analyze various compounds at cellular/subcellular level, such as desorption electrospray ionization (DESI) [120], probe-ESI [121], easy ambient sonic-spray ionization (EASI) [122]. Paper spray ionization, possessing both the characteristics of ESI and ambient ionization techniques, has made some significant progress.

To improve the efficiency and capability for live cell analysis, automatic multi-channel paper-based chip-MS was developed for direct MS cell analysis by Lin's group [123, 124]. As shown in Fig. 7.7a, Liu et al. developed a microdialysis-paper spray ionization as the interface to MS for online chemical monitoring of cell culture [123]. A homemade microdialysis hollow fiber module was constructed to selectively dialysis molecules of interest from cell culture medium. Microdroplets were then generated with controllable size and frequency through a syringe pump and were directly dropped on the paper substrate for online

MS detection. They further developed a multi-channel paper-based chip for cell metabolism study by ESI MS under ambient condition [125]. As shown in Fig. 7.7b, a multi-channel microfluidic device with the functions of a concentration gradient generator and cell culture chambers was used to generate samples with different stimulation conditions. Paper spray ionization was simultaneously employed for microsampling these samples and as the interface for direct MS analysis without any sample pretreatment. On this platform, they investigated the effects of hypoxia on lactate efflux from normal and cancer cells and the differential inhibitory effects and dose–response information of α-cyano-4-hydroxycinnamate on different types of cancer cells. Instead of using paper as the substrate for ionization spray, Wu et al. developed a glass spray-MS platform for direct cell-based drug assay under ambient pressure (Fig. 7.7c) [126]. The authors later developed a multi-channel glass spray chip-MS platform, in which cell co-culture, cell apoptosis assay, and MS detection could be simultaneously performed [127]. Chen et al. later developed a cell-compatible polycarbonate paper chip for in situ live cell components detection by paper spray MS [124]. However, this method is well suitable for large amount of cell analysis, but not appropriate for single-cell analysis. To perform in situ single-cell analysis, a Live Single-Cell Extractor (LSCE) was presented

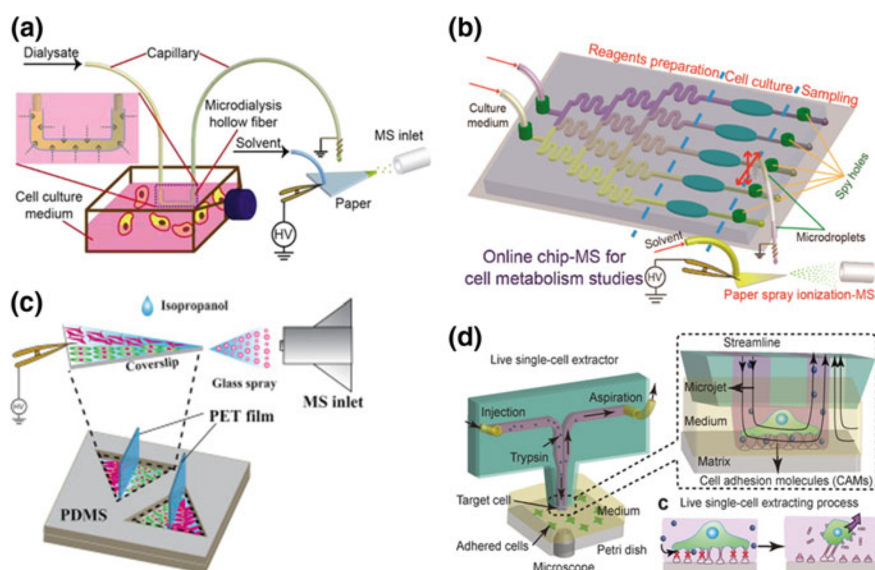

Fig. 7.7 Living single-cell analysis on microfluidic device. **a** Schematics of a microdialysis-paper spray ionization-MS system for online chemical monitoring of cell culture. Reprinted with permission from Ref. [123]. Copyright © 2014 American Chemical Society. **b** Schematic illustration of the online multi-channel microfluidic chip-MS platform for cell metabolism study. Reprinted with permission from Ref. [125]. Copyright © 2016 American Chemical Society. **c** A versatile glass spray-MS platform for direct drug assay. Reprinted with permission from Ref. [126]. Copyright © 2015 Elsevier B.V. **d** Microfluidic chip-based LSCE. Reprinted with permission from Ref. [128]. Copyright © 2018 John Wiley & Sons, Inc.

by Mao et al. for single-cell extraction, *cell adhesion*, *and cell heterogeneity analysis* (Fig. 7.7d) [128]. The tip of the LSCE was placed perpendicular to a petri dish with cell samples, and a stable microjet could be formed underneath the tip of the LSCE when the ratio between aspiration and injection flow rates is sufficiently high. They employed trypsin molecules as the injection liquid to selectively digest the adhered cell of interest. The collected cells can be further analyzed by mass spectrometry and other detection techniques.

