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Advances in Microfluidics Applied to Single Cell Operation

*Xu-Dong Zhu, Ju Chu, and Yong-Hong Wang**

The field of microbiology have traditionally been concerned with and focused on studies at the population level. Microfluidic platforms have emerged as important tools for biology research at a small scale, even down to a single cell level. The spatial and temporal control of cells and stimuli transported by microfluidic channels in well-designed microsystems realized the studies of specific cells in a controlled microenvironment. The true cellular physiology responses, which are obtained mostly by inference from population-level data, could be revealed in this way. Nowadays, significant applications like cell culture, analysis, sorting, genomics, and proteomics at the single cell level have been achieved in microfluidic chips. Highly integrated microfluidic systems with complete bio-analytic functions are also coming forth and of great promise for single cell related physiology, biomedical, and high throughput screening research. Herein, the leads of technologies applied to single cell operation are reviewed. Challenges and potentials of these works are also summarized, to highlight fields for further research.

1. Introduction

As the smallest functional unit of life, single cell is an important subject in the study of cell biology.[1] Appropriate techniques like flow cytometry and capillary electrophoresis have promoted the progress of single-cell research. However, neither the richness of information nor the throughput could fulfil the intensive research. Microfluidics are defined as systems based on microchannels (10–100 μm) and used for manipulation of small volume of the fluid (nL).[2–7] Crediting its perfectly matching with single cell at the length-scale and its ultra-high throughput, microfluidic systems have been widely used in cell biology, especially in the field of single cell.

Here, as shown in **Figure 1**, advanced microfluidic techniques and applications in aspect of single cell operation are reviewed. Firstly, we summarize the new technologies related to single cell operation, which include microdroplets operation, spatial manipulation of microparticles, and detection technologies. Then advanced applications of microfluidics for single cell including cell culturing, analyzing, sorting, and omics study are reviewed and discussed. We hope this review can stimulate more inspiration of research at single cell level.

Dr. X.-D. Zhu, Prof. J. Chu, Prof. Y.-H. Wang
National Engineering Centre for Biotechnology (Shanghai)
College of Biotechnology
East China University of Science and Technology
130 Meilong Road, Shanghai 200237, China
E-mail: yhwang@ecust.edu.cn

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2. Advanced Microfluidic Techniques

2.1. Microdroplet Operation Associated With Single Cells

Microdroplet technique is an important branch of microfluidic. It is of great value in the use of single cell for three major reasons: (1) In aspect of the length scale, the diameter of microdroplet is controllable and can easily match the size of single cells (10–1000 μm). (2) Single-cell encapsulation is easy to accomplish by a random encapsulation method[8] or at very high efficiency by ingenious design of microchannel[9] with a little time and materials consumption. (3) Acting as independent reactors, microdroplets are valuable in the analysis of extracellular products at single cell level because the extracellular products like proteins or metabolites are restricted in the droplet. With these features, microdroplet is very conducive to high-throughput analysis or screening.[10–12] Furthermore, microdroplet has some superiority including small volume, no cross-contamination and fast reaction kinetics over other technologies.[13] Nowadays microdroplet technique has developed multiple operating units, such as single-cell encapsulation and reagents introduction. Relevant innovations emerged are summarized below.[14]

2.1.1. Droplets Generation

T-junctions and flow-focusing[15,16] are the most commonly used chip configurations as their high efficiencies in droplets generation.[8,17] Both of them can reach a high droplets generation rate up to 10 kHz.[18] However, under a continuous flow mode, neither of them can change the composition of droplet and droplet size flexibly. Recently, a variety of active droplet generators have been constructed, which can achieve on demand droplets generation with real time changeable composition or size. For example, Collins used surface acoustic wave (SAW) to generate independent picoliter droplets whose volume were defined by the applied power, duration of the force, and

system geometry.[19] Here, several attractive droplets generation methods are summarized in **Table 1**.

Including the electric, thermodynamics, photology, and acoustics could directly or indirectly achieve real-time size changing. The droplets size can reach a submicrons level, which was hard to imagine in conventional droplet generation. However, few of these methods can step into cell research because the lack of biocompatibility of these methods.

When comes to increasing the flexibility of droplets compositions, few methods were reported during the droplets formation process.[20] The mainstream techniques are introducing reagents after the formation of the original droplets, which will be discussed below.

2.1.2. Single-Cell Encapsulation

For single-cell encapsulation, the most widely-used method is the random encapsulation, which utilizes cells at the highest droplet generation rates. The number of cells in droplets obeyed the Poisson's distribution. To avoid droplets with multiple cells, the cell suspension is usually highly diluted. This process will lead to lots of unwished empty droplets. In contrary, active methods such as active optical trapping,[21] acoustic package,[22] can precisely control the cell encapsulation. It is meaningful to realize selective encapsulation of some rare cells or microorganism. But they worked at a very low speed. There is no doubt that a method obtained both high throughput and efficiency is of great meaning, but has yet to find.

2.1.3. Reagents Introduction

When used as a micro-reactor, the droplets should be composed of a variety of components. Therefore, a variety of technology for reagents introduction has also developed in the microdroplet system. Before the formation of droplets, multiple components can be induced into droplets by converging[23] or drawing the reagents in order.[24] If droplets have formed, reagent injection[25,26] or droplet fusion are preferred. As mentioned above, the latter methods are more efficient because the former methods are weak in throughput.

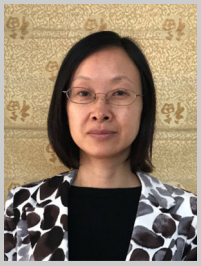
2.1.4. Droplets Fusion and Splitting

Fusion of droplets can greatly expand the application of microfluidic chip. It enables the redistribution of the reagents, dilution, or nutrition supply. Fusion occurs when the interface of two adjacent droplets was instable. The velocity, the properties of fluid and the collision angle of droplets have big influence on the droplets fusion.[27,28]

The passive fusion is usually based on the special channel structure, for example, extended channels are designed to generate velocity gradient and allow droplet approaching and oil film drainage between droplets.[29] Although the structures of these chips are simple, the precise control of flow velocity is required to prevent uncontrolled multi-droplet fusion. Yoon took advantage of the elasticity of PDMS to make two valves structure. By controlling the valves, integration of the different number of droplets was achieved.[30] In respect of active fusion, new techniques including electric fusion, optical trapping,[31,32] LED (light-emitting diode),[33] magnetic induced[34] droplet fusion were conducted. Electric fusion, for example, is based on the balance of surface tension, the viscosity of liquid within microchannel and the electric field.[35] Zagnoni showed us such a chip implementing on-demand fusion. It solved the problem of synchronization before droplet fusion. Pico-injection, injecting the required components into passing droplet, is also an effective way of droplet fusion.[26]

