Microfluidics for cell-based high throughput screening platforms : a review

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Review

Microfluidics for cell-based high throughput screening platforms—A review

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HIGHLIGHTS

- High throughput screening is a major instrument in drug discovery.
- This paper reviews the application of microfluidics to cell-based high throughput screening platforms.
- We describe different modes in microfluidic platforms for cell-based high throughput screening.
- We discuss the advantages and disadvantages of each screening mode.
- We also give a perspective of the future of microfluidics for cell-based high throughput screening platforms.

GRAPHICAL ABSTRACT

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ABSTRACT

In the last decades, the basic techniques of microfluidics for the study of cells such as cell culture, cell separation, and cell lysis, have been well developed. Based on cell handling techniques, microfluidics has been widely applied in the field of PCR (Polymerase Chain Reaction), immunoassays, organ-on-chip, stem cell research, and analysis and identification of circulating tumor cells. As a major step in drug discovery, high-throughput screening allows rapid analysis of thousands of chemical, biochemical, genetic or pharmacological tests in parallel. In this review, we summarize the application of microfluidics in cell-based high throughput screening. The screening methods mentioned in this paper include approaches using the perfusion flow mode, the droplet mode, and the microarray mode. We also discuss the future development of microfluidic based high throughput screening platform for drug discovery.

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Contents

1. Introduction	37
2. Cell-based high-throughput screening on microfluidic platforms	38
2.1. Perfusion flow mode high-throughput screening	38

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2.2. Droplet mode high-throughput screening	42
2.3. Microarray mode high-throughput screening	45
3. Conclusions	46
Acknowledgment	49
References	49

1. Introduction

Recently, the pace of drug discovery using high-throughput techniques has been accelerated by the advances in genomics, proteomics, celloomics and metabolomics [1–5]. This rapid progress has produced considerable numbers of pharmaceutically valuable lead compounds [6]. These new candidates or targets have increased the demand for experimental complexity of high-throughput screening, e.g. the organization of liquid handling, interpretation and utilization of experimental data, and the preparation of large number of libraries.

The process of high-throughput screening requires rapid analysis of thousands of reaction tests in parallel to identify specific compounds for a particular biological process. Most of high-throughput screening technologies involve robotics for liquid and plate handling, sensitive detectors and software for data processing and control. In the early times of high-throughput screening (1980s), it was mainly based on the assessment of mixtures of compound libraries in 96-well microtiter plates. The throughput of even a large system was <100 plates per day. Twenty years later, as shown in Fig. 1, most screenings were performed with millions of

compounds with high purity, and fully automatic robotic systems based on 384- and 1536-well plates. All the data was estimated automatically to prevent human error. Currently, most of the screenings can run continuously and without or with little human intervention [7].

Although the fast development of high throughput screening technologies in recent years has been proven successful, R&D productivity (in terms of approvals per R&D spent) in drug discovery has dropped [8,9]. There are several reasons for such decline. First of all, the complete systems of current high-throughput screening technologies, including liquid handling equipment, data acquisition, extensive robotic liquid and plate handling equipment are expensive. Many researchers, who are motivated to screen for potential small molecular targets in independent labs, are restricted by the high cost of the high-throughput screening systems [7]. Second, the cost of biological samples and drug libraries for drug screening is also high, and the current approaches to high-throughput screening make it difficult to further reduce the consumption of reagents. The well volume capacity for a 384-well plate has been reduced to 100 μ L, but further minimization of the well volume of microwell plates is restricted by

Fig. 1. A fully integrated multifunctional robotic screening system for high-throughput screening. The system is fully enclosed and comprises the following components: (1) 6-axis robot hand; (2) Caliper with interchangeable 96- or 384-tip pipetting head, an independent 8-channel pipettor, two bulk-reagent dispensers, plate gripper (2a), microtiter plate shaker (2b), positive-pressure filtration system (2c) and ultrasonic tip-wash station (2d); (3) fluorescence detection system; (4) microtiter plate storage capacity with humidity, temperature and CO₂ controls; (5) plate washer; (6) plate centrifuge; (7) high-capacity stacker to store tip boxes; (8) individual dispensing heads; (9) automatic barcode labeler; (10) barcode reader; (11) plate regrip station that changes the plate orientation to facilitate the interaction between the robot arm and individual components; (12) room temperature incubator that stores regular microtiter plates; (13) plate-lid-handling station; (14) shaker station that provides an independent plate-shaking operation (15) air purifier provides ultra-dust-free conditions for the enclosed system. Reproduced with permission from Ref. [7].

uncontrolled liquid evaporation. In addition, the decrease of the volumes handled by the dispensing systems is also limited by the difficulty of accurately dispensing very small volumes of liquids smaller than 1 μL [10,11]. Third, the failure rate in drug development is high in the clinical development phase. Clinical drug development (Phase I–III) takes approximately 63% of the total drug development costs [8,9]. The use of appropriate cell-based assays in an early, preclinical stage of drug discovery is expected to provide a more efficient way to eliminate possible false leads, due to low drug efficacy or high toxicity [12]. However, this strategy is difficult to be widely used for current high-throughput screening because cell-based assays are more expensive and require more complex liquid handling compared with cell-free assays. Therefore, a new high-throughput screening technology which requires only low sample and reagent consumption, has a low cost, is easy to handle, and supports cell-based screening assays is an urgent requirement for the current drug discovery industry.

Recently, microfluidic devices have been proposed as a potential platform for high-throughput screening technology because of their properties of low sample consumption, low analysis cost, easy handling of nanoliter-volumes of liquids and being suitable for cell-based assays [1–3,13]. Making use of these properties of microfluidics, a number of recent publications have proposed concrete microfluidic approaches related to drug discovery. Two representative examples are (1) a study of the structure of membrane proteins [14,15] (these proteins can regulate cell process and through them cells interact with their surrounding environments; most of drug design is based on the structure of the target protein membrane); and (2) the creation of organ-on-a-chip [16–18] (in these chips, multicellular tissues representing human organ function are studied; in the future, these organ-on-a-chip devices might be a substitute solution for animal or even be part of clinical test).

To date, different components for microfluidic based cell-based high throughput screening platforms have been developed, including cell culture [6,19–21], introduction and transportation of samples [22–25], and characterization of cell viability [26–29]. The microfluidic community has focused on the demonstration of integrating these different components into a single microfluidic device. Among current microfluidic platforms for cell based high throughput screening, there are three major complementary modes to manipulate microfluids: perfusion flow mode, droplet based mode and microarray mode [30]. In the following sections, various microfluidic screening platforms will be classified on the basis of the three modes, and their applications in cell-based high throughput screening will be discussed.

