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Cell-based drug screening on microfluidics

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ABSTRACT

With the inherent merits of a low reagent consumption, the small amount of sample needed and easy integration, a wide variety of microfluidic platforms (continuous-flow, droplet, digital and paper) have been developed to address the challenges (e.g. throughput, single-cell isolation, cost and flexibility) of cell-based drug screening. This review essentially summarises the pros and cons of the platforms for different applications, so as to shed light on the road ahead for drug screening research and discovery via the advance of microfluidics.

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1. Introduction

The pharmaceutical industry continues to be challenged by escalating cost and increasing time for new drug development [1–3]. It's estimated that $648.0 million required to bring a drug to market [4]. In addition, it's reported that it takes over a decade to develop a drug and the failure rate is more than 95%. Launching a new drug includes two stages: drug discovery in the preclinical stage and drug development in the clinical stage. Therefore, how to cut down the cost and shorten the time of the above two stages is always the big challenge in the pharmaceutical industry. Cell-based drug screening is generally used in the drug discovery period to eliminate and select drug candidates from hundreds of thousands of chemical compounds library [5]. Traditionally, this was carried out in 96-well plates. To parallel carrying out a large number of pharmacological tests, robotic fluid handling devices, sensitive detectors and data processing software are involved, which greatly raised the cost. To lower the cost or time consumption and increase the throughput, miniaturised microtiter plates, such as 384, 1536 and 3456-well plates, were developed and employed in the drug screening field [6,7]. The required reagent volume decreased from 10 μl in the 384-well plates to 1 μl in the 1536-well plates. Other reports have even claimed the use of 9600-well plates to further lower the volume requirement to 0.2 μl per assay [8,9]. However, microtiter plate-based screening still faces some technical hurdles, such as the uncontrolled evaporation of the dispensed liquids in open systems and the limitations in dispensing low volume liquid technologies [10,11].

Drug screening is critical not only for pharmaceutical industry, but also for precision medicine. Precision medicine in Oncology requires the most suitable therapeutic strategies for individual cancer patients. Although all clinically approved drugs or drug combinations have been tested *in vitro* using cell lines, *in vivo* using animal models and in clinical trials, there is no guarantee of success for any case, due to insufficient knowledge of cancer etiology, diversity of caner types, and metastasis etc. That calls for tests of drug susceptibility of primary tumour samples from an individual patient. However, the amount of biopsy samples is far from enough for drug screening using the traditional 96-well or 384-well plates. That puts microfluidics to be the best option, if not the only one, as the platform for drug screening given its merit of low sample input [12–16]. Microfluidics represents a technology which can precisely control and manipulate fluid that is geometrically constrained to the sub-millimetre scale [17–19]. Many progresses, such as high-throughput [20–22] and 3D cell culture-based drug screening [23], have been made in microfluidics. Although 3D cell culture techniques, which can simulate the natural extracellular

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microenvironment, have been used in traditional well plate-based assays, static culture maintenance has limited its applicability. In contrast, microfluidic devices, with the capability to replicate a physiologically relevant microenvironment by continuous perfusion, have been more popular for drug screening applications. By providing a microenvironment similar to the environment cells undergo in real organs or tissues, these microfluidic devices might replace animal or even part of clinical tests in the future.

There are various models used for drug screening, including protein structure-based, cell-based, animal test and clinical trials etc. Table 1 briefly summarised the pros and cons of each models. In this review, we introduce various microfluidic technologies related to cell-based drug screening. Different from a previous review paper on drug screening [24], which focused on the discussion of cell types and cell culture systems and cellular activities' detection methods in cell-based high-throughput drug screening, here we focus on the development of microfluidic technologies for drug screening. Various microfluidic platforms (continuous-flow, droplet microfluidics, digital microfluidics and paper microfluidics) for cell-based drug screening and future trends of these microfluidic technologies in the drug screening field are reviewed and discussed in details.

2. Cell-based drug screening on microfluidics

2.1. Cell-based drug screening on continuous-flow based microfluidics

Continuous-flow microfluidics is the manipulation of the flow of liquid through fabricated microchannels without breaking continuity. In this kind of microfluidics, the liquid flows continuously in the microchannel driven by external mechanical pumps [25,26] or by combinations of electro-kinetic mechanisms and capillary forces [27–30]. With the advantage of being easily implemented, continuous-flow is the most widely accepted microfluidic platform for simple biochemical [31–33] and biomedical applications [34–36].

For drug screening applications, in the initial stage, Gao et al. cultured four cell lines in localised arrays of microfluidic chambers and tested their responses to two drugs [37]. Khademhosseini et al. developed a method to guide different drugs to different cell types [38]. To make the platform more reliable for drug screening, the key is to adjust the drug concentrations flexibly and to construct 3D microstructures to mimic the natural extracellular microenvironment.

2.1.1. Drug dilution generator on continuous-flow based microfluidics

As in the drug screening field, the biological effects of a drug are closely related to its concentration. Dose-response screening is necessary for the evaluation of drug efficiency. Kim et al. proposed a serial dilution microfluidic chip to generate linear or logarithmic step-wise drug concentrations by adjusting the flow rates of two converging fluids at the channel junctions of the ladder network [39]. They tested the cytotoxicity of mitomycin C (an anticancer drug) to human breast cancer MCF-7 cells with serial drug concentrations generated on the designed microfluidic chip. Ye et al. further integrated drug dilution and micro-scale cell culture into a single device [40]. The device consisted of eight similarly structured units, with each one containing a drug dilution generator in the upstream and parallel cell culture chambers in the downstream. Eight units were connected to a central reservoir for solution perfusion. Each drug dilution generator was connected to the cell culture chamber array by a long exit microchannel to avoid cells entering the upstream dilution network during cell loading from the central reservoir. Based on the integrated microfluidic device, the cell toxicity of seven clinically established chemotherapeutic agents against HepG2 cells were tested. To broaden gradient dilution range, Wang et al. developed two serial dilution chips to form dilution rates of 1:1 and 1:4 by oscillatory methods on the basis of designing a series chamber [41]. The structural limitations and operation complexity hinders the application of conventional 2D and 3D microfluidics devices to multi-drug combinations

Table 1
The performance of each target-based drug screening technologies.