Splitting of the droplet can also be categorized into passive and active methodologies. The passive one relies mainly on the structure of chips. The size of the divided droplet is inversely proportional to the flow resistance of branch channel.[36] In Yoon's work, two pneumatic micro valve were used to change the flow resistance of two branch channels, so as to realize arbitrary proportion of the split droplets size.[37] Although devices of active split were more complex and difficult to operate, they could control the droplets division more precisely. Link applied an electric field in the T-shaped bifurcation of the chip. When the

Ju Chu is the professor in the school of Biotechnology at East China University of Science and Technology (ECUST), PR China. She received her PhD degree from ECUST in 1996. Her research interests cover biochemical engineering, metabolic engineering as well as high throughput screening of high performance strains. She took charge of many domestic and international collaborative research projects on industrial biotechnology, especially on bioprocess optimization and rational scale up. She has won the Chinese National Award for Science and Technology Progress for three times. Recently, she also supervised her PhD student to carry out the microfluidics studies to realize the ultra-high throughput screening of high lactic acid producer strain.



Yong-Hong Wang is a professor in the School of Biotechnology at East China University of Science and Technology and a fellow of the State Key Laboratory of Bioreactor Engineering (Ministry of Science and Technology, China). Her research interests cover high throughput screening technology for enzyme and microorganism, and microorganism bioprocess engineering. She has received the Second Prize of National Award for Science and Technology Progress (Ministry of Science and Technology, China, 2011).

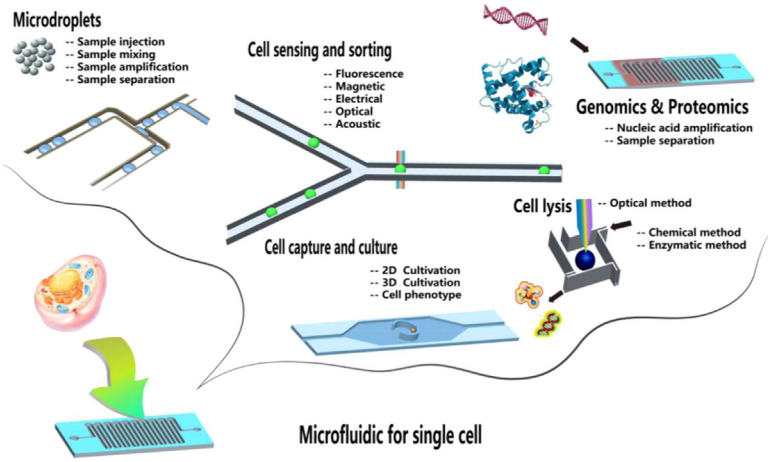


Figure 1. Microfluidics applied to single cell. Operation units of microfluidic for single cell: microdroplets, cell capture, cultivation, sensing, sorting, lysis, and omics analysis.

Table 1. Different methods of droplet generation.

Principle of generation	Advantages	Disadvantages	Generation frequency	System complexity	Ref	Application
Slotted-vial array sample	i) High integrity, simple structure	i) The slotted-vial is non-standard accessories which has difficulty in quality control	24 Hz	Medium	[24]	Protein crystallization conditions screening
	ii) High compositions flexibility					
Deposit samples	i) Generates a sequence of droplets with compositions programmed by the user	i) Low generation frequency	3 Hz	Medium	[98]	Determining the minimum inhibitory concentration and pair-wise interactions of ampicillin, tetracycline, and chloramphenicol against *E. coli*
	ii) Precise (<1% in standard deviation) control of liquid volumes	ii) Need to precisely control the flow rate of the four channels to achieve droplet fusion				
Electric	i) Flexible size controlled by the electric field in microchannels.	i) The liquid should be slightly conducting to form a Taylor cone	1 kHz	High	[99]	N/A
	ii) Periodic formation of a Taylor cone under an ac electric field, and the droplet size produced by the Taylor cone is less than 1 μm.	ii) Due to the application of high voltage, there is a doubt in the biocompatibility				
Thermodynamics	i) Exploits the dependency on temperature of viscosities and interfacial tension to adjust the droplets size.	i) Size changing is limited in a small range	10 kHz	Low	[100]	N/A
Photology	i) Droplets with controllable volumes in the range 1 pL and 150 pL with less than 1% volume variation	i) The generation of the droplets is based on a cavitation mechanism that can be harmful for species being encapsulated	10 kHz	High	[101]	N/A
	ii) High generation frequency	ii) Absence of spatial selectivity				
Acoustics	i) Produce individual picoliter-scale droplets on-demand	i) Low generation frequency	N/A	High	[19]	Particle concentration and encapsulation
	ii) Pre-concentrated particles simultaneously with droplet production					

droplet arrived at the fork, under the affection of electric field, it was polarized. Then the polarized droplet was pulled in opposite direction by electric field force and finally split into two droplets.[38]

2.2. "Micro-Particles" Spatial Manipulation

Both individual cells and microdroplets can be regarded as "micro-particles." To analyze the particles of this sort, researchers need to immobilize particles or make particles move by the set route. Present technology arose in the field of manipulation including electric field, steric hindrance,[39,40] fluid mechanics,[41] acoustic wave,[42] and so on.