2. Cell-based high-throughput screening on microfluidic platforms

2.1. Perfusion flow mode high-throughput screening

Microfluidic devices can perform screening assays in a perfusion flow mode. Such screening devices require a series of generic components for introducing reagents and samples, transferring fluids within a microchannel network, and combining and mixing reactants.

In microfluidics based high-throughput screening, one challenge is to guide different chemical reagents to different cell types. By using the technique of reversible sealing of elastomeric polydimethylsiloxane (PDMS), Ali et al. [31] fabricated a PDMS substrate layer containing microwells combined with a PDMS cover layer with arrays of microchannels, see Fig. 2. Multiple cell types, such as hepatocytes, fibroblasts, and embryonic stem cells, were guided by microchannels by the first PDMS cover layer, and captured within microwells in the PDMS substrate layer. After formation of the cell

arrays on the substrate, the first PDMS cover layer was removed. A second PDMS cover layer was orthogonally aligned attached to the microwell substrate to deliver different fluids to the patterned cells. Although this approach demonstrates a simple microfluidic system for cell-based cytotoxicity assays, the throughput of this system is not sufficiently high for drug discovery. Gao et al. [32] used a microfluidic channel combined with vacuum actuated chambers for one-step cell seeding and anticancer drug testing.

Pneumatic pumping is another way to guide different mediums to different cell types [33]. Wang et al. [27] reported a multilayer pneumatic pump based microfluidic array platform for cell cytotoxicity screening of mammalian cell lines, see Fig. 3. The microfluidic channels were isolated by pneumatically actuated elastomeric valves into parallel rows for cell seeding and, subsequently, into parallel columns for toxin exposure. Hence, the microfluidic channels for cell seeding were orthogonal to the channels for toxin exposure. Cells were trapped in circular chambers by trapping sieves. This platform contains 576 chambers, and it was demonstrated using three different cell types (BALB/3T3, HeLa, and bovine endothelial cells) and five toxins (digitonin, saponin, CoCl_2 , NiCl_2 , and acrolein).

To reduce the cross-contamination between different chambers, Park et al. [34] performed screening in a 2×4 addressable array chamber by combining elastomeric valves with a bridge-and-underpass microfluidic channel architecture. With the similar principle, Kane et al. [35] developed a microfluidic device with 8×8 chambers for co-culture based drug screening.

Kim et al. [36] further developed a similar microfluidic device with 64 individually addressable cell culture chambers, in which on-chip generation of drug concentrations is possible, enabling drug combination screening applications with pair-wise combinations of drug concentrations and parallel culture of cells.

Another way of on-chip drug screening is based on the controlled diffusive mixing of solutions in continuous laminar flow inside a network of microfluidic channels, i.e. at low Reynolds number, which is a simple and versatile approach to generate (drug) concentration gradients [37]. As shown in Fig. 4, by integrating 8 such gradient generators with parallel cell culture chambers, Ye et al. [38,39] presented a high content multiparametric screening method (plasma membrane permeability, nuclear size, mitochondrial transmembrane potential and intracellular redox states) for human liver carcinoma (HepG2) responding to multiple anti-cancer drugs with different concentrations. This device provides a possible solution for cancer treatment screening by rapidly extracting tumor cell response to several drugs with little sample consumption. Combining the gradient generator with poly(ethylene glycol) diacrylate hydrogels, Ostrovidov et al. [40] investigated cell viability through the spatially controlled release of drug from a hydrogel covering on the microfluidic gradient generator, which also has the potential to be applied in drug discovery. Chung et al. [41] developed a microfluidic device for high throughput capture and imaging of thousands of single cells by shear force in a continuous flow channel. Combined with gradient generator, this device was used to study heterogeneity in calcium oscillatory behavior in genetically identical cells and monitor kinetic cellular response to chemical stimuli.

For perfusion culture chips equipped with a microfluidic concentration generator as described above, the possible concentration range is narrow and the generated concentration profiles are linear, which makes such microfluidic design difficult to be applied in a practical pharmacological drug dose response assay in which a logarithmic concentration profile spanning 5–7 orders of magnitude is usually required. Sugiura et al. [42] presented a serial dilution microfluidic network which is capable of generating a logarithmic concentration profile spanning 6 orders of magnitude

Fig. 2. Schematic diagram of reversible sealing of microfluidic arrays onto microwell patterned substrates to fabricate multiphenotype cell arrays. Reproduced with permission from Ref. [31].

Fig. 3. Schematic and image of the multilayer pneumatic pump based microfluidic array platform for cell cytotoxicity screening of mammalian cell lines. Reproduced with permission from Ref. [27].

automatically and performing an on-chip cell viability assay. Besides a wide concentration profile, Selimović et al. [43] described a microfluidic device for generating nonlinear concentration gradients for cell assays by using an asymmetrical network in microfluidic concentration generators. Also, a high throughput study of cell apoptosis by drug combinatorial concentrations was performed by using a microfluidic concentration generator with repeated splitting-and-mixing of the source solutions in a radial channel network [44].

Maintaining steady flow for a long time period (hours to days) is necessary for cellular studies in perfusion culture, especially in cell-based high throughput screening. Besides commercial syringe

pumps which are used in most microfluidic devices for the generation of flows, using gravity driven flow is another possible solution. Zhu et al. [45] proposed an improved and simple gravity driven system with horizontally-oriented tubes maintaining a constant hydraulic pressure drop across microfluidic channels. With a similar principle, a pressure-control based microfluidic system has been applied in a three-dimensional cell culture platform for cell-based drug testing in 30 microbioreactors [46,47].

The characterization of the metabolic activity of live cells, as well as the performance of a live/dead cell assay is also important for high-throughput screening. Most of current live/dead cell assays are based on commercial fluorescence kits. Also, qualitative and

Fig. 4. Schematic of the integrated microfluidic device with channel networks that can generate concentration gradients for cell-based high-throughput screening. Reproduced with permission from Ref. [38].

quantitative analysis of cell-based screening plays an increasingly important role in drug discovery. Cultured cells transduce and transmit a variety of chemical and physical signals, through the production of specific substances and proteins. These cellular signals can be used as parameters to monitor chemical information to build drug efficacy profiles.