Target	Characteristics/Advantages/Disadvantages
Protein Structure	By using computational methods to investigate the ligand-protein binding interactions. • Reducing the time and cost. • No need for physically existing compounds screening process. • The screening tools are not in general cases. • Weak receptor structural flexibility in docking computations. • Scoring is challenging in predicting the correct binding pose accurately and the compounds ranking. • False positive/negative results from homology protein structures' modeling.
Cell	By using a series of vitro assays to investigate cell proliferation, toxicity, morphology changes, etc. • With low cost and easy operation to identify drug candidates. • The cells do not respond the same way as *in vivo* because of the different surrounding environment.
Animal	By using implanted human tumour xenografts model to investigate drug effects. • A validated model to select drug candidates for clinical evaluation. • Time-consuming and expensive • Animal ethical issues • A general lack of metastatic from primary implanted node. • The stromal compartment include vascular endothelial cells that are murine origin.
Organ	By using multichannel 3D microfluidic and other microfabricated structures to simulate human tissues/organs to investigate drug effects. • "Simulating the activities and physiological responses of human organ and reducing costly reagents consumption. • Can't perform biochemical analysis. • Losing the original organ functional activity because of the constructing cell sources (immortal cell lines). • Ignoring the effects of other organs on it.
Body	By connecting several organs (vitro tissue cultures) on a chip via fluidic streams in a physiologically relevant manner to investigate drug effects. • Predicting the drugs' efficacy, potential toxic side effects and dose response with high accuracy. • Providing human metabolic environment. • Promoting individualized medical development. • Difficult to design and create devices that simulate the human metabolism with high authenticity, • Difficult to increase the throughput, • Difficult to integrate with online analytics/sensors, • Difficult to develop a common medium.

screening. 3D printing solves this problem by fabricating complicated and interconnected microfluidic channels [42–45]. Chen et al. engineered the first 3D printed microfluidic chips with a helical structure for rapid mixing of solutions and tested four drugs combinations of 36 different concentrations to the viability of A549 cells [45], as shown in Fig. 1.

An integrated and portable multilayer microdevice has been developed by Liu et al. to minimise the cell damage from fluid shear stress in high-throughput drug screening [46]. In this design, a drug dilution generator, a vertical perfusion-based cell culture array and pneumatic microvalves were all integrated onto a single chip. By turning the microvalves on/off, the cell suspension was loaded into the microchambers. Then, a series of drug concentrations generated by the drug dilution generator flowed into the corresponding culture chambers for cell culture. Using this integrated microfluidic device, they conducted the cytotoxicity experiments of cisplatin on the cancer cell line A549/DDP. The results demonstrated the feasibility of multilayer microdevices for high-throughput drug screening.

The on-chip drug concentration generator was also integrated by Kim et al. into a fully automatic and programmable microfluidic cell array (Fig. 2) with a parallel cell culture for drug screening [47]. The microfluidic device had 64 independent cell culture chambers in which cells could be cultured and treated with different drugs, either sequentially or simultaneously, of 64 pair-wise concentration combinations. LabVIEW was used as the interface to automatically control the system. They successfully utilised this platform for the cell viability evaluation of prostate cancer cells (PC3) using combinations of either mitoxantrone or doxorubicin with TRAIL (TNF-alpha related apoptosis inducing ligand) in a sequential or simultaneous format, showing the power of the microfluidic platform for optimising fully automatically combinatorial drug treatments.

2.1.2. 3D cell culture-based drug screening on continuous-flow based microfluidics

A 2D monolayer cell culture lacks the complex microenvironment *in vivo*, such as cell-cell communications and cell-matrix interactions, which often results in an effective drug dose in the 2D mode which is ineffective when treating patients [48,49]. Therefore, a 3D cell culture, which can simulate the physiological fluid flow conditions and complex tissue structures, attracts more and more attention among researchers [26,50–55].

Toh et al. developed a 3D hepatocyte chip for the drug toxicity test by constructing a 3D microenvironment within the microfluidic channels based on continuous-flow microfluidics [52]. With a drug concentration gradient generator, 8 parallel cell culture channels were fabricated to be operated individually. Comparable results to those found *in vivo* have been demonstrated on this chip in simulating functional hepatocytes.

Different from building 3D microstructures in microchannels, Torisawa et al. developed a spheroid device, which comprised of a micro-hole array in a silicon chip along with elastomeric microchannels, for monitoring cellular activity and drug sensitivity [53]. The spheroids could be precisely located within the microstructures for holding cells injected through the flow inlet. Liver-specific functions can be maintained long-term (2 weeks) as the cell culture medium in the microstructure can be replaced by a fresh medium via the microfluidic channel. A 3D cell culture in a hydrogel on-chip was also developed by Sung et al. to reproduce the multiorgan interactions by co-culturing colon cancer, liver and bone marrow cells [26]. To mimic the mode of blood flow in humans, every chamber was interconnected with channels. Hepatoma cells and colon cancer cells were embedded in the matrigel. Due to their greater mobility, myeloblasts were embedded in alginate. The device was utilised to test the cytotoxic effect of tegafur, an oral prodrug of 5-fluorourail (a cancer drug), showed consistency with other published animal and human clinical studies.