2.2.1. Microfluidic Traps

Using advanced multilayer lithography technology, microfluidic traps with various structures (like U type, comb type, and half ring) can be designed and fabricated on microfluidic chip.[43–45] When micro-particles come across these specially designed traps, they are captured. The sizes of traps usually only accommodate one single particle. The advantage of this microfluidic trap has small influence in biological activity. However, the capture is unselective. Various examples microfluidic traps are listed in **Figure 2**A and B.[46–51]

2.2.2. Dielectrophoresis Capture

Dielectrophoresis occurs when a neutral particle is placed in a non-uniform electric field and experiences a translational force due to the polarization effect induced in the particle.[52] Micro and nano particles suspended in the liquid can then be manipulated using dielectrophoretic (DEP) and electrophoretic forces which are produced upon the application of voltages to these electrodes. Yun designed and manufactured a microfluidic chip combined with dielectrophoresis to manipulate a cell and a microbead. Combining with deflective dielectrophoretic barriers, the controlled liquid flows allows the accurate control of a cell or microbead in suspensions (Figure 2C).[53] For dielectrophoresis capture, its advantage is that it enables frequency-selective capture of viable cells and microorganism due to polarization based on their shape, distinguishing size, and subcellular composition. However, its drawbacks in aspect of throughput, and requiring of media with low conductivity challenge the progress of this technique. A recent review from Fernandez well summarized the microbial analysis in dielectrophoretic microfluidic systems.[54]

2.2.3. Optical Manipulation

Optical trapping have been widely used on microfluidic chips because of its high resolution (~50 nm), and powerful trapping (100–2000 pN).[55] Thus far, various optical trapping methods have been evaluated for cell trapping from free-space beam-based optical tweezers to near-field optical trapping.[56] For example, Li demonstrated a microfluidic chip which utilized optical tweezers to manipulate the behavior of *Bacillus subtilis*. Besides the immobilizing ability, their system could also provide control over the particle's motion in the 2D focal plane of the optical tweezers (Figure 2D).[57] Daly reviewed the advance in optical trapping and manipulation which covered lots of representative applications in cell research.[58]

2.3. Detection Technologies

Unlike the traditional detection techniques, sensors of microfluidic chip should possess features of high sensitivity, fast

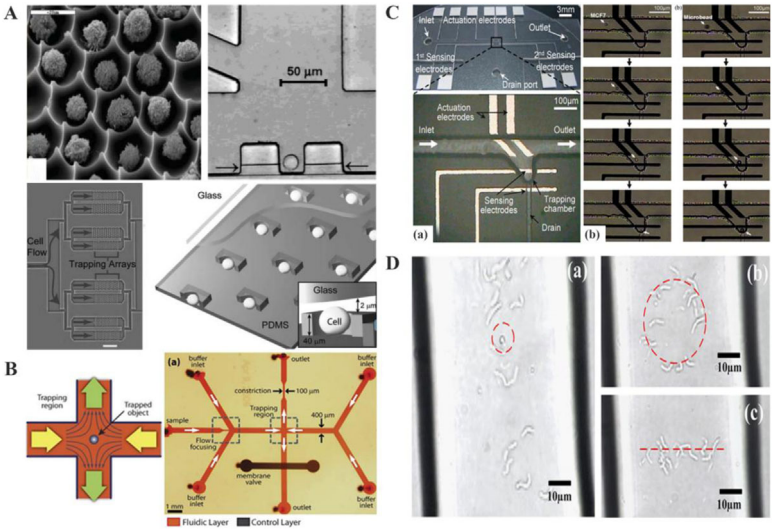


Figure 2. A) Micro-traps with different structure; (B) A microfluidic-based hydrodynamic trap. Reproduced with permission.[41] Copyright 2011, Royal Society of Chemistry; (C-a) The image of the fabricated microfluidic chip and magnified view. C-b) Sequence of capturing (left) single-cell (MCF7) and (right) microbead in trapping chamber. Reproduced with permission.[53] Copyright 2010, Elsevier; (D) Microfluidic chip utilizing optical tweezers drive the cells drawing a circle and a line. Reproduced with permission.[57] Copyright 2014, Elsevier.

response, and low cost. Several techniques are highlighted recently, which can be categorized into optical sensors, electrochemical sensors, mass spectrometry sensors, and Raman spectroscopy sensors. Several superior reviews of microfluidic detection technology have been reported.[59,60] Here, we pay a special attention to the development in recent 3 years. Evaluation of different detection techniques and their characteristics are listed in **Table 2**.

3. Advanced Microfluidic Applications

3.1. Cell Culture and Living Cells Detection

With the help of micro-perfusion system, microfluidic chip can realize dynamic cell culture as a chemostat. At present, dozens of cells and microorganisms including stem cells,[61–63] cancer cells,[64] bacteria, fungi,[65] and viruses[66] have been successfully cultured within the microfluidic chip. Meanwhile, interactions between cells also can be simulated. In recent years, microfluidic cell culture equipment has been used in tissue engineering,[67,68] diagnostics, drug screening,[69] immunology, cancer research, and stem cells proliferation and differentiation.[61,70–72] Taylor introduced the first microfluidic system for complete mammalian cell culture. It integrated all the necessary steps for mammalian cell culture, including cell seeding, cell culture and re-seeding to fresh surface. Several cell lines were seeded and repeatedly subcultured for weeks in 150 nL droplets. Moreover, the morphology and characteristics of growth was comparable to standard tissue culture vessels[73] (**Figure 3**A1). Quake built a fully automated cell culture and screening microfluidic platform with 96 independent culture chambers (Figure 3A2). Each chamber can be filled with arbitrary culture media imaged by time-lapse microscopy independently. With this device, they quantified the influence of transient stimulation on the proliferation, osteogenic differentiation, and motility of human primary mesenchymal stem cells.[74] Besides, using serial microfluidic mass sensor arrays, Cermak presented an approach to precisely and rapidly measure growth rates of many individual cells simultaneously.[75]

3.2. Cell Separation and High-Throughput Cell-Based Screening

In cell biology, highly efficient methods to separate components from a mixture in fluid are always needed. A clinically and

Table 2. Different detection techniques.

