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Recent advances in microfluidics for drug screening

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ABSTRACT

With ever increasing drug resistance and emergence of new diseases, demand for new drug development is at an unprecedented urgency. This fact has led to extensive recent efforts to develop new drugs and novel techniques for efficient drug screening. However, new drug development is commonly hindered by cost and time span. Thus, developing more accessible, cost-effective methods for drug screening is necessary. Compared with conventional drug screening methods, a microfluidic-based system has superior advantages in sample consumption, reaction time, and cost of the operation. In this paper, the advantages of microfluidic technology in drug screening as well as the critical factors for device design are described. The strategies and applications of microfluidics for drug screening are reviewed. Moreover, current limitations and future prospects for a drug screening microdevice are also discussed.

I. INTRODUCTION

The recent development of a wide variety of drugs improves the quality and prolongs the life of humankind. Pharmaceuticals continue to help us fight against infections, treat diseases, and alleviate afflictions. In addition, the development of new and effective drugs promotes drastic healthcare cost reductions.[1] Problematically, growing drug resistance is driving the need to constantly develop new drugs. However, the development of new, efficient drugs entails a tedious process, in which millions of dollars are spent over a period of more than a decade.[2]

The fundamental aims of the drug development process are to maximize bioavailability and specificity of its active ingredient, while minimizing the side effects from toxicity to the metabolic system. Among drug discovery approaches, target-based biochemical screening[3–5] and phenotypic cell-based screening are widely adopted to identify the therapeutic effects of a drug. In target-based screening, purified proteins are used to measure the engagement of a drug with its target.[6] However, on a cellular level, the relevance of these proteins is questionable. In cell-based screening, changes in cell physiology during drug delivery are monitored without validating the drug's target or identifying the mechanism of action. Due to the need for repeated testing, these tests are iterated, which renders the discovery of a new drug a daunting and lengthy challenge, taking over a decade in most cases with a failure rate of 95%.[7]

Animal models play a vital role in drug screening and development. However, due to rising ethical concerns, the scientific community is decreasing its use of animal testing. As an alternative, human derived cells are used for *in vitro* tests, which act as an effective means for preliminary screening. Conventionally, drug screening is carried out on simple platforms, such as 96 well plates. However, this methodology requires time-consuming manual labor. In addition, the cells cultured in a semistatic environment do not accurately imitate the cellular microenvironment. Hence, an *in vitro* drug's response is seldom representative of the human body.[8] Recent developments to quicken high-throughput screening include automated machinery, data processing software, and sensitive detectors for pharmacological tests. To reduce time and cost and improve throughput, microtiter plates of 384, 1536, 3854, and even 9600 wells have been introduced. Although this decreases the amount of reagent used by many folds, problems associated with these miniaturized setups arise: the uncontrolled liquid evaporation and limitation of low volume technologies.[9] Another drawback of the current drug development technique is its use of monolayer cell cultures, in opposition to the 3D cell structures present *in vivo*. The treatment results of the drug in later steps (i.e., clinical stage of drug development) are affected by the differences between the *in vitro* 2D cell culture models and the *in vivo* 3D cell structures.[10] New technologies that allow drug testing in 3D structures are thereby necessary. The above-mentioned limitations can be overcome with the use of microfluidic technology.

Microfluidic chip technology developed in recent years has become an ideal choice for drug screening at the cellular level.[11] Microfluidic devices are composed of microstructures, which closely mimic the extracellular environment and provide flexible adjustment of drug concentration.[12] Furthermore, they allow easy serial dilutions and thus help in monitoring the dose-response of a drug, as microfluidics easily generate laminar flow, by minimizing cell damage caused by fluid turbulence.[13] Microfluidic devices are automated and multiplexed, where cells are simultaneously cultured on the same chip, thereby enabling biochemical tests without the use of many reagents.[14]

II. ADVANTAGES OF MICROFLUIDIC-BASED DRUG SCREENING DEVICES

Microfluidic devices provide an ideal controlled environment for cell culture and drug testing on a wide variety of cells, such as cancer, hepatic, and bacteria.[15] This controlled environment allows the monitoring of cell cultures over long periods of time.[16] Moreover, microfluidic devices are able to isolate single cells for further culturing. Normally, there is a broad heterogenic response among same-type cells when reacting to a specific drug. However, this heterogeneity cannot be observed in traditional drug screening methods, since the response information is an average of all cells. The use of microfluidic chips enables the analysis of single cell's antidrug response. Microfluidic chips are also used to analyze drug response of a cancer cell line with different phenotypes,[17] showing a clear distinction in drug response as a function of a specific phenotype. This ability to discern different responses of a same cell line against the same component provides valuable information for the development of new drugs.