The development of reliable, human-derived and low-cost 3D cellular models [56,57] is a necessary step to bring precision medicine into fruition and to speed up drug discovery. Mulholland [57] et al. developed a microfluidic platform with self-generating nutrients and drug concentration gradients perfusion (equipment-free) to screen a panel of drugs for cancer cell-enriched multicellular spheroids derived from primary human prostate cancer cells tumour biopsies. Electrical sensing is a simple, inexpensive, rapid, label-free and sensitive modality to detect cell growth conditions on the electrodes surface [58–60]. Pandyaa et al. engineered a microfluidic platform (Fig. 3) real-time detect the response of cancer cells seeded in the 3D gel matrix to a dynamic delivering chemotherapeutic drugs with

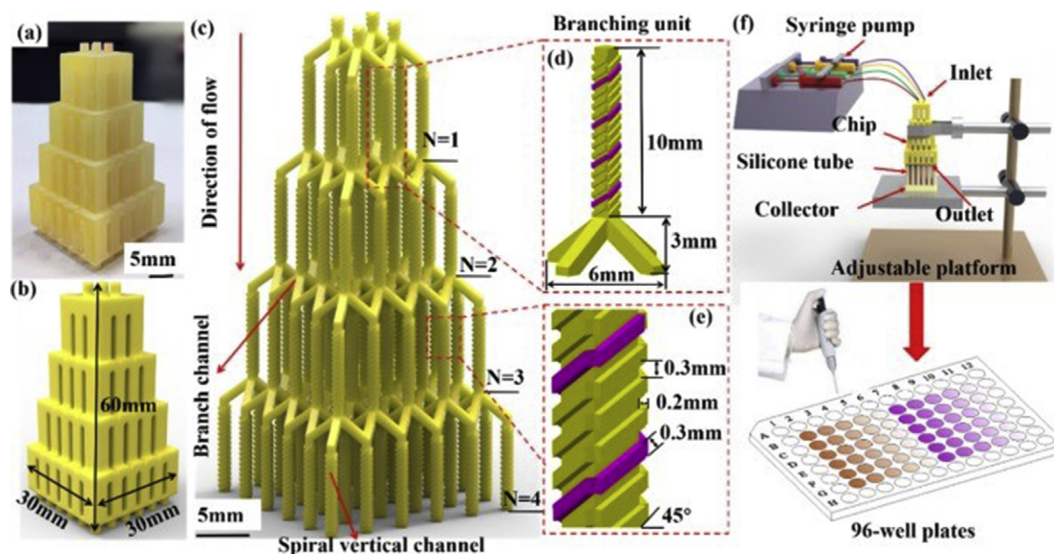

Fig. 1. (a) The schematic 3D printed prototype of microfluidic chip. (b) CAD model. (c) Interconnected 3D printed multi-drug combination gradient generator microchannel network architecture. (d) Details of the tree-shaped branch structure. (e) The spiral vertical channel. (f) The microfluidic system. Reproduced with permission of Elsevier from Ref. [45].

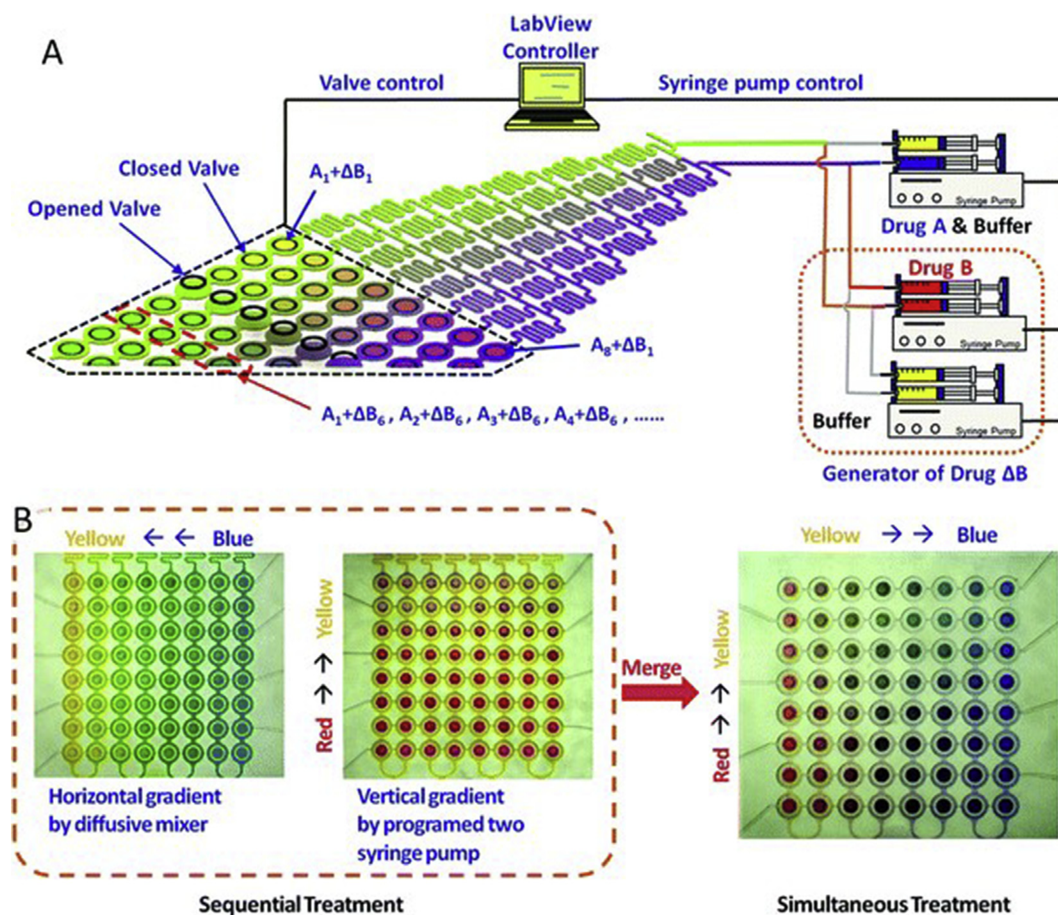

Fig. 2. Schematic of the microfluidic array. The generation of different concentrations of drugs A and B to perfuse cells cultured in the downstream microchambers. The operation is controlled by a LabVIEW interface. Reproduced with permission of Royal Society of Chemistry from Ref. [47].

interdigitated microelectrodes in less than 12 h and validated the results with traditional cell viability assay [58].

All the investigations above have given the 3D cell culture microfluidic devices a great prospect in the pharmaceutical industry given its ability of emulating the physiological behaviour.