Principle of detection	Characteristic	Analyte(s)	LOD (limit of detection)	Ref
Fluorescence	High sensitivity and high selectivity for cellular and molecular sensing;	FFA uptake rates	$3.5 \pm 0.2 \times 10^{-15}$ M cell^{-1}	[102]
	Analytes extended from synthetic dyes, fluorescent proteins, DNA, and miRNA to nanoparticles	miR-21 DNA	10^{-9} M	[103]
		miRNA210	0.03 fM	[104]
		Cy3-labeled DNA probes	0.2 nM	[105]
		H_2O_2, GSH, and Cys (Simultaneously)	0.492 aM, 12.4 fM, and 838 aM,	[106]
Absorbancy	Lable free, generality and sensitivity;	A formazan dye from coupled assay	10 μM	[107]
	Adequate detection limit.	Methylene blue	13 μM	[108]
		Bromophenol blue	29 fM	[109]
Chemiluminiscence	No need excitation light source;	Ractopamine (RCT) in swine urine	0.97 ng mL^{-1}	[110]
	Appropriate for miniaturization of the on-line detection system;	Cancer biomarker proteins prostate specific antigen (PSA) and platelet factor 4 (PF-4)	0.5 pg mL^{-1}	[111]
	Limited to some special reaction reagents	Pseudoephedrine (PSE)	5.7 ng mL^{-1}	[112]
Raman spectrum	Probing the fingerprint of molecules;	Carcinoembryonic antigen (CEA)	10^{-12} M	[113]
	Molecular quantity is important for reliable and reproducible results and precise quantitative measurement	Melamine in milk	203 ppm	[114]
		Borate capping agent for detecting drugs	4.5–13 ng mL^{-1}	[115]
Electrochemistry	Low cost, small size, high sensitivity and high speed;	Reporter group p−aminophenol (p-AP)	1 μM	[116]
	Ease of integrating with microchips	Nitric oxide	840 pM	[117]
		Nitrite and ascorbate	713.5 pA mM^{-1}, 255.4 pAmM^{-1}	[118]
Mass spectrum	Lable free, high selective, and sensitive;	Arginine vasopressin (AVP)	1 pM	[119]
	Simultaneous detection of multiple analytes	Dopamine (DA), serotonin (5-HT), Asp, Glu	42 nM, 49 Nm, 38 nM, 32 nM	[120]
		Cytochrome c	10 μg mL^{-1}	[121]

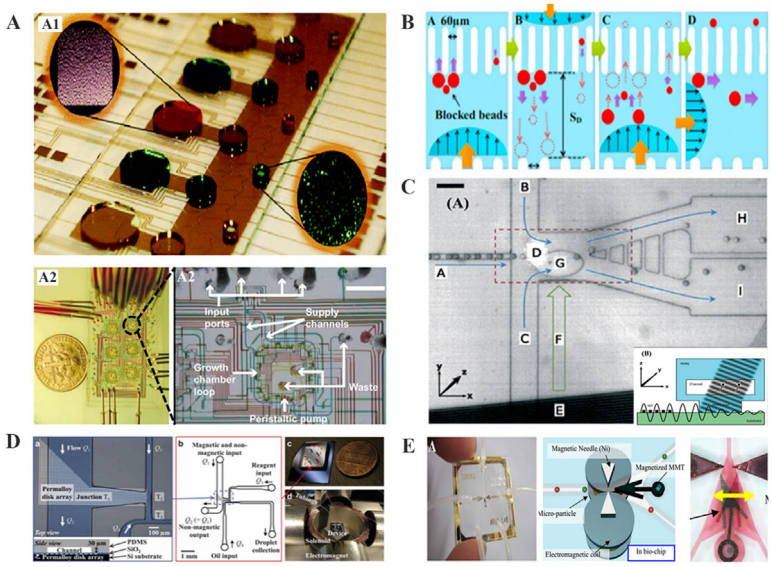


Figure 3. A) Two microfluidic based cell culture platforms. A1) the first microfluidic system for complete mammalian cell culture. It integrated all the necessary steps for mammalian cell culture, including cell seeding, cell culture, and re-seeding to fresh surface. Reproduced with permission.[73] Copyright 2010, Royal Society of Chemistry. A2) a fully automated PDMS-based microfluidic cell culture system consisting of 96 individually addressable cell culture chambers. Reproduced with permission.[74] Copyright 2007, ACS Publications. B) Working principle of cell size-based sorter. C) Acoustic microfluidic fluorescence-activated cell sorter. Reproduced with permission.[82] Copyright 2014, Royal Society of Chemistry. D and E) High throughput microfluidic cell sorters by magnetic force. D) Reproduced with permission.[86] Copyright 2013, Royal Society of Chemistry. E) Reproduced with permission.[87] Copyright 2011, Springer.

common example is the isolation and capture of cells from whole blood,[76,77] especially the separation of cancer cells and rare bacteria.[78,79] Warkiani used a label-free spiral microfluidic device to allow size-based isolation of viable cells by hydrodynamic forces that are present in curvilinear microchannels.[80] Tan carefully designed a micro-sieve to separate and sort cells by size, as shown in Figure 3B.[81] The spatial manipulation technology using microfluidic traps arrays or hydrodynamic are widely used in this application mainly because these chips are characterized by simple structure, low cost, and little damage to cell vitality.

Unlike cell separation, which usually means isolation one type of cell from a mixture of different cells, cell based screening is inclined to pick improved clones from isogeny library. The former pays more attention to the purity of separation, but the latter mainly focuses on high throughput screening. Implementation of high-throughput screening (HTS) in lab-on-chip essentially needs the cooperation of the manipulation and detection technology in well-designed channel. For instance, the fluorescent-induced HTS is similar to flow cytometry theoretically. The fluorescence labeled cells flows through the detection area, and the fluorescence signal intensity can be used to trigger the follow-up manipulation mode (like micro valve, electric field, etc.)[82,83] to complete the cell sorting. Wang et al. built a flexible high-throughput screening system based on the different fluorescence intensity of monodisperse nanoliter aqueous droplets to activate the high-voltage pulse and made the cell migrated and separated.[84] Schmid designed a fluorescence induced acoustics HTS chip. It carried out the sorting of the droplets or other particles ignoring the size or physical properties of objects. Here, the screening rate of microdroplets can reach 3000 events s^{-1} under 15 dBm acoustics energy. Furthermore, this system can also directly sort melanoma cells without encapsulation[82] (Figure 3C).

Theoretically all the detection means mentioned in this review (Table 2) can be used to trigger a downstream spatial manipulation, as reviewed in preceding text. For instance, dielectrophoresis based raman spectroscopy could implement single-cell sorting on chip for carotenoid-producing yeast concentration.[85] Meanwhile sorting methods are not limited to what we mentioned, other techniques including micro valve or electromagnetic control (Figure 3D and E)[86,87] are also meaningful. Nowadays, cell-based HTS is drastically increasing in microfluidic chip for increasing rate of monodisperse microdroplets generation up to ~ 10 kHz.

3.3. Cell Lysis and Omics Analysis

In addition to the operations applied to cells, microfluidic chip is also applicable to cell lysis and subcellular structure analysis. Recently, lots of reports have appeared on the analysis of the contents of individual cells with microfluidics. In some specially designed microfluidic devices, cells can be limited in a chamber individually. Then, by introducing lysis and derivative reagent, researchers could complete cell lysis and chemical derivatization.[88] Compared with capillary electrophoresis, net-structure of microfluidic chip can greatly speed up the rate of sample introduction and cell lysis. Meanwhile, exerting a powerful electric field on the channel could quickly and efficiently complete the electrophoresis separation. The only limitation is the difficulty of chips fabrication.