Mass spectrometry has emerged as one of the most powerful tools for the identification and characterization of cell metabolites and biomarkers. Chen et al. [48] reported a platform based on stable isotope labeling carried out in a microfluidic chip with electrospray ionization mass spectrometry (SIL-chip ESI-MS) for qualitative and quantitative analysis of the metabolism of cells treated by drugs. This platform, shown in Fig. 5, has integrated cell

culture chambers, on-chip sample preparation (*i.e.* a solid phase extraction (SPE) module) and ESI-MS. This platform has the potential to be used as an on-line multiparameter cell metabolism analysis platform for high throughput drug screening.

Being able to monitor cell death is an important aspect of many high throughput screening devices, and one way to detect cell death is to observe the cell's morphological properties. Therefore, morphology-based examination provides a possible solution for cell death detection. With a morphology-based image cytometric analysis approach, Kim et al. [49] developed a microfluidic platform for label-free in situ monitoring of Cd^{2+} induced cell apoptosis (Fig. 6).

Electrical properties of cells have been used as well for cell

Fig. 5. Schematic diagram of the chip-ESI-MS platform for qualitative and quantitative analysis of the metabolic activity of cells exposed to drugs. Reproduced with permission from Ref. [48].

Fig. 6. (a) Schematic of the microfluidic device to detect cell death by cell's morphological properties and (b) optical image of adherent cells cultured in microchannel and the curve of cell viability quantified by the device under different chemical concentration. Reproduced with permission from Ref. [49].

analysis in microfluidics-based high-throughput screening technology. Cell-based impedance spectroscopy is a label-free and non-destructive method to measure dielectric properties of biological samples. Electric signals are convenient parameters for recording and processing compared to other techniques such as optical detection, since no light source and optical detectors are required. By investigating impedance changes during cell apoptosis, Meissner et al. [50] developed a microelectronics-based microfluidic system for the real time investigation of cell changes during drug induced cytotoxicity (Fig. 7). Hsiung et al. [51] reported a dielectrophoresis (DEP)-based microfluidic chip for cell perfusion culture, which was used to study anticancer drug induced cell apoptosis. Microfluidic chips with integrated microelectrodes also can be used to monitor neurotransmitter release from neural cells and to study drug effects on neural cells, which is important to discover drugs

for diseases related to brain functions [52].

Most of the research using high-throughput screening technologies is based on the 2D culture of mammalian cells on microfluidic channel surfaces, however, the situation in which cells are growing in a 3D culture environment is regarded as a better representation of the *in vivo* environment. Such systems would have more biological or clinical relevance compared with conventional systems based on 2D cell culture [53]. Toh et al. [54] achieved a 3D cell perfusion culture by creating an array of micropillars in a microfluidic channel. Different types of cells including Carcinoma cell lines (HepG2, MCF7), primary differentiated cells (hepatocytes) and primary progenitor cells (bone marrow mesenchymal stem cells) can be perfusion-cultured in this system for 72 h to 1 week with preserved 3D cyto-architecture. Based on this system, Toh et al. [55] developed a multi-channel 3D hepatocyte cell culture

Fig. 7. (a) Photograph of the microfluidic device detecting cell apoptosis by investigating impedance changes. (b) The microfluidic channel and (c) schematic of cell area implemented with electrodes for impedance recording. Reproduced with permission from Ref. [50].

system for simultaneous administration of multiple drug doses to functional primary hepatocytes, see Fig. 8. Development of such 3D culture approaches opened the possibility to create *in vitro* models mimicking 3D organ-level structures, which in the future may be used to study drug toxicity as a (partial) replacement of animal testing.

Due to the development of separated components, e.g. fluid handling, cell culture, or cell analysis, for high-throughput screening, microfluidics also has potential in developing integrated systems capable of performing automatic cell culture, drug release, cell activity detection for drug discovery. Welton et al. [56] presented a microfluidic system for drug screening applications by on-line monitoring human cancer cell metabolism process, see Fig. 9. With fully integrated chemo- and biosensors, 4 different parameters (pH, oxygen, lactate and glucose) of cell activity could be monitored.

2.2. Droplet mode high-throughput screening

Next to the perfusion flow microfluidic platforms described in the previous section, in more recent times microfluidic droplet (*i.e.*

droplet-based microfluidic or multiphase microfluidic) platforms have been introduced which can perform a wide range of experimental operations for chemistry and biology screening. The key feature of microfluidic droplet systems is the use of water-in-oil emulsion droplets to compartmentalize reagents into nanoliter to picoliter volumes. The oil that separates the aqueous phase droplets can prevent cross-contamination between reagents in neighboring droplets, and reduce the non-specific binding between channel surface and the reagents. Also, droplets can be split and/or merged to start or stop reactions, or to perform washing steps.

Clausell-Tormos et al. [57] first described a droplet-based microfluidic platform for growing cells and multicellular organisms (*C. elegans*). With the help of novel biocompatible surfactants and a gas permeable storage system made of PDMS, the cells or organism in the aqueous micro-compartments can survive and proliferate for more than several days. This system opens a new way for cell-based high throughput screening with a volume that is over a 1000-fold smaller, and with a throughput that is 500 times higher than those of conventional microplate assays. However, in this continuous droplet flow micro-compartment system, it is difficult to perform the draining of toxic metabolites from cells and

Fig. 8. (a) (Top) schematic and (bottom) photograph of a 3D cell perfusion culture system for simultaneous administration of multiple drug concentrations. (b) Schematic of a single cell culture channel of the 3D cell culture chip. (c) The curve of cell viability with different exposed drug concentration. Reproduced with permission from Ref. [55].

Fig. 9. The microfluidic system for the detection of cell metabolism. (a) Illustration of chip layout. (b) Photograph of the microfluidic chip. (c) Illustration of the system components and electric interface. (d) A microscopy image of cells growing in the chamber. (e) Photograph of pH and oxygen sensor electrodes. Reproduced with permission from Ref. [56].

the addition of nutrients, which restricts the application of this system in cell-based research.

Brouzes et al. [58] presented a complete microfluidic droplet screening workflow for high throughput cytotoxicity screening of single mammalian cells, shown schematically in Fig. 10. This system integrated all important manipulations for droplet-based microfluidic drug screening at a frequency of more than 100 Hz. The cells were encapsulated in individual aqueous microdroplets, and merged with optically-coded droplets from a library enabling to

identify drug composition and drug concentration in each droplet. After 24 h off-chip incubation, the droplets were reinjected into the chip and merged with fluorescent dye droplets for staining live/dead cells to enable an on-chip fluorescence assay.