Microfluidic devices can be adapted for specific applications. Therefore, they provide a platform to study 3D structures of cells. The more semblance an *in vitro* sample bears to the *in vivo* sample it is based on, the more representative the results are. In the form of organoids, different types of cells, such as hepatic[18] or tumor cells,[10] can be cultured within microfluidic devices, thereby performing drug screening in a more reliable environment. This increase in similarity with *in vivo* circumstances provides more accurate data, which lead to an increased efficiency in drug testing. Nevertheless, methods that apply 3D-structured cell cultures are not as common as expected due to their complex building and manipulation required. However, Au *et al.*[19] demonstrated that digital microfluidics[20] provides the needed tools for easy manipulation and drug screening of hepatic organoids, thereby also demonstrating the potential of microfluidic devices in drug screening at an earlier stage.

In an *in vivo* environment, the concentration gradient of biomolecules regulates cell behavior, such as cell growth and differentiation. Studies show that there is a close relationship between the concentration of cytokines and the invasion and metastasis of cancer cells.[21] Such characteristics are also used to develop anticancer drugs. Traditional drug concentration gradient experiments are primarily performed in multiwell plates. With the advancement of microfluidic technology and wide applications of microchips in biological research, concentration gradient technology based on microfluidic control principles is being vigorously developed, which can save time, energy, and money. The microfluidic device can be used to realize high-throughput drug screening, simulate *in vivo* environment, and improve screening efficiency. In summary, there are several advantages of manufacturing a concentration gradient for drug screening in microfluidic chips.[22] First, a wider concentration range can be generated, and the concentration gradient is spatially more finely divided. Second, the gradient environment is more stable, allowing real-time monitoring. Third, the gradient can change dynamically over time to meet different needs. In addition, microfluidic devices integrate various components for a low cost, high throughput, short amount of time, and reproducible drug screening. Compared with drug tests using animals, a microfluidic system reduces animal death and avoids ethical issues.

Another key approach for the use of microfluidic devices for drug screening is the development of combination therapies. Empirically, some drugs work better when in tandem with other drugs rather than isolated.[23,24] Ascertaining the proper amount and type of drug is thereby essential to develop efficient combination therapies. Microfluidic chips can realize this function by using its special fluid properties. Drug screening devices have been reported for the determination of potential concentrations and combinations of multiple reagents. Kim *et al.*[25] proposed a device composed of 64 isolated cell culture chambers. In each, different concentrations of two drugs were used as input. Within a single microfluidic chip, 64 different cell-drug reactions are screened, not only reducing the costs but also increasing the time efficiency of testing.

III. DESIGN OF MICROFLUIDIC-BASED DRUG SCREENIG DEVICES

The development of microelectromechanical systems and biochemical analysis systems in micromachining technology provides the basis for the application of microfluidics in chemical and biochemical analysis, specifically with sensors for detection.[26,27] With the rapid development of genomics, proteomics, combinatorial chemistry, and bioinformatics, a large number of medicinal compounds are discovered. These drugs require an appropriate analytical protocol to effectively screen and validate their efficacy, cytotoxicity, and potential side effects during the initial stages of drug discovery.[28] Drug screening requires the use of *in vivo* animal models, but due to its high cost, low throughput, and long analysis time, microfluidic systems are increasingly used to simulate *in vivo* conditions, such as chip tissue and chip organs.[29] The primary task of drug screening using a microfluidic system is to design and manufacture microfluidic devices based on the desired target's function.[30]

A. Critical factors on designing a microfluidic-based drug screening system

To design a microfluidic-based drug screening system, cell culture, which involves the maintenance and growth of cells in a controlled laboratory environment, should be considered.[31,32] Critical factors on designing a microfluidic cell culture platform include biocompatible materials and surface modifications.[33,34] Biocompatible materials can simply be understood as materials that do not harm living tissue.

1. Manufacturing process and biocompatible materials

The earliest substrates of microfluidic devices are silicon and glass, which can be fabricated by standard photolithography, surface micromachining, bulk micromachining, laser direct writing, and chemical etching.[35] Inorganic materials, such as silicon and glass, have high chemical stability, great strength, and thermal conductivity. However, the opaqueness of silicon and the cost of glass materials limit their wide adaptation to microfluidic devices.[36] Versatile, reproducible, and cheap polymers are developed as an alternative material. From the late 1990s, substantial fabrication methods, such as injection molding, hot embossing, soft-lithography, direct laser plotting, laser photoablation, X-ray lithography, and 3D printing, are generated based on polymers.[36] In the past two decades, polydimethylsiloxane (PDMS) became the most popular substrate in the microfluidic device.[37] PDMS is a polymer that is transparent, elastomeric, chemically inert, thermally stable, biocompatible, flexible, permeable, and UV resistant, making it preferable for analytic chemistry and biological applications. Soft photolithography, which was introduced by Xia and Whitesides in the late 1990s, is widely used for rapid prototyping of a PDMS device due to its low cost and fast process.[38]