There were also reports of cell lysis in microdroplet mode.[21] But the derivative compounds after cell lysis are naturally limited in the droplets. It is hard to separate different compounds after cell lysis, and difficult to dock with analytical instruments. For most bioresearch, the primary goal of cell lysis is to conduct a

subsequent omics analysis, that is why we put these two topics in the same section. Actually, microfluidic chip has had lots of applications in genomics[89,90] and proteomics, here, we illustrate a few representative examples.

In genomics, Tsuji constructed a phospholipid-coated water-in-oil microdroplet (20–60 μm in diameter), and successively monitored the progress of transcription reaction by fluorescence resonance energy transfer.[91] Ottesen used microfluidic digital PCR to amplify multiple, various genes from bacterial cells and used a gene involved in termites mutualistic symbiosis to discover the unknown ribosomal RNA-based species identity of several symbionts.[92] Enzyme-free detection of mRNA using specific staining and immobilization of the target molecules via a double hybridization approach thereby avoiding bias due to enzymatic processes like reverse transcription and PCR amplification from very few cells was also realized by Haider.[93] Zhang reviewed the advances and challenges on continuous-flow microfluidic PCR in droplets.[94] In the process of commercialization, genes related microfluidic technic, such as droplet digital PCR is developing rapidly. Examples from Bio-Rad, RainDance, and Oxford Nanopore Technology could offer the relative technologies.

In aspect of proteomics, protein analysis, especially quantitative analysis in single cell, is difficult because of the vanishingly small amount of protein and the enormous complexity of the proteome in a single cell. And it is hard to be amplified like genes. But microfluidics can provide the potential for extremely high detection sensitivity and high-throughput screening. Recently, an increasing number of related researches appeared. Four kinds of microfluidic platforms including microfluidic fluorescent flow cytometry, droplet based microfluidic flow cytometry, large-array micro wells and large-array micro chambers were emerging to enable the quantification of single-cell proteins. It was well summarized by Fan[95] and Kannan.[96] With increasing feasibility of the integration between high sensitivity analysis method and microfluidic, we believe microfluidics will play an important role in the single-cell proteomics research.

4. Conclusions

Herein, we reviewed advanced technology and application of microfluidic chip applied to single cell in recent years. As we can see, along with the maturity of key technologies associated with microfluidic, the versatility of the microfluidic devices and the control precision are highly improved. These progresses naturally expand the application of microfluidic chip in cell biology constantly. However, there still exist portions needs further development: (1) The gap between the microfluidic microenvironments and the in vivo situations. The micro-environments of single cells trapped in microfluidic array or droplets may be considerably different from in vivo situations, such as nutrient supply and gas transfer. It will be doubtable when directly use the results onto the normal metabolism status. (2) Highly integrated microfluidic chip is valuable. But in most cases, with the improvement of versatility of the integrated microfluidic chip, the throughput of analysis usually decreases because of the existence of rate-determining step. For instance, a chip integrated with cell capture, culture, lysis, and analysis will no longer have the advantages of high throughput probably for the time-consuming steps in cell lysis. So it is important to improve the throughput of each operation units of an integrated device. (3) The microfluidic chips with complex 3D structure are of great value. However, the most commonly used soft lithography are suitable for planar microfluidic device, or limited non-planar devices by stacking. But it is unable to make complex 3D structures. There is an urge of novel techniques like 3D printing with high-precision to easily construct 3D devices.[97] (4) Another challenge for the microfluidics community is to find ways of simplifying the use of microfluidic devices. Although the utility of microfluidic devices is becoming increasingly evident, such devices will not be adopted by researchers unless they can be used off the shelf without the aid of engineers. If all these requirements are achieved, it will be of great meaning and may carry forward the cell on chip to tissue or organ on chip. At that time, the microfluidic technology will bring more convenience to people's daily life and health.

Abbreviations

5-HT, serotonin; AVP, arginine vasopressin; CEA, carcinoembryonic antigen; CYS, cysteine; DA, dopamine; FFA, free fatty acid; GSH, glutathione; HTS, high-throughput screening; LOD, limit of detection; RCT, ractopamine; p-AP, p−aminophenol; PDMS, polydimethylsiloxane; PF-4, platelet factor 4; PSA, prostate specific antigen; PSE, pseudoephedrine; SAW, surface acoustic wave.

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Conflict of Interest

The authors declare no financial or commercial conflict of interest.

Keywords

high throughput, Lab on chip, microfluidic, single cell

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[1] A. Schmid, H. Kortmann, P. S. Dittrich, L. M. Blank, *Curr. Opin. Biotechnol.* **2010**, *21*, 12.
[2] X. Sun, W. D. Weinlandt, H. Patel, M. Wu, C. J. Hernandez, *Lab Chip* **2014**, *14*, 2491.
[3] K. Eyer, P. Kuhn, S. Stratz, P. S. Dittrich, *J. Vis. Exp.* **2013**, *80*, 50618.
[4] A. J. Hughes, A. E. Herr, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 21450.
[5] S. A. Vanapalli, M. H. Duits, F. Mugele, *Biomicrofluidics* **2009**, *3*, 12006.
[6] F. V. Ignatovich, D. Topham, L. Novotny, *IEEE J. Sel. Top. Quantum Electron.* **2006**, *12*, 1292.