To simulate *in vivo* tumors within their microenvironment, 3D multicellular aggregates are used, which provide more complexity than the standard monolayer culture environment. For this purpose, cell encapsulation and 3D culture were performed in a droplet-based microfluidic system [59]. In the 3D droplet system,

Fig. 10. Workflow of a droplet-based microfluidic drug screening platform. The droplet screening consists of 4 steps. (a) A reformatting step to emulsify compound-coded droplets and pool them into a droplet library. (b) Merging each library member with one of the cell-containing droplets that are continuously generated. (c) Incubation. (d) Merged droplets with fluorescent dyes are reinjected into an assay chip to identify each compound via their code and assess their specific effect on cells. Reproduced with permission from Ref. [58].

alginate beads with entrapped breast tumor cells were formed and trapped for cell culture in a continuous flow system. The cells were proliferated in the alginate beads for several days to form 3D multicellular aggregates, after which the drugs were loaded and a cell viability assay was performed. In the multicellular aggregate droplets, cells showed a higher resistance compared to the system using standard monolayer culture.

Another application of droplet-based high-throughput cell screening is the diagnosis of sepsis due to bacterial infections, which causes more than 130,000 deaths in the USA every year as a result of infections by drug-resistant strains of bacteria. However, with the current diagnostic approaches it takes more than one day to diagnose for the presence of the bacteria and determine the minimal inhibitory concentration of an antibiotic. Shortening the diagnosis time to identify specific antibiotics to treat bacterial infections could decrease the patient mortality, and reduce the cost of patient treatment. Boedicker et al. [60] described a plug-based microfluidic technique that enabled to characterize the drug

sensitivity of bacteria in samples and measure the minimal inhibitory concentration of antibiotics. By confining the cells in nanoliter droplets, the cell density was increased without preincubation and the time required to detect the bacteria was reduced. In this system, the detailed functional characterization of a bacterial sample could be achieved in less than 7 h. A similar principle was used by Baraban et al. [61], who presented a microfluidic droplet analyzer to measure the minimal inhibitory concentration of antibiotics by monitoring dynamic populations of bacterial strains in thousands of nanoliter droplets. In their system, the detection is based on light scattering instead of fluorescence, which extends the application of current microfluidic devices for strains without fluorescent markers.

Jakiela et al. [62] reported a microfluidic droplet system which can fully manipulate and monitor the dynamics of bacteria populations in a series of addressable microdroplets over hundreds of generations. This microfluidic droplet system integrates all the steps needed for the characterization of the dynamics of bacterial

populations: (1) formation of droplets containing cells and growth medium; (2) cycling droplets back and forth in a straight channel for cell incubation and cell density measurement; and (3) splitting and fusing of droplets to control the concentration of chemical factors over time.

Trivedi et al. [63] presented a hand-assembled microfluidic droplet system for cell based drug screening using polytetrafluoroethylene (PTFE) tubing modules, see Fig. 11. This system integrates steps needed for high throughput drug screening, including on-line droplet generation, storage, serial mixing and optical detection based on commercially available cross-junctions and optical fibers. The cells were captured in alginate solutions and cross-linked by BaCl_2 at a T-junction. The cells inside the tubing can be simultaneously cultured with high viability, sufficient for drug screening and toxicity assays, due to the high gas permeability of PTFE.

The fast and precise determination of effective combinations of antibiotics to combat bacteria is particularly interesting for toxicological investigations and inhibitor studies. Cao et al. [64] presented a microfluidic droplet device for the generation of multi-dimensional concentrations of antibiotics, to achieve an assay for toxic effects of *Escherichia coli*, see Fig. 12. More than 5000 distinct experiments with different combinations of antibiotic concentrations could be realized in a single experimental run. Compared with conventional toxicological methods, this microfluidic droplet system is suitable for the evaluation of effects under different conditions with the advantages of reduced experimental complexity and higher information density.

Digital microfluidics (DMF) refers generally to another fluid-handling technique for droplets. Rather than creating and handling droplets in channels or in tubing, DMF platforms manipulate nanoliter to microliter droplets on chip surfaces by dielectric effects introduced through arrays of electrodes. The key mechanisms is that the droplet contact angle depends on the electric field applied between the droplet and the chip surface. Recently, DMF has been applied in the study of cell apoptosis as a function of staurosporine concentration, achieving a 33-fold reduction in reagent consumption compared with conventional methods [65]. Another advantage of DMF is that it is compatible with conventional high-throughput screening instruments, but it can result in lower detection limits and larger dynamic range because apoptotic cells are much less likely to delaminate when exposed to droplet manipulation by DMF relative to pipetting/aspiration in multiwell plates.

2.3. Microarray mode high-throughput screening

High-throughput screening systems based on microarrays enable assays with a large number of biological samples on a 2D solid substrate. This approach has been widely used in assays of drug screening, gene expression, and protein analysis. Microarrays can screen for thousands of different samples simultaneously in one single experiment.

Lee et al. [66] developed a miniaturized 3D cell-culture array (called DataChip) for high-throughput toxicity screening of drug candidates, illustrated in Fig. 13. Human cells were capsulated and 3D-cultured in 20 nL alginate gels on a functionalized glass substrate, after which this substrate was placed on another glass substrates pre-spotted with spatially addressable multiple compounds (i.e. a microarray structure). This system could integrate 1080 individual different cell arrays on one single DataChip, with a nearly 2000-fold reduction in reagent consumption; the cytotoxicity response was identical to that with a conventional microplate assay.

Kwon et al. [67] designed another kind of microarray platform based on a standard microscope slide integrated with 2100 individual cell-based assays on one chip. This straightforward microarray platform for cell-based screening can be fabricated in standard microfabrication lab. Cells were loaded in multiple microwells in one substrate. A microarray of chemical-laden hydrogels was printed on another substrate, matched with the array of cell-laden microwells. To demonstrate the screening up to the extent of apoptosis and necrosis, MCF-7 breast cancer cells were sealed in the microwells and exposed to a small library of chemical compounds.

The use of hanging drops on the underside of culture plate lids is a typical method to generate 3D cellular spheroids. 3D cell spheroid culture allows for cellular self-organization and enables straightforward monitoring. As a result, the method can provide valuable information that is physiologically more relevant than 2D cell culture. By using a commercial 384-well plate and liquid handling robot, Hung et al. [68] achieved a hanging droplet microarray system for drug testing in cellular spheroid formation, as shown in Fig. 14. This platform significantly simplified the experimental process for cell culture and cellular formation in hanging droplets.

Although the recently developed microarray technologies provide a high throughput method for cell-based drug screening, specific liquid pumps and liquid handling equipment for accurate array printing are still required in these systems. Based on a PDMS

Fig. 11. Schematic of cell based drug screening using PTFE tubing modules. Reproduced with permission from Ref. [63].