2.2. Cell-based drug screening on droplet microfluidics

.Unlike continuous-flow microfluidics, droplet-based microfluidics focuses on generating and manipulating discrete droplets with the use of immiscible multiphase flows inside the microchannels [61–63]. This method allows for manipulating each droplet as a microreactor which can be transported, mixed and analysed, individually [64,65]. With the advantage of parallel processing and experimentation, droplet-based microfluidics has the potential of increasing throughput and scalability without increasing the size or complexity of the microdevices. In recent years, droplet-based microfluidics has been used in many research fields, such as materials synthesis [66–69], bio-chemical analysis [70–73] and biomedical applications [74–77]. As in the drug screening field, many new techniques, such as drug concentration generator, high-throughput drug screening, single cell drug screening and 3D cell-culture-based drug screening have emerged to promote drug discovery.

2.2.1. Generation of concentration gradients for dose-response studies

By changing the relative flow rates of samples and buffers continuously, the concentration gradients of drugs can be easily generated on droplet-based microfluidics. 7–10 drug concentration data points were typically chosen in the primary screening stage [78,79]. Also, this can be done with more than one drug.

Cao et al. generated 1150 distinct mixtures of ampicillin, captopril and caffeine, and tested their effects on the growth of *E. coli*. The dose response study not only enabled the identification of the optimal antibiotic concentration and mixture, but also revealed the antagonistic effects between these compounds [80]. However, the drug toxicity value was quite sensitive to the data quality, considering the non-duplicated data points and four adjustable non-linear parameters [81]. To increase the data points, Yager's group reported a rapid microfluidic method for measuring enzyme inhibition. The assay utilised a two-inlet microfluidic device. One inlet was pumped with the substrate, while the other was pumped with the same concentration of the substrate plus the inhibitor. When the two fluid streams travelled through the *y* dimension of the device, an inhibitor gradient was formed in the *x* dimension *via* diffusion. A fluorogenic or chromogenic substrate was used to make the production of enzymatic activity visible. After inhibition with various gradients of the encountered immobilised enzyme on one surface of the microchannel, different fluorescent signals lit up at different locations along the width of the device [72]. Due to the large amount of data collected, this method was more accurate and reproducible. Miller's group also developed a system with a high-throughput for the precise measurement of drug dose-response relationships. The system (Fig. 4) exploited the Taylor-Aris dispersion to create 10,000 drug concentrations through the combination of continuous and droplet-based microfluidic systems [82].

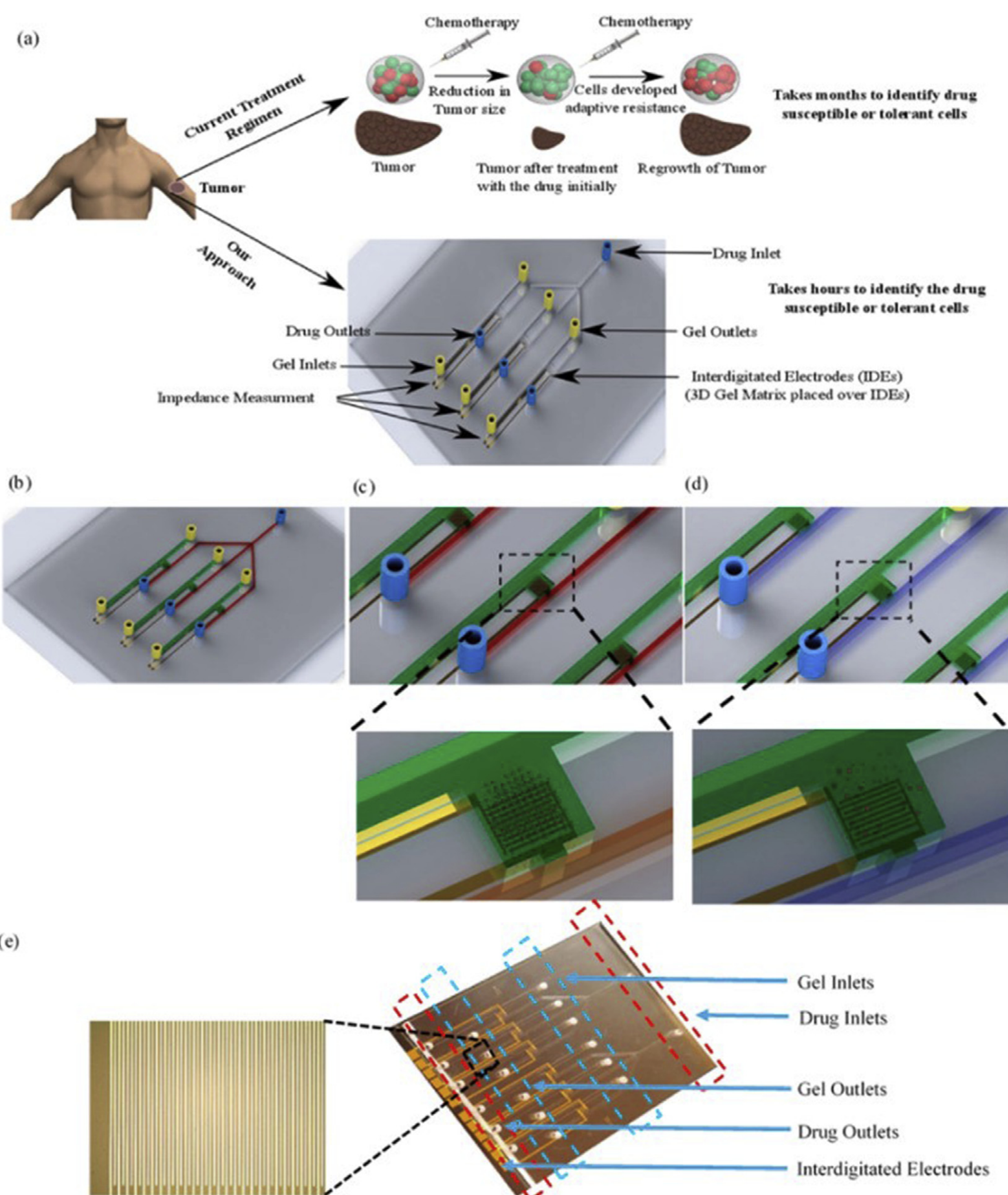

Fig. 3. Lab-on-a-Chip for chemotherapy drug response screening. (a) 3D schematic of the presented platform for multiplex drug susceptibility testing, (b) Cancer cells are placed in a 3D matrix in a separate microchannel (green) that is connected to a parallel microchannel for drug flow (red), (c) Cancer cells are intact and viable in the 3D gel structure and before the introduction of drugs. In the presence of effective drug flow, the cancer cells respond (lyse), (d) The number of intact cancer cells reduced after introducing effective drug flow in the chip, (e) Image of a fabricated device. The chip involves interdigitated electrodes with 10 μm width and spacing. Reproduced with permission of Elsevier from Ref. [58].