[7] E. K. Sackmann, A. L. Fulton, D. J. Beebe, *Nature* **2014**, *507*, 181.
[8] M. Hosokawa, Y. Hoshino, Y. Nishikawa, T. Hirose, D. H. Yoon, T. Mori, T. Sekiguchi, S. Shoji, H. Takeyama, *Biosens. Bioelectron.* **2015**, *67*, 379.
[9] M. Sauzade, E. Brouzes, *Lab Chip* **2017**, *17*, 2186.
[10] S. L. Sjostrom, Y. P. Bai, M. T. Huang, Z. H. Liu, J. Nielsen, H. N. Joensson, H. A. Svahn, *Lab Chip* **2014**, *14*, 806.
[11] T. C. Scanlon, S. M. Dostal, K. E. Griswold, *Biotechnol. Bioeng.* **2014**, *111*, 232.
[12] X. Niu, F. Gielen, J. B. Edel, A. J. deMello, *Nat. Chem.* **2011**, *3*, 437.
[13] S. Zeng, X. Liu, H. Xie, B. Lin, *Microfluidics: Technol. Appl.* **2011**, 69.
[14] P. Day, A. Manz, Y. Zhang, *Microdroplet technology: Principles and emerging applications in biology and chemistry*, Springer Science & Business Media, Berlin **2012**.
[15] L. Granieri, J. C. Baret, A. D. Griffiths, C. A. Merten, *Chem. Biol.* **2010**, *17*, 229.
[16] J. C. Baret, O. J. Miller, V. Taly, M. Ryckelynck, A. El-Harrak, L. Frenz, C. Rick, M. L. Samuels, J. B. Hutchison, J. J. Agresti, D. R. Link, D. A. Weitz, A. D. Griffiths, *Lab Chip* **2009**, *9*, 1850.
[17] S. Koster, F. E. Angile, H. Duan, J. J. Agresti, A. Wintner, C. Schmitz, A. C. Rowat, C. A. Merten, D. Pisignano, A. D. Griffiths, D. A. Weitz, *Lab Chip* **2008**, *8*, 1110.
[18] M. Chabert, J. L. Viovy, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 3191.
[19] D. J. Collins, T. Alan, K. Helmerson, A. Neild, *Lab Chip* **2013**, *13*, 3225.
[20] W. B. Du, M. Sun, S. Q. Gu, Y. Zhu, Q. Fang, *Anal. Chem.* **2010**, *82*, 9941.
[21] M. Y. He, J. S. Edgar, G. D. M. Jeffries, R. M. Lorenz, J. P. Shelby, D. T. Chiu, *Anal. Chem.* **2005**, *77*, 1539.
[22] U. Demirci, G. Montesano, *Lab Chip* **2007**, *7*, 1139.
[23] M. Huang, Y. Bai, S. L. Sjostrom, B. M. Hallstrom, Z. Liu, D. Petranovic, M. Uhlen, H. N. Joensson, H. Andersson-Svahn, J. Nielsen, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E4689.
[24] S. Meng, F. Qun, *Lab Chip* **2010**, *10*, 2864.
[25] Y. N. Sun, X. D. Chen, X. G. Zhou, J. B. Zhu, Y. D. Yu, *Lab Chip* **2015**, *15*, 2429.
[26] A. R. Abate, T. Hung, P. Mary, J. J. Agresti, D. A. Weitz, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 19163.
[27] K. Wang, Y. Lu, L. Yang, G. Luo, *AICHE J.* **2013**, *59*, 643.
[28] K. Wang, Y. Lu, C. P. Tostado, L. Yang, G. Luo, *Chem. Eng. J.* **2013**, *227*, 90.
[29] L. H. Hung, K. M. Choi, W. Y. Tseng, Y. C. Tan, K. J. Shea, A. P. Lee, *Lab Chip* **2006**, *6*, 174.
[30] D. H. Yoon, A. Jamshaid, J. Ito, A. Nakahara, D. Tanaka, T. Akitsu, T. Sekiguchi, S. Shoji, *Lab Chip* **2014**, *14*, 3050.
[31] R. M. Lorenz, J. S. Edgar, G. D. Jeffries, Y. Zhao, D. McGloin, D. T. Chiu, *Anal. Chem.* **2007**, *79*, 224.
[32] Z. G. Li, K. Ando, J. Q. Yu, A. Q. Liu, J. B. Zhang, C. D. Ohl, *Lab Chip* **2011**, *11*, 1879.
[33] A. Venancio-Marques, D. Baigl, *Langmuir* **2014**, *30*, 4207.
[34] H. Tsuchiya, M. Okochi, N. Nagao, M. Shikida, H. Honda, *Sens. Actuators B Chem.* **2008**, *130*, 583.
[35] M. Zagnoni, J. M. Cooper, *Lab Chip* **2009**, *9*, 2652.
[36] B. P. Zhou, C. Wang, X. Xiao, Y. S. Hui, Y. L. Cao, W. J. Wen, *Rsc Adv.* **2015**, *5*, 10365.
[37] D. H. Yoon, J. Ito, T. Sekiguchi, S. Shoji, *Micromachines* **2013**, *4*, 197.
[38] D. R. Link, E. Grasland-Mongrain, A. Duri, F. Sarrazin, Z. Cheng, G. Cristobal, M. Marquez, D. A. Weitz, *Angew. Chem. Int. Ed. Engl.* **2006**, *45*, 2556.
[39] L. Bell, A. Seshia, D. Lando, E. Laue, M. Palayret, S. F. Lee, D. Klenerman, *Sens. Actuators B Chem.* **2014**, *192*, 36.
[40] A. A. Banaeiyan, D. Ahmadpour, C. B. Adiels, M. Goksor, *Micromachines* **2013**, *4*, 414.
[41] M. Tanyeri, M. Ranka, N. Sittipolkul, C. M. Schroeder, *Lab Chip* **2011**, *11*, 1786.