Fig. 12. Schematic diagram of the experimental set-up of a droplet-based microfluidic system for testing combinations of antibiotics. Reproduced with permission from Ref. [64].

liquid pipet chip, Zhou et al. [69] developed a PDMS printing system controlled by pneumatic pump. This system can achieve cell seeding, cell transferring and cell stimulation by drugs in each spot.

The combination of two or more existing drugs, administered either simultaneously or sequentially, can improve therapeutic efficacy, as well as reduce drug toxicity and drug resistance in clinical treatment due to its multi-target treatment mechanisms. Furthermore, it is regarded as an effective way to increase the efficiency of drug discovery as most drug combinations are carried out using existing drugs which have passed through the strict clinical and safety examinations [70]. Based on the sequential operation droplet array (SODA) technique [71], Du et al. [72] achieved multi-step operations for drug combination screening involving cell culture, medium changing, schedule-dependent drug dosage and stimulation, and cell viability testing in an oil-covered nanoliter-scale droplet array system, by using multiple droplet manipulations including liquid metering, aspirating, depositing, mixing, and transferring, as shown in Fig. 15. The drug consumption for each screening test was substantially decreased to 5 ng–5 µg, leading to a 10–1000 fold reduction compared with traditional drug screening systems.

3. Conclusions

In this paper, we have reviewed the application of microfluidics in cell-based high-throughput drug screening, including screening performed in perfusion flow, in droplet-based flow and in an array platform. Compared with traditional high-throughput assays, microfluidic high-throughput technology shows advantages of lower reagent consumption, and better ability to control cellular microenvironments. The performance of different modes of microfluidics for cell-based high throughput screening platforms is summarized in Table 1.

Today, high throughput screening libraries always contain millions of compounds, which are routinely screened in 384-, 1536- and 3456-well formats. The throughput of microfluidic technology has been proven to reach the million level [58], however, the throughput is not the only limitation of microfluidic technology currently applied in drug discovery. In microfluidic cell-based screening systems, the size of each cell assay unit could be scaled down to the submicroliter range, in which the cell number could be reduced to hundreds cells, or further to single cell level. However, such a miniaturization is restricted because too few cells may have a nonphysiological behavior due to lacking contact with neighbors, which may reduce the predictability of the assay. On the other

Fig. 13. Schematic of the DataChip platform for direct multiparameter testing of compound toxicity. Reproduced with permission from Ref. [66].

hand, some necessary steps, *e.g.* library preparation and data handling, are still not integrated in most of microfluidic cell-based high throughput screening platforms. With the progress of industry development for microfluidic technology, we believe that microfluidic platforms have the potential to screening the whole compound library on the cell based level, not only the secondary screening but also the primary screening. Therefore, there is an urgent need to develop a strategy to integrate microfluidic high-throughput screening with other components, *e.g.* clinical study and data analysis, in drug discovery.

Moreover, choosing the right chip material is also an important factor for the application of microfluidic technology in high throughput screening. PDMS is a widely used material in the research of microfluidics due to its low cost and ease of micro-fabrication. And PDMS is gas permeable and compatible for cell culture, which is a significant advantage for cell-based screening. However, PDMS has limitations to be applied in industrial uses. PDMS is not compatible with most organic solvents, and is able to absorb small hydrophobic molecules. Although various strategies have been introduced to modify PDMS surfaces [73–76], its drawbacks still cannot be fully resolved for high-throughput industrial uses. Therefore, for industrial uses, other more chemical stable or more compatible with organic solvents, *e.g.* glass, poly(methyl methacrylate) (PMMA) or cyclic olefin copolymer (COC), are used.

The scale-up of screening throughput for microfluidic platforms to millions of assays is a challenging task. There are some industrial applications showing the potential of microfluidic technology to be scaled up to millions of screening. In the perfusion flow mode, highly integrated microfluidic devices developed by Fluidigm, in

which the fluids are controlled by micromechanical valves in micrometer-sized channels and millimeter-sized chambers, have showed great potential for biological and biochemical research [77,78]. An integrated chip having 256 subnanoliter reaction chambers requires 2056 microvalves to control. However, the further scale-up of the screening throughput for cell-based drug screening might be limited by the number of microvalve in this system. Based on the microfluidic droplet mode, Biomillenia now can reach 30 million strain variants in a single day, which has been applied in the directed evolution of enzyme. However, the droplet mode lacks a versatile way for compound delivery into droplets and cell growth inside droplets, which has limited this mode to be further applied in cell-based high-throughput screening. The microarray mode is more compatible with the current drug screening system. Curiox Biosystems has applied microarray technology in stem cell research and immunoassay development.

Combined with the development of other microfluidic technologies, *e.g.* organs-on-a-chip and human-on-a-chip [79,80], drug discovery and development can be further advanced. Although at the current stage, it is unlikely that organs-on-a-chip devices can be used on short notice in actual high throughput drug screening contexts due to their complicated structure and high cost, such a strategy can possibly be used in the early drug discovery to bridge the animal models and conventional cell-based models, and to produce more reliable and predictive data in early phases of drug discovery. Organs-on-a-chip devices will eventually reduce the need for animal testing and facilitate the development of safer and more effective drugs. Therefore, microfluidic technology will be a major step towards the aim of fully-automatic drug discovery.

Fig. 14. (a) Illustration of the 384 hanging drop for drug testing in cellular spheroid formation, and its cross-sectional view. (b) Photo of the array plate. (c) Schematic of the hanging drop formation process in the array plate. (d) Photo of the 384 hanging drop array plate operated with liquid handling robot capable of simultaneously pipetting 96 cell culture sites. (e) Schematic of the final humidification chamber used to culture 3D spheroids in the hanging drop array plate. Reproduced with permission from Ref. [68].

Fig. 15. Illustration of drug combination assay in the droplet array system. (a) Cell seeding; (b) Addition of the 1st drug; (c) Addition of the 2nd drug; (d) Cell culture; (e) Addition of the fluorescent dye. Reproduced with permission from Ref. [72].

Table 1

The performance of different modes of microfluidics for cell-based high throughput screening platforms.