2.2.2. Single-cell drug screening on droplet microfluidics

Due to the cell heterogeneity, different cells may have a different response to a certain drug. This raises a high demand in single-cell drug screening. Brouzes's group developed an integrated droplet microfluidic platform that enables high-throughput single cell drug screening [83]. On this platform, the drug concentration library was emulsified with a unique fluorescent label and combined with droplets containing single cells. The combined emulsion (containing a single cell and a drug with a certain concentration) were incubated for 24 h. It was then reinjected into a second device to merge with the droplets containing cell viability fluorescent dyes and incubated on a chip briefly before fluorescence analysis. The cytotoxicity study of the chip showed consistent results with those obtained using traditional methods. Bithi et al. developed a microfluidic platform integrated with pipettes to create cell-laden nanoliter-sized droplets arrays for the analysis of single-cell and cell clusters response to chemotherapy drug doxorubicin [84].

To expand the applications of single-cell drug screening in different fields, Baret et al. developed a droplet-based screening platform for the gene assay. Different concentrations of 20-hydroxyecdysone (a naturally ecdysteroid hormone) were generated by on-chip dilution and were encoded with a fluorescent label, to mix with single *Bombyx mori* cell. The study enabled a concurrent analysis of thousands of single cells with 10 fluorescent-labelled hormone concentrations [85]. For high-throughput screening, Clausell-Tormos et al. combined an auto-sampler and a tree-shaped splitter on-chip, raising the screening conditions up to 100 on an enzyme inhibition assay. In this study, fluid plugs with

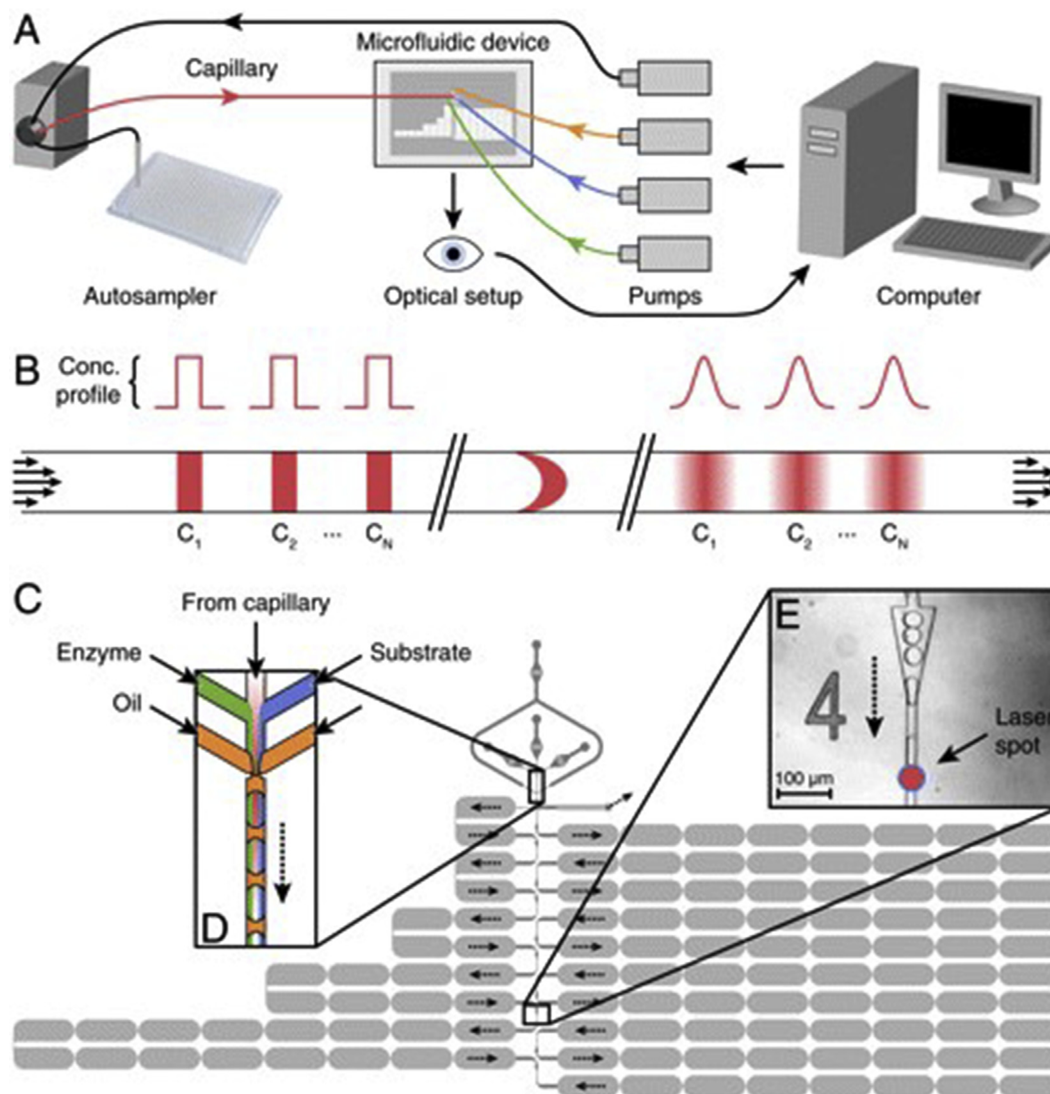

Fig. 4. The microfluidic screening system. (A) The overview of the system. (B) Schematic showing the injections of different compounds sequentially transformed to smooth pulses by the Taylor-Aris dispersion. (C) The design of the microfluidic channel. (D) Graphic illustration of the droplet generation region on the device. (E) Optical micrograph of one of the 10 analysis points. Reproduced with permission of Proceedings of the National Academy of Sciences of the United States of America from Ref. [82].

different inhibitor concentrations were obtained from a prepared 96-well plate, by the auto-sampler, and were split in the tree-shaped splitter on-chip. Then they were merged with the enzyme and its fluorogenic substrate. After incubation, the plugs were analysed by measuring the fluorescence, for the 96 inhibition curves [71].