[42] J. S. Jeong, J. W. Lee, C. Y. Lee, S. Y. Teh, A. Lee, K. K. Shung, *Biomed. Microdevices* **2011**, *13*, 779.
[43] M. J. Delince, J. B. Bureau, A. T. Lopez-Jimenez, P. Cosson, T. Soldati, J. D. McKinney, *Lab Chip* **2016**, *16*, 3276.
[44] S. H. Jin, S. C. Jang, B. Lee, H. H. Jeong, S. G. Jeong, S. S. Lee, K. P. Kim, C. S. Lee, *Lab Chip* **2016**, *16*, 1358.
[45] Y. Zhou, S. Basu, K. J. Wohlfahrt, S. F. Lee, D. Klenerman, E. D. Laue, A. A. Seshia, *Sens. Actuators B Chem.* **2016**, *232*, 680.
[46] A. R. Wheeler, W. R. Throndset, R. J. Whelan, A. M. Leach, R. N. Zare, Y. H. Liao, K. Farrell, I. D. Manger, A. Daridon, *Anal. Chem.* **2003**, *75*, 6.
[47] M. Deutsch, A. Deutsch, O. Shirihai, I. Hurevich, E. Afrimzon, Y. Shafran, N. Zurgil, *Lab Chip* **2006**, *6*, 995.
[48] D. Di Carlo, L. Y. Wu, L. P. Lee, *Lab Chip* **2006**, *6*, 1445.
[49] J. Kim, J. Erath, A. Rodriguez, C. Yang, *Lab Chip* **2014**, *14*, 2480.
[50] A. Benavente-Babace, D. Gallego-Perez, D. J. Hansford, S. Arana, E. Perez-Lorenzo, M. Mujika, *Biosens. Bioelectron.* **2014**, *61*, 298.
[51] D. Jin, B. Deng, J. X. Li, W. Cai, L. Tu, J. Chen, Q. Wu, W. H. Wang, *Biomicrofluidics* **2015**, *9*, 014101.
[52] C. Zhang, K. Khoshmanesh, A. Mitchell, K. Kalantar-zadeh, *Anal. Bioanal. Chem.* **2010**, *396*, 401.
[53] H. Park, D. Kim, K.-S. Yun, *Sens. Actuators B Chem.* **2010**, *150*, 167.
[54] R. E. Fernandez, A. Rohani, V. Farmehini, N. S. Swami, *Anal. Chim. Acta* **2017**, *966*, 11.
[55] J. Nilsson, M. Evander, B. Hammarstrom, T. Laurell, *Anal. Chim. Acta* **2009**, *649*, 141.
[56] A. Ashkin, *Phys. Rev. Lett.* **1970**, *24*, 156.
[57] Y. Li, Z. Guo, S. Qu, *Opt. Lasers Eng.* **2014**, *55*, 150.
[58] M. Daly, M. Sergides, S. N. Chormaic, *Laser Photonics Rev.* **2015**, *9*, 309.
[59] K. Kleparnik, F. Foret, *Anal. Chim. Acta* **2013**, *800*, 12.
[60] Y. Zhu, Q. Fang, *Anal. Chim. Acta* **2013**, *787*, 24.
[61] T. Matsumura, K. Tatsumi, Y. Noda, N. Nakanishi, A. Okonogi, K. Hirano, L. Li, T. Osumi, T. Tada, H. Kotera, *Biochem. Biophys. Res. Commun.* **2014**, *453*, 131.
[62] C. H. Lin, H. C. Chang, C. H. Hsu, *JoVE* **2016**, e54105.
[63] Z. Tatarova, J. P. Abbuehl, S. Maerkl, J. Huelsken, *Lab Chip* **2016**, *16*, 1934.
[64] K. E. Sung, D. J. Beebe, *Adv. Drug Deliver. Rev.* **2014**, *79–80*, 68.
[65] S. Schlimpert, K. Flardh, M. Buttner, *JoVE* **2016**, 53863.
[66] A. E. Fischer, S. K. Wu, J. B. G. Proescher, A. Rotem, C. B. Chang, H. Zhang, Y. Tao, T. S. Mehoke, P. M. Thielen, A. O. Kolawole, T. J. Smith, C. E. Wobus, D. A. Weitz, J. S. Lin, A. B. Feldman, J. T. Wolfe, *J. Virol. Methods* **2015**, *213*, 111.
[67] E. M. Materne, I. Maschmeyer, A. K. Lorenz, R. Horland, K. M. S. Schimek, M. Busek, F. Sonntag, R. Lauster, U. Marx, *J. Vis. Exp.* **2015**, *98*, 52526.
[68] Y. Yamagishi, T. Masuda, M. Matsusaki, M. Akashi, U. Yokoyama, F. Arai, *Biomicrofluidics* **2014**, *8*, 064113.
[69] J. Pihl, M. Karlsson, D. T. Chiu, *Drug Discov. Today* **2005**, *10*, 1377.
[70] A. Valero, J. N. Post, J. W. van Nieuwkasteele, P. M. ter Braak, W. Kruijer, A. van den Berg, *Lab Chip* **2008**, *8*, 62.
[71] S. A. Kobel, O. Burri, A. Griffa, M. Girotra, A. Seitz, M. P. Lutolf, *Lab Chip* **2012**, *12*, 2843.
[72] C. Y. Jiang, L. Dong, J. K. Zhao, X. Hu, C. Shen, Y. Qiao, X. Zhang, Y. Wang, R. F. Ismagilov, S. J. Liu, W. Du, *Appl. Environ. Microbiol.* **2016**, *82*, 2210.
[73] I. Barbulovic-Nad, S. H. Au, A. R. Wheeler, *Lab Chip* **2010**, *10*, 1536.
[74] R. Gomez-Sjoeberg, A. A. Leyrat, D. M. Pirone, C. S. Chen, S. R. Quake, *Anal. Chem.* **2007**, *79*, 8557.
[75] N. Cermak, S. Olcum, F. F. Delgado, S. C. Wasserman, K. R. Payer, M. Murakami, S. M. Knudsen, R. J. Kimmerling, M. M. Stevens,