	Performance
Continuous flow mode	<ul style="list-style-type: none"> • Easily integrated with steps of library preparation • Easily coupled with different detection methods: fluorescence, absorption, mass spectrometry, electrical properties of cells • Difficult to achieve high throughput screening for different samples
Droplet mode	<ul style="list-style-type: none"> • High throughput screening for large number of droplets up to millions • Low sample and reagent consumption in the microliter range • Difficult to perform multi-step liquid handling for droplets • Difficult to carry out long-term in-droplet cell culture • Difficult to analyze droplet by other detection methods besides fluorescence and absorption spectroscopy
Array mode	<ul style="list-style-type: none"> • Easy to screen large number of different samples • Easy to be coupled with current industry high-throughput screening technologies • Precise and bulk equipment required to handle small volume liquid in parallel

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References

- [1] P.S. Dittich, A. Manz, Lab-on-a-chip: microfluidics in drug discovery, *Nat. Rev. Drug Discov.* 5 (2006) 210–218.
- [2] P. Neuzi, S. Giselbrecht, K. Länge, T.J. Huang, A. Manz, Revisiting lab-on-a-chip technology for drug discovery, *Nat. Rev. Drug Discov.* 11 (2012) 620–632.
- [3] J. Hong, J.B. Edel, A.J. deMello, Micro- and nanofluidic systems for high-throughput biological screening, *Drug Discov. Today* 14 (2009) 134–146.
- [4] L. Chin, J.N. Andersen, P.A. Futreal, Cancer genomics: from discovery science to personalized medicine, *Nat. Med.* 17 (2011) 297–303.
- [5] G. Emilien, M. Ponchon, C. Caldas, O. Isacson, J.M. Maloteaux, Impact of genomics on drug discovery and clinical medicine, *QJM* 93 (2000) 391–423.
- [6] M.-H. Wu, S.-B. Huang, G.-B. Lee, Microfluidic cell culture systems for drug research, *Lab. Chip* 10 (2010) 939–956.
- [7] G. Wu, S.K. Doberstein, HTS technologies in biopharmaceutical discovery, *Drug Discov. Today* 11 (2006) 718–724.
- [8] S.M. Paul, D.S. Mytelka, C.T. Dunwiddie, C.C. Persinger, B.H. Munos, S.R. Lindborg, A.L. Schacht, How to improve R&D productivity: the pharmaceutical industry's grand challenge, *Nat. Rev. Drug Discov.* 9 (2010) 203–214.
- [9] F. Pammolli, L. Magazzini, M. Riccaboni, The productivity crisis in pharmaceutical R&D, *Nat. Rev. Drug Discov.* 10 (2011) 428–438.
- [10] G. Wu, J. Irvine, C. Luft, D. Pressley, H.C. Nicholas, B. Janzen, Assay development and high-throughput screening of caspases in microfluidic format, *Comb. Chem. High. Throughput Screen* 6 (2003) 303–312.
- [11] L. Kang, B.G. Chung, R. Langer, A. Khademhosseini, Microfluidics for drug discovery and development: from target selection to product lifecycle management, *Drug Discov. Today* 13 (2008) 1–13.
- [12] K. Bhadriraju, C.S. Chen, Engineering cellular microenvironments to cell-based drug testing improve, *Drug Discov. Today* 7 (2002) 612–620.
- [13] E. Michelini, L. Cevenini, L. Mezzanotte, A. Coppa, A. Roda, Cell-based assays: fuelling drug discovery, *Anal. Bioanal. Chem.* 398 (2010) 227–238.
- [14] S. Einav, D. Gerber, P.D. Bryson, E.H. Sklan, M. Elazar, S.J. Maerkl, J.S. Glenn, S.R. Quake, Discovery of a hepatitis C target and its pharmacological inhibitors by microfluidic affinity analysis, *Nat. Biotechnol.* 26 (2008) 1019–1027.
- [15] L. Li, D. Mustafi, Q. Fu, V. Tereshko, D.L. Chen, J.D. Tice, R.F. Ismagilov, Nanoliter microfluidic hybrid method for simultaneous screening and optimization validated with crystallization of membrane proteins, *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 19243–19248.
- [16] D. Huh, G.A. Hamilton, D.E. Ingber, From 3D cell culture to organs-on-chips, *Trends Cell Biol.* 21 (2011) 745–754.
- [17] A.M. Ghaemmaghami, M.J. Hancock, H. Harrington, H. Kaji, A. Khademhosseini, Biomimetic tissues on a chip for drug discovery, *Drug Discov. Today* 17 (2012) 173–181.
- [18] M.B. Esch, T.L. King, M.L. Shuler, The role of body-on-a-chip devices in drug and toxicity studies, *Annu. Rev. Biomed. Eng.* 13 (2011) 55–72.
- [19] R.S. Kane, S. Takayama, E. Ostuni, D.E. Ingber, G.M. Whitesides, Patterning proteins and cells using soft lithography, *Biomaterials* 20 (1999) 2363–2376.
- [20] J. El-Ali, P.K. Sorger, K.F. Jensen, Cells on chips, *Nature* 442 (2006) 403–411.