The rapid assays of illicit drugs *are* becoming more urgent in Europe and North America. Espy et al. presented the determination of eight drugs of abuse in blood using paper spray or extraction spray MS in less than 2 min *of* minimal sample preparation [129]. Compared with conventional blood sampling methodologies, this method showed the potential for rapid, high-throughput, and quantitative assays of multi-abused drugs.

7.3.7 Mass Cytometry

Single cell mass cytometry, a technique of coupling flow cytometry with ICP MS, is firstly proposed by Garry Nolan's group at Stanford University [130]. In this technique, cells are labeled with epitope-specific antibodies conjugated to transition element isotope reporters. In contrast to the fluorophores used in conventional flow cytometry, the stained individual cells are ionized and sensitively detected with cytometry and time-of-flight ICP MS. Up to 34 parameters could be simultaneously detected by this mass cytometry technique. Later, Nolan's group applied this technique to other applications, such as delineating cell cycle stages [131] and identifying in vivo skeletal muscle stem cell [132]. However, the combination of mass cytometry with microfluidics has not been reported yet. Due to the multi-parameter analytical ability for cells as well as the flexible manipulation property for single cells, their advantages may promote the development of their combination system for the application in biological research field.

7.4 Applications in MS-Based Single-Cell Analysis

7.4.1 Nucleic Acids

Single-cell genetic analysis plays a critical role in the study of disease diagnosis, embryonic development, microbe detection, and so on. With the development of highly sensitive technologies, nucleic acid detection can be realized at single-molecule resolution. Recently, whole-genome evaluation is becoming increasingly popular at the single-cell level. Reverse transcriptase polymerase chain reaction (RT-PCR)-based approach is probably the most widely used technique to

amplify the transcribed target RNA strands, and the amplification steps were generally monitored by optical methods such as imaging via chemiluminescence or fluorescent labeling, aiming to reveal the heterogeneity of different cell types. For single-cell gene analysis, microfluidic-based RT-PCR *enabled* high-throughput sequencing applications and parallel analysis of multiple single cells. Microfluidic approaches also provide a flexible platform for sensitive detection of gene *with the measurement precision better than* conventional methods [133, 134]. For example, sample pretreatment procedures can be integrated on the microfluidics to remove some PCR inhibitors to reduce false-negative results [135].

7.4.2 Proteins

Single-cell-based qualitative and quantitative analysis of proteins play an essential role in *the* understanding of cellular functions and revealing protein heterogeneity, which is extremely important in biomarker discovery, disease diagnostics, pathology, and therapy [136]. Protein analysis at the single-cell level is challenging due to the low abundance of proteins in single cells, the large dynamic range of many protein constituents, and the temporary existence of cellular proteins responding to external stimulation [137, 138]. Microfluidic-based MS analysis *provides* an effective approach for multi-parameter, specific, high-throughput, and automated proteins studies. Many essential functions for protein identification, such as enzymatic digestion, separation, and sample infusion, can be integrated on the microfluidic device. For example, Zhu et al. developed a microfluidic-based approach termed nanoPOTS (nano-droplet processing in one-pot for trace samples) to increase sample processing efficiency for single-cell analysis (Fig. 7.8a) [139]. nanoPOTS uses robotic nanoliter liquid handling to dispense cells and reagents *into nanowells* on a standard microscope slide. Cell suspension, MS-compatible surfactant, reducing reagent, alkylating agent, and multiple proteases were subsequently added into each nanowell with the total volume of 200 nL. To minimize evaporation during reaction incubation procedures, a layer of 30 μm PDMS was reversibly sealed to the nanowell chip. By combination with ultrasensitive nanoLC-MS, over 3000 proteins were confidently identified from just 10 HeLa cells.