2.2.3. 3D cell culture-based drug screening on droplet microfluidics

Considering that the cells are affected by the surrounding extracellular matrix and have interactions with cells and stroma [86], various 3D cell culture platforms have been developed in droplet microfluidics to simulate the microenvironment *in vivo* and to obtain more information [87–93]. In a study by Yu et al. [91], alginate beads encapsulated breast tumour cells were trapped in micro-sieve structures for spheroid formation over several days in a continuous perfusion system. An anticancer drug, doxorubicin, was used as a model to explore the cell toxicity effects towards the formed tumour spheroids. The results suggest that cells in the tumour spheroids had a higher resistance than those cultured in the monolayer manner. However, the size and morphology of the tumour spheroids formed in this method was not very uniform. Utilising matrigel and alginate mixed hydrogel beads, Wang et al. realised a uniform size distribution of multicellular tumour spheroids and tested it on the drug sensitivity of HeLa cells' to vincristine [92].

For the applications of the preclinical drug and therapeutic testing, a method for the generation of high-throughput hydrogel-based multicellular tumour spheroids is needed. Sabhachandani et al. developed a droplet-based microfluidic platform for generating up to 1000 multicellular spheroids. Using this platform, they encapsulated MCF-7 breast cancer cells sensitive, or resistant, to doxorubicin (Dox) in alginate to perform the formation of multicellular tumour spheroids. They also co-encapsulated stromal fibroblasts and tumour cells in the spheroids, which were further exposed to a series of Dox concentrations or a drug combination of paclitaxel (PCT) and Dox, to explore the drug screening on this platform. This platform not only permitted large-scaled spheroids to be processed simultaneously but also observed the spatio-temporal dynamics in each spheroid given the known locations of the entrapment of the spheroids [93]. To establish a more

physiologically relevant tumour model for drug testing considering that tumour is consisted of core tumour cell sand the shell dibroblast cells, Sun et al. reconstituted core (tumour cells)–shell (stromal fibroblast) tumour structures with uniform size and demonstrated similar drug resistance as those prepared with traditional methods [94]. To simplify the system set up, decrease the cost and make the operation flexibly. Zhao et al. developed a droplet-based 3D cell culture method with capillary-based liquid handling system (Fig. 5) to perform multiple cell-based assay operations and tested the effect of doxorubicin (an anticancer drug) to 3D HepG2 cell spheroids [95].

2.3. Cell-based drug screening on digital microfluidics

Other than handling the droplets in the channels, digital microfluidics manipulates discrete microdroplets on the chip surfaces by the electric control of the electrode arrays. The mechanism behind this technology is electrowetting-on-dielectric (EWOD), which changes the interfacial tension between the droplet and the dielectric layer, therefore, changing the contact angle of the drop when an electric field was applied across the droplet [96]. With the advantage of having each droplet isolated from its surroundings, easy intelligent control with real-time feedback and automatic analysis [97], digital microfluidics has been used for POCT diagnostics [98,99] and many other biomedical applications [100–104].

For cell-based drug screening, Bogojevic et al. developed a DMF platform investigating the cell toxicity effects by monitoring cell apoptosis [105]. The authors seeded and cultured HeLa cells on the DMF chip overnight. They then incubated them with a series doses of staurosporine (a cytotoxic agent) and determined the degree of cell apoptosis by capase-3 activity (an apoptosis marker to indicate the activity by the fluorescence emitted from its substrate). The results demonstrated a larger dynamic range and approximately a 33-fold lower reagent consumption on the digital microfluidic chip compared with conventional methods. Wheeler's group also developed a digital microfluidic system that can generate arrays of hepatic organoids using co-cultures of NIH-3T3 and HepG2 cells in hydrogel matrices [106]. In this study, they loaded 6.0 μl of collagen–cell suspensions onto the reservoirs on-chip. Droplets dispensed from the reservoirs formed organoids over an hour of incubation. Using a general droplet exchange procedure, the hepatic organoids were “fed” with the culture media to explore the toxicity effects under a series of concentrations of acetaminophen. Another digital microfluidic platform was developed by Aijian et al. to promote the formation and automatic analysis of multicellular spheroids in a hanging drop culture [107]. Using this digital microfluidic platform, a drug screening model (insulin-induced drug resistance) was carried out on human colorectal adenocarcinoma spheroids. All of these studies have promoted automatic cell spheroid culture and analysis on digital microfluidics.

2.4. Cell-based drug screening on paper microfluidics

Paper-based microfluidic devices are typically composed of a series of nitrocellulose fibres [108,109] or hydrophilic cellulose [110,111] to guide the liquid from an inlet to a certain outlet by imbibition. Paper-based microfluidic devices can be manufactured by technologies such as wax printing [112,113], photolithography [114,115] or plasma treatment [116], etc. Due to its extremely low cost, flexibility, lightness and low thickness, paper-based microfluidics has been used in many research fields, such as point-of-care disease diagnostics [117–119], food inspection [120–122] and environmental monitoring [123–125].