- [21] T.H. Park, M.L. Shuler, Integration of cell culture and microfabrication technology, *Biotechnol. Prog.* 19 (2003) 243–253.
- [22] H.A. Stone, A.D. Stroock, A. Ajdari, Engineering flows in small devices: Microfluidics toward a lab-on-a-chip, *Annu. Rev. Fluid Mech.* 36 (2004) 381–411.
- [23] J. Melin, S.R. Quake, Microfluidic large-scale integration: the evolution of design rules for biological automation, *Annu. Rev. Biophys. Biomol. Struct.* 36 (2007) 213–231.
- [24] T.M. Squires, S.R. Quake, Microfluidics: fluid physics at the nanoliter scale, *Rev. Mod. Phys.* 77 (2005) 977–1026.
- [25] D.J. Beebe, G.A. Mensing, G.M. Walker, Physics and applications of microfluidics in biology, *Annu. Rev. Biomed. Eng.* 4 (2002) 261–286.
- [26] I. Barbulovic-Nad, H. Yang, P.S. Park, A.R. Wheeler, Digital microfluidics for cell-based assays, *Lab. Chip* 8 (2008) 519–526.
- [27] Z.H. Wang, M.C. Kim, M. Marquez, T. Thorsen, High-density microfluidic arrays for cell cytotoxicity analysis, *Lab. Chip* 7 (2007) 740–745.
- [28] R.N. Zare, S. Kim, Microfluidic platforms for single-cell analysis, *Annu. Rev. Biomed. Eng.* 12 (2010) 187–201.
- [29] R. Gomez-Sjoeberg, A.A. Leyrat, D.M. Pirone, C.S. Chen, S.R. Quake, Versatile, fully automated, microfluidic cell culture system, *Anal. Chem.* 79 (2007) 8557–8563.
- [30] O.J. Dressler, R.M. Maceiczky, S.-I. Chang, A.J. deMello, Droplet-based microfluidics enabling impact on drug discovery, *J. Biomol. Screen* 19 (2014) 483–496.
- [31] A. Khademhosseini, J. Yeh, G. Eng, J. Karp, H. Kaji, J. Borenstein, O.C. Farokhzad, R. Langer, Cell docking inside microwells within reversibly sealed microfluidic channels for fabricating multiphenotype cell arrays, *Lab. Chip* 5 (2005) 1380–1386.
- [32] Y. Gao, P. Li, D. Pappas, A microfluidic localized, multiple cell culture array using vacuum actuated cell seeding: integrated anticancer drug testing, *Biomicrofluidics* 15 (2013) 907–915.
- [33] M.A. Unger, H.P. Chou, T. Thorsen, A. Scherer, S.R. Quake, Monolithic micro-fabricated valves and pumps by multilayer soft lithography, *Science* 288 (2000) 113–116.
- [34] E.S. Park, A.C. Brown, M.A. DiFeo, T.H. Barker, H. Lu, Continuously perfused, non-cross-contaminating microfluidic chamber array for studying cellular responses to orthogonal combinations of matrix and soluble signals, *Lab. Chip* 10 (2010) 571–580.
- [35] B.J. Kane, M.J. Zinner, M.L. Yarmush, M. Toner, Liver-specific functional studies in a microfluidic array of primary mammalian hepatocytes, *Anal. Chem.* 78 (2006) 4291–4298.
- [36] J. Kim, D. Taylor, N. Agrawal, H. Wang, H. Kim, A. Han, K. Rege, A. Jayaraman, A programmable microfluidic cell array for combinatorial drug screening, *Lab. Chip* 12 (2012) 1813–1822.
- [37] N.L. Jeon, S.K. Dertinger, D.T. Chiu, I.S. Choi, A.D. Stroock, G.M. Whitesides, Generation of solution and surface gradients using microfluidic systems, *Langmuir* 16 (2000) 8311–8316.
- [38] N.N. Ye, J.H. Qin, W.W. Shi, X. Liu, B.C. Lin, Cell-based high content screening using an integrated microfluidic device, *Lab. Chip* 7 (2007) 1696–1704.
- [39] J. Qin, N. Ye, X. Liu, B. Lin, Microfluidic devices for the analysis of apoptosis, *Electrophoresis* 26 (2005) 3780–3788.
- [40] S. Ostrovidov, N. Annabi, A. Seidi, M. Ramalingam, F. Dehghani, H. Kaji, A. Khademhosseini, Controlled release of drugs from gradient hydrogels for high-throughput analysis of cell-drug interactions, *Anal. Chem.* 84 (2012) 1302–1309.
- [41] K. Chung, C.A. Rivet, M.L. Kemp, H. Lu, Imaging single-cell signaling dynamics with a deterministic high-density single-cell trap array, *Anal. Chem.* 83 (2011) 7044–7052.
- [42] S. Sugiura, K. Hattori, T. Kanamori, Microfluidic serial dilution cell-based assay for analyzing drug dose response over a wide concentration range, *Anal. Chem.* 82 (2010) 8278–8282.
- [43] S.E. Selimovic, W.Y. Sim, S.B. Kim, Y.H. Jang, W.G. Lee, M. Khabiry, H. Bae, S. Jambovane, J.W. Hong, A. Khademhosseini, Generating nonlinear concentration gradients in microfluidic devices for cell studies, *Anal. Chem.* 83 (2011) 2020–2028.
- [44] C.-G. Yang, Y.-F. Wu, Z.-R. Xu, J.-H. Wang, A radial microfluidic concentration gradient generator with high-density channels for cell apoptosis assay, *Lab. Chip* 11 (2011) 3305–3312.
- [45] X.Y. Zhu, L.Y. Chu, B.H. Chueh, M.W. Shen, B. Hazarika, N. Phadke, S. Takayama,