Quantitative analysis of specific proteins is helpful for the diagnosis of early disease more accurately like cancer. Isobaric tags for relative and absolute quantitation (iTRAQ) is a widely used isobaric labeling method for quantitative proteomics by MS analysis. Recently, Ros et al. presented a reversible PDMS microfluidic system for relative and absolute quantification of targeted proteins by MALDI-MS/MS [140]. As shown in Fig. 7.8b, a two-layer device contained a fluid layer and a control layer *forming* a set of defined wells. An ITO-coated glass slide was reversible sealing with the two *layers* for fluid treatment and also served as the conductive MALDI-MS sample plate. In order to realize quantification analysis, the authors used iTRAQ labeling strategy to label proteins on *the* microfluidic device

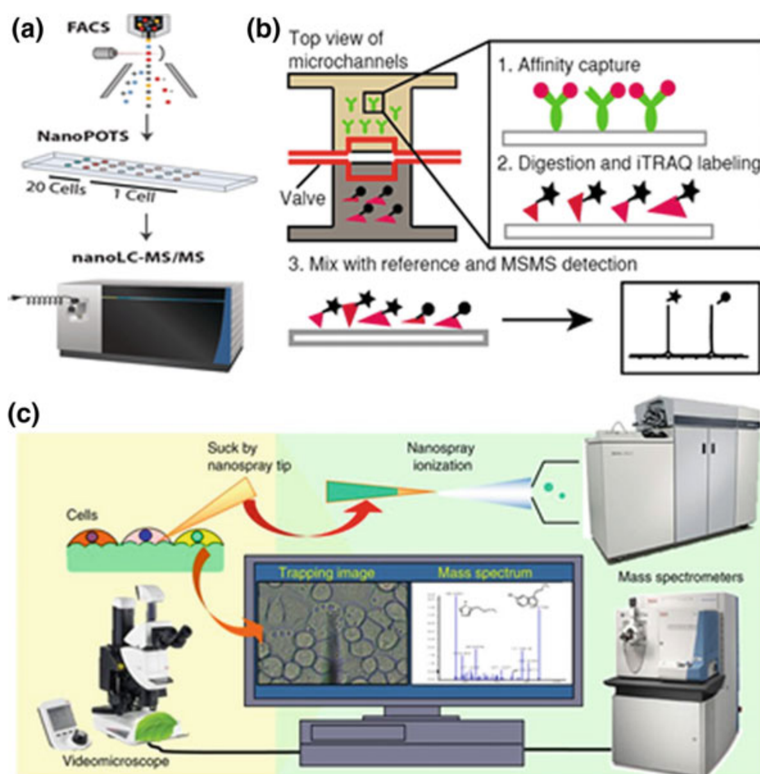

Fig. 7.8 Protein and small molecules analysis in single cells on microfluidic device. **a** *Schematic* of FACS–nanoPOTS coupling for quantitative proteomic analysis in single mammalian cells. Reprinted with permission from Ref. [139]. Copyright © 2018 John Wiley & Sons, Inc. **b** A quantitative approach employing isobaric tags with *MALDI-MS* realized with a microfluidic platform. Reprinted with permission from Ref. [140]. Copyright © 2016 American Chemical Society. **c** Schematic principle of live plant single-cell MS. Reprinted with permission from Ref. [144]. Copyright © 2015 Springer Nature Limited

for the first time. They also integrated all the necessary manipulation procedures on the chip, such as protein digestion, labeling, as well the matrix delivery. After finishing all liquid handling steps, the PDMS layer was removed, remaining analyte-matrix co-crystallized on the ITO glass surface for MALDI-MS detection. The apoptosis-related protein Bcl-2 was successfully detected, and the number of Bcl-2 molecules was quantitatively assessed.

Among proteins analysis, proteomics has attracted an increasing interest, with the aim to study the complete or subset of proteins present in a species under a certain condition. It is very useful for the investigation of the relationship between diseases and clinical diagnostics [141]. However, to develop a high-throughput method for single-cell proteomics by mass spectrometry, two major challenges should be resolved. Firstly, protein losses had to be minimized when delivering the

proteome of a single cell into a MS instrument. Secondly, peptides from single-cell samples *need* to be identified and quantified simultaneously. To overcome the above difficulties, Slavov et al. developed a single cell ProtEomics by MS (SCoPE-MS) to identify distinct human cancer cell types based on their proteomes [142]. In this method, they manually picked live single cells under a microscope and lysed them mechanically which could obviate significant protein losses during LC/MS analysis. Besides, they used tandem mass tags (TMT) to quantify the levels of each TMT-labeled peptide and to increase analytical throughput. However, the single-cell processing is still done manually, and the introduction of microfluidic techniques into this system will greatly enhance the analytical capabilities for single cells.

7.4.3 Small Molecules/Metabolomics

The analysis of intercellular small molecules like metabolites and small molecule intermediates is extremely important. They are widely involved in signal pathways and have a close relationship with physiological and pathological processes. For example, glutathione (GSH) is one of the most important and potent antioxidants in our body. Wang et al. developed a Ag-Au nanoparticle-modified porous silicon chip with the surface as matrix to assist ionization LDI MS and could specially capture and analysis of thiol compounds through Au-S binding [108]. The array-patterned silicon chip showed high-throughput analytical ability and also had great potential for more efficient analysis of small thiol biomarkers in complex biological samples.