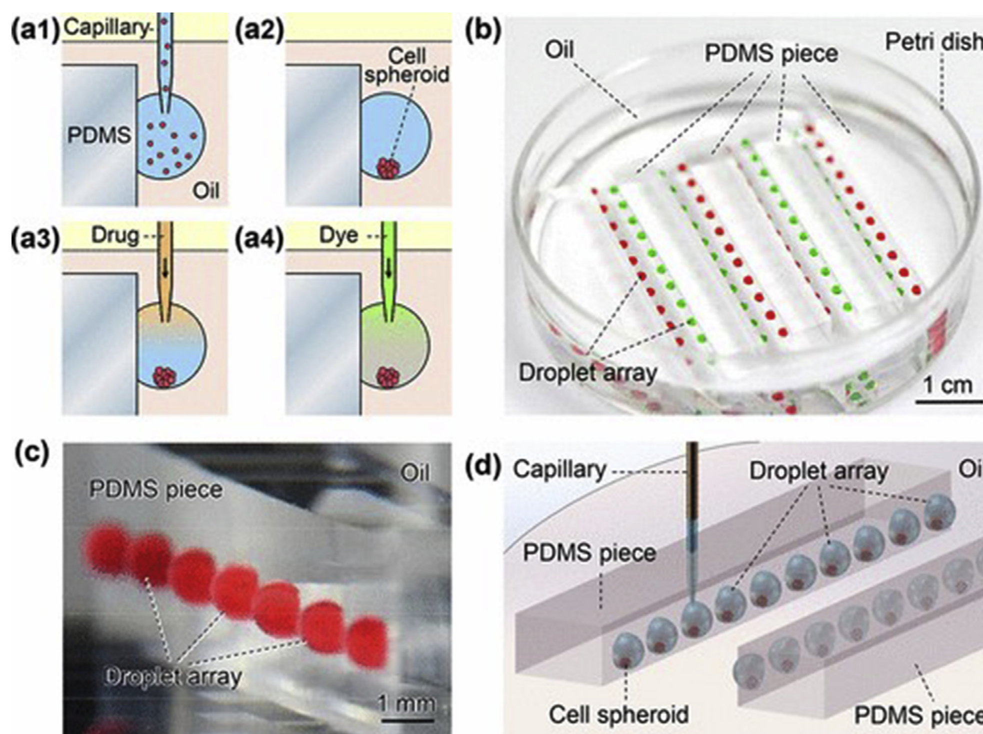

Fig. 5. (a) Illustration of cell culture and drug testing process in the present sidewall-attached droplet array: (1) Cell seeding; (2) Cell spheroid formation in a droplet; (3) Drug addition; (4) Fluorescence dye addition to stain the cell spheroid. (b) Photograph of a sidewall-attached droplet array with red and green dye droplets. (c) Detail view of red dye droplets array attached on the PDMS sidewall. (d) Schematic diagram of cell spheroids forming in a sidewall-attached droplet array system. Reproduced with permission of American Chemical Society from Ref. [95].

A 3D cell culture on the microfluidics has been reported [126,127]. Chen et al. designed paper chips for the 2D and 3D cell culture for the drug toxicity tests [128]. The paper chips were made of polycarbonate scaffolds, which can promote cell suspension to seed and to be held there easily. HepG2 cells and fibroblasts were assembled by layers for the 3D cell culture. Based on the designed paper device, they carried out toxicity testing of various anticancer drug concentrations on the HepG2 cells. Inspired by the 3D cell culture of the paper microfluidics, Hong et al. developed an integrated paper microfluidic device for high-throughput drug screening [129]. A drug dilution generator was constructed on the device to generate a series of concentrations of the drug diffused model through the paper fibres. HeLa cells were cultured in a collagen hydrogel filled microarray as a 3D scaffold. After the HeLa cells were exposed to the drug for 8 h, they evaluated the cell viability and found that the drug inhibition efficiency was positively correlated with the drug concentration. Lei et al. also developed a simple high throughput drug screening platform on paper microfluidics by designing microwells on the paper substrate [130]. Two human hepatoma cell lines encapsulated in the hydrogel were cultured in the microwells and immersed in the drug containing culture medium for 2 days and then the cell viability were quantified by impedance measurement with the paper substrate placed on the measurement electrodes. The results disclosed different drug responses of the two cell lines, as shown in Fig. 6.

The paper microfluidics was also integrated with the electrochemical device [131] for inexpensive, fast and sensitive early cancer detection [132]. In this device, aptamers (oligonucleotide or peptide molecules that bind to a specific target molecule) were modified on the 3D microporous Au-paper for specific cancer cell capture and the sequential 3D cell culture. The results demonstrated that the device was able to detect living cancer cells with specificity, sensitivity and a low detection limit in a broad linear range.

3. Discussion and outlook

For drug screening, various models have been involved, either *in vitro* or *in vivo*. The models used for drug screening include protein structure-based (computer aided drug screening), cells, animals, organs and body (organs and body are referred in microfluidics field). The performance of these target-based drug screening is summarised in Table 1. The process from drug discovery to appear in the market is time-consuming and complicated since cytotoxicity, efficacy and side effects of the new drugs should be validated in each step to ensure reliability of the results. In order to obtain expected therapeutic effects on humans, ideally, drug screening tests should be performed within vivo models. Animal models, organ and body on chip are all the representatives of vivo models. However, these models are expensive and time-consuming. Organelle-based screening has its advantages of simulating the microenvironment in bodies. Nevertheless, after screening biochemical analysis is difficult to perform with those 3D structured organelle. As such, cell-based drug screening prior to vivo models help to eliminate and select from thousands of candidates, thus a lot of time and expense will be saved.

In the past few years, great progress has been made in cell-based drug screening. Among the progress, utilising cells and further constructing multicellular spheroids even organ-on a chip from cancer patients' biopsies for drug screening is a great breakthrough compared to the traditional used commercial cell lines. The operation with physiologically relevant patients' three-dimensional (3D) tumour models will promote the development of personalized medicine.