- Arrays of horizontally-oriented mini-reservoirs generate steady microfluidic flows for continuous perfusion cell culture and gradient generation, *Analyst* 129 (2004) 1026–1031.
- [46] M.-H. Wu, S.-B. Huang, Z. Cui, Z. Cui, G.-B. Lee, Development of perfusion-based micro 3-D cell culture platform and its application for high throughput drug testing, *Sens. Actuators B Chem.* 129 (2008) 231–240.
- [47] M.-H. Wu, S.-B. Huang, Z. Cui, Z. Cui, G.-B. Lee, A high throughput perfusion-based microbio-reactor platform integrated with pneumatic micropumps for three-dimensional cell culture, *Biomed. Microdevices* 10 (2008) 309–319.
- [48] Q.S. Chen, J. Wu, Y.D. Zhang, J.M. Lin, Qualitative and quantitative analysis of tumor cell metabolism via stable isotope labeling assisted microfluidic chip electrospray ionization mass spectrometry, *Anal. Chem.* 84 (2012) 1695–1701.
- [49] M.J. Kim, K.H. Lim, H.J. Yoo, S.W. Rhee, T.H. Yoon, Morphology-based assessment of Cd²⁺ cytotoxicity using microfluidic image cytometry (μ FC), *Lab. Chip* 10 (2010) 415–417.
- [50] R. Meissner, B. Eker, H. Kasi, A. Bertsch, P. Renaud, Distinguishing drug-induced minor morphological changes from major cellular damage via label-free impedimetric toxicity screening, *Lab. Chip* 11 (2011) 2352–2361.
- [51] L.C. Hsiung, C.L. Chiang, C.H. Wang, Y.H. Huang, C.T. Kuo, J.Y. Cheng, C.H. Lin, V. Wu, H.Y. Chou, D.S. Jong, H. Lee, A.M. Wo, Dielectrophoresis-based cellular microarray chip for anticancer drug screening in perfusion microenvironments, *Lab. Chip* 11 (2011) 2333–2342.
- [52] Y. Chen, C. Guo, L. Lim, S. Cheong, Q. Zhang, K. Tang, J. Reboud, Compact microelectrode array system: tool for in situ monitoring of drug effects on neurotransmitter release from neural cells, *Anal. Chem.* 80 (2008) 1133–1140.
- [53] A. Abbott, Cell culture: biology's new dimension, *Nature* 424 (2003) 870–872.
- [54] Y.-C. Toh, C. Zhang, J. Zhang, Y.M. Khong, S. Chang, V.D. Samper, D. van Noort, D.W. Huttmacher, H. Yu, A novel 3D mammalian cell perfusion-culture system in microfluidic channels, *Lab. Chip* 7 (2007) 302–309.
- [55] Y.-C. Toh, T.C. Lim, D. Tai, G. Xiao, D. van Noort, H. Yu, A microfluidic 3D hepatocyte chip for drug toxicity testing, *Lab. Chip* 9 (2009) 2026–2035.
- [56] A. Weltin, K. Slotwinski, J. Kieninger, I. Moser, G. Jobst, M. Wego, R. Ehret, G.A. Urban, Cell culture monitoring for drug screening and cancer research: a transparent, microfluidic, multi-sensor microsystem, *Lab. Chip* 14 (2014) 138–146.
- [57] J. Clausell-Tormos, D. Lieber, J.C. Baret, A. El-Harrak, O.J. Miller, L. Frenz, J. Blouwolf, K.J. Humphry, S. Koster, H. Duan, C. Holtze, D.A. Weitz, A.D. Griffiths, C.A. Merten, Droplet-based microfluidic platforms for the encapsulation and screening of mammalian cells and multicellular organisms, *Chem. Biol.* 15 (2008) 427–437.
- [58] E. Brouzes, M. Medkova, N. Savenelli, D. Marran, M. Twardowski, J.B. Hutchison, J.M. Rothberg, D.R. Link, N. Perrimon, M.L. Samuels, Droplet microfluidic technology for single-cell high-throughput screening, *Proc. Natl. Acad. Sci. U. S. A.* 106 (2009) 14195–14200.
- [59] L. Yu, M.C. Chen, K.C. Cheung, Droplet-based microfluidic system for multicellular tumor spheroid formation and anticancer drug testing, *Lab. Chip* 10 (2010) 2424–2432.
- [60] J.Q. Boedicker, L. Li, T.R. Kline, R.F. Ismagilov, Detecting bacteria and determining their susceptibility to antibiotics by stochastic confinement in nanoliter droplets using plug-based microfluidics, *Lab. Chip* 8 (2008) 1265–1272.
- [61] L. Baraban, F. Bertholle, M.L. Salverda, N. Bremond, P. Panizza, J. Baudry, J.A.G. de Visser, J. Bibette, Millifluidic droplet analyser for microbiology, *Lab. Chip* 11 (2011) 4057–4062.
- [62] S. Jakiela, T.S. Kaminski, O. Cybulski, D.B. Weibel, P. Garstecki, Bacterial growth and adaptation in microdroplet chemostats, *Angew. Chem. Int. Ed.* 52 (2013) 8908–8911.
- [63] V. Trivedi, A. Doshi, G. Kurup, E. Ereifej, P. Vandevord, A.S. Basu, A modular approach for the generation, storage, mixing, and detection of droplet libraries for high throughput screening, *Lab. Chip* 10 (2010) 2433–2442.
- [64] J. Cao, D. Kürsten, S. Schneider, A. Knauer, P.M. Günther, J.M. Köhler, Uncovering toxicological complexity by multi-dimensional screenings in micro-segmented flow: modulation of antibiotic interference by nanoparticles, *Lab. Chip* 12 (2012) 474–484.
- [65] D. Bogojevic, M.D. Chamberlain, I. Barbulovic-Nad, A.R. Wheeler, A digital microfluidic method for multiplexed cell-based apoptosis assays, *Lab. Chip* 12 (2012) 627–634.
- [66] M.-Y. Lee, R.A. Kumar, S.M. Sukumaran, M.G. Hogg, D.S. Clark, J.S. Dordick, Three-dimensional cellular microarray for high-throughput toxicology assays, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 59–63.
- [67] C.H. Kwon, I. Wheeldon, N.N. Kachouie, S.H. Lee, H. Bae, S. Sant, J. Fukuda, J.W. Kang, A. Khademhosseini, Drug-eluting microarrays for cell-based screening of chemical-induced apoptosis, *Anal. Chem.* 83 (2011) 4118–4125.
- [68] Y.-C. Tung, A.Y. Hsiao, S.G. Allen, Y.-S. Torisawa, M. Ho, S. Takayama, High-throughput 3D spheroid culture and drug testing using a 384 hanging drop array, *Analyst* 136 (2011) 473–478.
- [69] Y. Zhou, Y.H. Pang, Y.Y. Huang, Openly accessible microfluidic liquid handlers for automated high-throughput nanoliter cell culture, *Anal. Chem.* 84 (2012) 2576–2584.
- [70] T.T. Ashburn, K.B. Thor, Drug repositioning: identifying and developing new uses for existing drugs, *Nat. Rev. Drug Discov.* 3 (2004) 673–683.
- [71] Y. Zhu, Y.-X. Zhang, L.-F. Cai, Q. Fang, Sequential operation droplet array: an automated microfluidic platform for picoliter-scale liquid handling, analysis, and screening, *Anal. Chem.* 85 (2013) 6723–6731.
- [72] G.-S. Du, J.-Z. Pan, S.-P. Zhao, Y. Zhu, J.M. den Toonder, Q. Fang, Cell-based drug combination screening with a microfluidic droplet array system, *Anal. Chem.* 85 (2013) 6740–6747.
- [73] J. Zhou, A.V. Ellis, N.H. Voelcker, Recent developments in PDMS surface modification for microfluidic devices, *Electrophoresis* 31 (2010) 2–16.
- [74] H. Hillborg, N. Tomczak, A. Olah, H. Schönherr, G.J. Vancso, Nanoscale hydrophobic recovery: a chemical force microscopy study of UV/ozone-treated cross-linked poly(dimethylsiloxane), *Langmuir* 20 (2004) 785–794.
- [75] K. Ren, Y. Zhao, J. Su, D. Ryan, H. Wu, Convenient method for modifying poly(dimethylsiloxane) to be airtight and resistive against absorption of small molecules, *Anal. Chem.* 82 (2010) 5965–5971.
- [76] J. Zhou, H. Yan, K. Ren, W. Dai, H. Wu, Convenient method for modifying poly(dimethylsiloxane) with poly(ethylene glycol) in microfluidics, *Anal. Chem.* 81 (2009) 6627–6632.
- [77] T. Thorsen, S.J. Maerkl, S.R. Quake, Microfluidic large-scale integration, *Science* 298 (2002) 580–584.
- [78] J.W. Hong, S.R. Quake, Integrated nanoliter systems, *Nat. Biotechnol.* 21 (2003) 1179–1183.
- [79] C. Luni, E. Serena, N. Elvassore, Human-on-chip for therapy development and fundamental science, *Curr. Opin. Biotechnol.* 25 (2014) 45–50.
- [80] D.J. Beebe, D.E. Ingber, J.M.J. den Toonder, Organs on chips 2013, *Lab. Chip* 13 (2013) 3447–3448.

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