Y. Kikuchi, A. Sandikci, M. Ogawa, V. Agache, F. Baléras, D. M. Weinstock, S. R. Manalis, *Nat. Biotechnol*. **2016**, *34*, 1052.
[76] J. Chen, D. Chen, T. Yuan, Y. Xie, X. Chen, *Biomicrofluidics* **2013**, *7*, 34106.
[77] J. Kim, H. Cho, S. I. Han, K. H. Han, *Anal. Chem*. **2016**, *88*, 4857.
[78] S. Nagrath, L. V. Sequist, S. Maheswaran, D. W. Bell, D. Irimia, L. Ulkus, M. R. Smith, E. L. Kwak, S. Digumarthy, A. Muzikansky, P. Ryan, U. J. Balis, R. G. Tompkins, D. A. Haber, M. Toner, *Nature* **2007**, *450*, 1235.
[79] J. Zhang, K. Chen, Z. H. Fan, *Adv. Clin. Chem*. **2016**, *75*, 1.
[80] M. E. Warkiani, B. L. Khoo, L. Wu, A. K. Tay, A. A. Bhagat, J. Han, C. T. Lim, *Nat. Protoc*. **2016**, *11*, 134.
[81] S. M. McFaul, B. K. Lin, H. Ma, *Lab Chip* **2012**, *12*, 2369.
[82] L. Schmid, D. A. Weitz, T. Franke, *Lab Chip* **2014**, *14*, 3710.
[83] T. C. Scanlon, S. M. Dostal, K. E. Griswold, *Biotechnol. Bioeng*. **2014**, *111*, 232.
[84] B. L. Wang, A. Ghaderi, H. Zhou, J. Agresti, D. A. Weitz, G. R. Fink, G. Stephanopoulos, *Nat. Biotechnol*. **2014**, *32*, 473.
[85] P. Zhang, L. Ren, X. Zhang, Y. Shan, Y. Wang, Y. Ji, H. Yin, W. E. Huang, J. Xu, B. Ma, *Anal. Chem*. **2015**, *87*, 2282.
[86] A. Chen, T. Byvank, W.-J. Chang, A. Bharde, G. Vieira, B. L. Miller, J. J. Chalmers, R. Bashir, R. Sooryakumar, *Lab Chip* **2013**, *13*, 1172.
[87] T. F. Kong, H. Shin, H. S. Sugiarto, H. F. Liew, X. H. Wang, W. S. Lew, N. T. Nguyen, Y. Chen, *Microfluid. Nanofluid*. **2011**, *10*, 1069.
[88] H. Wu, A. Wheeler, R. N. Zare, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12809.
[89] T. Kalisky, S. R. Quake, *Nat. Meth*. **2011**, *8*, 311.
[90] A. Reece, B. Xia, Z. Jiang, B. Noren, R. McBride, J. Oakey, *Curr. Opin. Biotechnol*. **2016**, *40*, 90.
[91] A. Tsuji, K. Yoshikawa, *ChemBioChem* **2010**, *11*, 351.
[92] E. A. Ottesen, J. W. Hong, S. R. Quake, J. R. Leadbetter, *Science* **2006**, *314*, 1464.
[93] M. Haider, B. Ji, T. Haselgrubler, A. Sonnleitner, F. Aberger, J. Hesse, *Biosens. Bioelectron*. **2016**, *86*, 20.
[94] Y. Zhang, H. R. Jiang, *Anal. Chim. Acta* **2016**, *914*, 7.
[95] B. Fan, X. Li, D. Chen, H. Peng, J. Wang, J. Chen, *Sensors* **2016**, *16*, 232.
[96] S. Kannan, *MOJ Proteom. Bioinform*. **2016**, *3*, 00094.
[97] H. Lee, D. W. Cho, *Lab Chip* **2016**, *16*, 2618.
[98] K. Churski, T. S. Kaminski, S. Jakiela, W. Kamysz, W. Baranska-Rybak, D. B. Weibel, P. Garstecki, *Lab Chip* **2012**, *12*, 1629.
[99] H. Kim, D. Luo, D. Link, D. A. Weitz, M. Marquez, Z. Cheng, *Appl. Phys. Lett*. **2007**, *91*, 133106.
[100] N. T. Nguyen, T. H. Ting, Y. F. Yap, T. N. Wong, J. C. Chai, W. Kong, L. J. Zhou, S. H. Tan, L. Yobas, *Appl. Phys. Lett*. **2007**, *91*, 084102.
[101] S. Y. Park, T. H. Wu, Y. Chen, M. A. Teitell, P. Y. Chiou, *Lab Chip* **2011**, *11*, 1010.
[102] J. T. Negou, L. A. Avila, X. Li, T. M. Hagos, C. J. Easley, *Anal. Chem*. **2017**.
[103] Z. Wang, S. Zong, Z. Wang, L. Wu, P. Chen, B. Yun, Y. Cui, *Nanotechnology* **2017**, *28*, 105501.
[104] L. Liang, F. Lan, X. Yin, S. Ge, J. Yu, M. Yan, *Biosens. Bioelectron*. **2017**, *95*, 181.
[105] T. Q. Hung, W. H. Chin, Y. Sun, A. Wolff, D. D. Bang, *Biosens. Bioelectron*. **2017**, *90*, 217.
[106] Q. Li, P. Chen, Y. Fan, X. Wang, K. Xu, L. Li, B. Tang, *Anal. Chem*. **2016**, *88*, 8610.
[107] F. Gielen, R. Hours, S. Emond, M. Fischlechner, U. Schell, F. Hollfelder, *Proc. Natl. Acad. Sci. USA* **2016**, *113*, E7383.
[108] W. Song, J. Yang, *Lab Chip* **2012**, *12*, 1251.
[109] K. S. Deal, C. J. Easley, *Anal. Chem*. **2012**, *84*, 1510.
[110] S. Wang, Q. Chen, X. Wei, J. Wu, C. Wang, J. Liu, L. Zhang, Y. Dong, *Electrophoresis* **2017**, *38*, 368.
[111] C. K. Tang, A. Vaze, J. F. Rusling, *Lab Chip* **2017**, *17*, 484.
[112] A. M. Kadavilpparampu, H. A. Al-Lawati, F. O. Suliman, S. M. Al Kindy, *Luminescence* **2015**, *30*, 1242.
[113] K. Zou, Z. Gao, Q. Deng, Y. Luo, L. Zou, Y. Lu, W. Zhao, B. Lin, *Electrophoresis* **2016**, *37*, 786.
[114] J. W. Martin, M. K. Nieuwoudt, M. J. T. Vargas, O. L. C. Bodley, T. S. Yohendiran, R. N. Oosterbeek, D. E. Williams, M. Cather Simpson, *Analyst* **2017**, *142*, 1682.
[115] N. D. Kline, A. Tripathi, R. Mirsafavi, I. Pardoe, M. Moskovits, C. Meinhart, J. A. Guicheteau, S. D. Christesen, A. W. Fountain, 3rd, *Anal. Chem*. **2016**, *88*, 10513.
[116] T. S. Safaei, R. M. Mohamadi, E. H. Sargent, S. O. Kelley, *ACS Appl. Mater. Interfaces* **2015**, *7*, 14165.
[117] R. A. Hunter, B. J. Privett, W. H. Henley, E. R. Breed, Z. Liang, R. Mittal, B. P. Yoseph, J. E. McDunn, E. M. Burd, C. M. Coopersmith, J. M. Ramsey, M. H. Schoenfisch, *Anal. Chem*. **2013**, *85*, 6066.
[118] J. M. Petroni, B. G. Lucca, V. S. Ferreira, *Anal. Chim. Acta* **2017**, *954*, 88.
[119] J. Yang, J. Zhu, R. Pei, J. A. Oliver, D. W. Landry, M. N. Stojanovic, Q. Lin, *Anal. Methods* **2016**, *8*, 5190.
[120] X. Li, H. Hu, S. Zhao, Y. M. Liu, *Anal. Chem*. **2016**, *88*, 5338.
[121] X. Hu, Y. Dong, Q. He, H. Chen, Z. Zhu, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*. **2015**, *990*, 96.