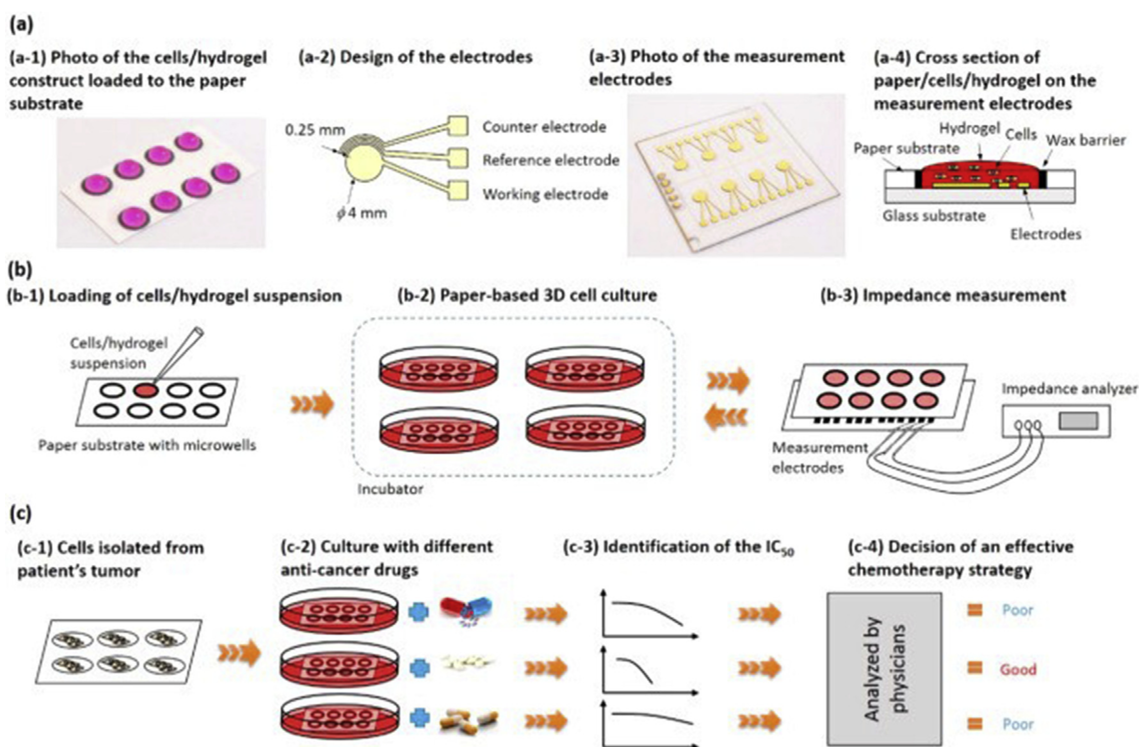

Fig. 6. Flow diagram of the paper-based cell culture and quantification platform. (a-1) Photo of the cells/hydrogel construct loaded to the microwells of the paper substrate. (a-2) The designed electrodes. (a-3) Photo of the glass substrate with the measurement electrodes. (a-4) Cross section of paper/cells/hydrogel on the measurement electrodes. (b) Experimental procedure of the impedimetric screening of drug sensitivity of cells suspended in the hydrogel. (c) Assessment of the chemosensitivity of the cells isolated from the patient's tissue and decision of an effective chemotherapy strategy. Reproduced with permission of Elsevier from Ref. [130].

Table 2
The performance of different microfluidic platforms for cell-based drug screening.

Microtluidic platforms	Continuous-flow- platforms based microfluidies	Droplet microfluidics	Digital micro fluidics	Paper microfluidics
Multi-step liquid handling	√	×	√	√
High-throughput Screening	√	√	–	–
Single cell drug screening	×	√	–	–
3D cell-based drug screening	√	√	√	√
Low cost	×	×	×	√
Flexibility	×	×	×	√

(√ represents yes, × represents 110, - represents no literature reported).

In this review, we summarised cell-based drug screening based on several main platforms of microfluidics: continuous-flow based microfluidics, droplet microfluidics, digital microfluidics and paper microfluidics. The performance of each platform is summarised in Table 2.

The ultimate goal of cell-based drug screening on microfluidics in research field is to promote its applications even replace the traditional well plate-based methods in pharmaceutical industry. Up to now, with the superiority of high-throughput experiments, continuous flow and droplet microfluidics are the most potential platforms for applications in pharmaceutical industry. However, many issues should be addressed before mass production. The limitations for the referred two platforms include the following aspects. First, high-throughput microfluidics chips preparation needs to be realised. Photolithography is the most widely used method for chip preparation nowadays. However, it's low-throughput, time-consuming and expensive. 3D printing may be a potential solution to resolve the throughput issue. Before realising chips mass production via 3D printing, printing resolution, the biocompatibility of materials with the cells and material compatibility with drugs need to be improved. Second, automating the whole process needs to be implemented. Sample waste is a big problem during drug screening. If the whole process can be automated and the samples can be prepared on demand, then a lot of money and labours can be saved. It's also challenging for automatic data analysis. The common method for data analysis is imaging then analyzing by imaging software, which is time consuming. Intelligent analysis would be better for the promotion and popularization of microfluidics. Additionally, specific matters should be settled as to droplet microfluidics, such as drug delivery into droplets and cell growth inside droplets. For industry applications, thousands of compounds are screening for drug candidates. Therefore, high throughput is a necessary standard for any techniques considering for commercialization. For digital microfluidics, there are few reports about high throughput applications, puts it as an investigation topic for future work. Other issues include integrating data acquisition and analysis system on the chip. For paper microfluidics, the major advantages of extremely low cost and easy operation promote their availability even at resource-limited countries. However, some problems hinder their application in pharmaceutical industry. Fluorescent imaging is the common method for drug screening results analysis on microfluidics chip. The auto-fluorescence interference from paper substrates causes inaccuracy for either quantitation or semi-quantitation analysis which needs to be overcome. In addition, integration with sensitive and easy-to-operate detection methods with the paper substrate also should be overcome.

In the future, some new potential research directions can be tried to further promote the applications of microfluidics in drug related field. (1) Currently, almost all of the microfluidic platforms depend on the large detection instruments to realise the subsequent results acquisition and data analysis. It is very important to integrate detector and data analysis systems into the microfluidic devices. (2) Droplet microfluidics is the most investigated method to achieve drug screening throughput up to now. However, multi-step liquid handling is difficult to realise in droplet microfluidics. It will be more applicable if it can be integrated with other techniques, such as digital microfluidics. (3) Single cell drug screening can help to identify the cells in the heterogeneous population and provide guidance to precision medicine. Droplet microfluidics has shown a good performance in realising single cell drug screening. Single cell drug screening on other microfluidic platforms, such as digital microfluidics, is also prospective and can be investigated. With no doubt, microfluidics will play more and more roles in cell analysis and drug screening in the future. With no doubt, microfluidics will play more and more roles in cell analysis and drug screening in the future.

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