Single-cell metabolomics analysis is of great interest to biochemistry and clinical medicine scientists. It helps address fundamental biological questions and allows for the observation of metabolic phenomenon-related phenotypic heterogeneity in single cells. However, metabolomics analysis at the single-cell level is still a challenge because of its complex microenvironment and low content. Sensitive and high-throughput methods for single-cell metabolomics analysis are still in urgent need. Microfluidic techniques provide a promising platform for single-cell metabolomics. MS, a label-free and *having the ability* to provide structural information of chemicals, has become a key enabling tool in the field of metabolomics [143, 144]. Many studies have presented the combination system of microfluidics and MS for single-cell metabolites analysis. For example, Korenaga et al. developed an ink-jet automated single cells and matrices printing system to directly positioning of single cells at defined sites of ITO glass substrate for MALDI-MS analysis, and intercellular phospholipids from single or several cells were successfully detected [50]. In this work, microarrays of cells *through* the developed ink-jet printing system is extremely important because it provides the ability for imaging and automatic analysis, which opens the way to higher-throughput measurements. Masujima and his colleagues developed a live single-cell MS platform for single plant and animal cell analysis, especially for embryo cells [145–147]. In this technology, they used microspray tips or other microcapillary tips to insert into a

single cell to sample a small amount of cell's contents [144] (Fig. 7.8c). A microliter of ionization solvent was then added to the opposite end of the tip, *and* the trapped intercellular metabolites were finally sprayed into the mass spectrometer by applying a high voltage between the tip and the inlet of the MS.

7.4.4 Pharmacological Analysis

Tumor–endothelial cell interaction plays an important role in many physiological and pathological processes, such as cancer metastasis, angiogenesis, and colonization. Majority of researches have showed that crosstalk between tumor cells and endothelial cells via paracrine/juxtacrine action has a significant impact on tumor growth, progression, and drug efficiency. To better understand their interaction, Lin et al. developed an integrated microfluidic device to probe the interaction between tumor and endothelial cells and its application in drug screening, in which all the necessary procedures including cell co-culture, protein detection, micro-solid-phase extraction unit for drug metabolites, and online MS detection could be performed [148]. Cervical carcinoma cells (CaSki cells) and human umbilical vein endothelial cells (HUVECs) were co-cultured in the cell co-culture component, which allowed for real-time monitoring of paclitaxel-induced apoptosis, the contents of intercellular ROS and GSH. After stimulation with paclitaxel, cell culture medium was introduced into the specific aptamer-precoated reaction chamber through a connecting tube for cell-secreted proteins detection by fluorescent. Whereas for cell metabolites detection, cell co-culture and the pretreatment components were connected for desalting and purification of paclitaxel metabolites and *then online* detected by ESI MS. This integrated microfluidic platform *provides* a promising tool for drug screening using the in vitro cell co-culture model.

7.5 Conclusion and Outlook

Coupling of microfluidics with MS takes advantages of both technologies, with the purpose to improve efficiency, sensitivity, and throughput. In this chapter, we documented the significant improvements in microfluidic technologies for single-cell analysis with mass spectrometry detection over the past few years, covering the recent development in microfluidic manipulation techniques for single cells, innovations in the coupling of microfluidic chips with different types of MS, and their applications in biological research and drug development. The microfluidics has become increasingly integrated which contained multiple necessary functional units for single-cell capture, separation, and detection. Several approaches have been reported for single-cell generation, including microwells, micropatterns, traps, and droplets. Their applications have tended to multi-targets or even omics components. Although some advances have been made in single-cell

analysis by microfluidics and MS combination system, there are still some demands needed to be improved like more efficient single-cell manipulation and sample pretreatment abilities to compatible with the requirement of MS detector. With the increasing requirement of analytical throughput for single cells, computational methods with powerful data processing abilities *are* also needed to be simultaneously improved. Because of the complexity of cellular process and the detection limitation of one MS technique, two or more types of MS detectors can be used together to detect more intercellular components, which will be helpful for more comprehensive understanding of the function and regulation mechanism of intercellular molecules in biological processes. Moreover, developing a new methodology to maintain the single-cell viability is a significant and essential issue in the future to perform single-cell analysis in its original state. With the development of integrated microfluidics and MS combination systems for automated single-cell manipulation, sample pretreatment, and MS detection, more applications of microfluidics-MS systems in clinical diagnosis can be expected in the near future.

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