In addition to silicon elastomers, microfluidic chips can also be created from thermosets (e.g., SU-8), thermoplastics (e.g., PMMA, PA), gels, and papers. Compared to glass, silicon, PMMA, and PA, PDMS has good biocompatibility, high gas permeability, a hydrophobic surface, and excellent optical transparency, which facilitates gas exchange, cell adhesion, and real-time observation of the cell culture. However, the hydrophobicity of PDMS leads to nonspecific absorption of drugs or biomolecules that are present within a biological environment. In addition, microfluidics can simulate the cellular biological microenvironment, which allow cell cultures to be transformed from 2D to 3D. Based on the complex microstructure design and precise fluid control, microfluidic technology provides a powerful research platform for 3D cell cultures. The platform helps to maintain physiologically relevant cell morphology and phenotypes, providing important techniques and tools for cellular metabolic activity and function research.[39] Advances in hydrogel materials have facilitated the development of more physiologically relevant 3D cell culture techniques. Figure 1 illustrates an overview of materials for microfluidic chip fabrication.[40]

2. 3D cell culture

The most common strategy for 3D cell culture is to enclose cells in 3D hydrogel materials, such as collagen, agarose, and various synthetic hydrogels. Hydrogels mimic the interaction between cells and the matrix. With good permeability, hydrogels enable diffusion and exchange of oxygen, nutrients, and metabolites to maintain cell growth and function. In 2013, Eun *et al.*[41] developed a microfluidic chip platform to study the effects of human mammary fibroblasts (HMFs) on the infiltration and metastasis of breast cancer cells (MCF-DCIS) in both 2D and 3D culture conditions. The authors wrapped MCF-DCIS in a mixture of collagen and matrigel, and cocultured it with HMF, which grew in a 3D matrix and on the surface of a 2D matrix. The results show that HMF with the 3D culture produced more paracrine signal molecules and promoted the invasive growth of breast cancer cells by enhancing the interaction

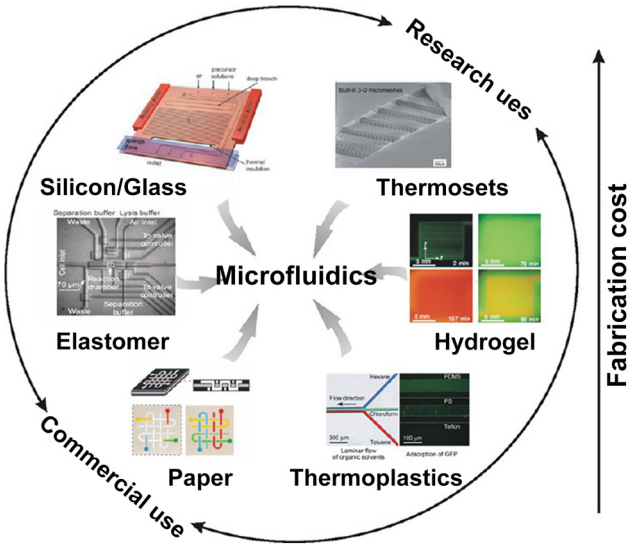

FIG. 1. Microfluidic chip fabrication materials. Reproduced with permission from Ren *et al.*, Acc. Chem. Res. **46**, 11, 2396–2406 (2013). Copyright 2013 American Chemical Society.

of HGF/c-Met. In 2016, Chen *et al.*[42] used the high-throughput microfluidic chip droplet platform to form a large number of monodispersed 3D liver micromodels via assembling hepatocytes and fibroblasts in a nuclear-shell hydrogel scaffold.

B. Drug screening devices

In recent years, microfluidic-based drug screening platforms are rapidly developing.[43,44] Latest designs for drug screening microfluidic platforms include concentration gradient-based microfluidic devices,[45,46] droplet microfluidic-based devices,[47] and other methods,[48] such as SlipChip, printer-based chip, and electrowetting-based chip.[49]

1. Continuous flow, diffusion, and concentration gradient-based microfluidic devices

One major advantage of using a microfluidic device to screen drugs is its ability to conduct high-throughput analysis or high content analysis. In conventional methods, one flask or one animal model can only test the property of one compound with a fixed concentration. However, simultaneous multiparametric measurement of cellular responses could be performed with the concentration gradient generator followed by a series of cell culture chambers [Fig. 2(a)].[50] This microfluidic device is capable of simultaneously testing 64 parallel samples. By controlling the flow rate of the medium and the drug solution, two aqueous solutions are mixed in different ratios, thus diluting the drugs to produce 8 different concentration levels. The activity and toxicity of each concentration are tested in the downstream cell culture chamber. The concentration gradient generator presents an excellent capability to generate well-defined concentration gradients. The relative standard deviation of

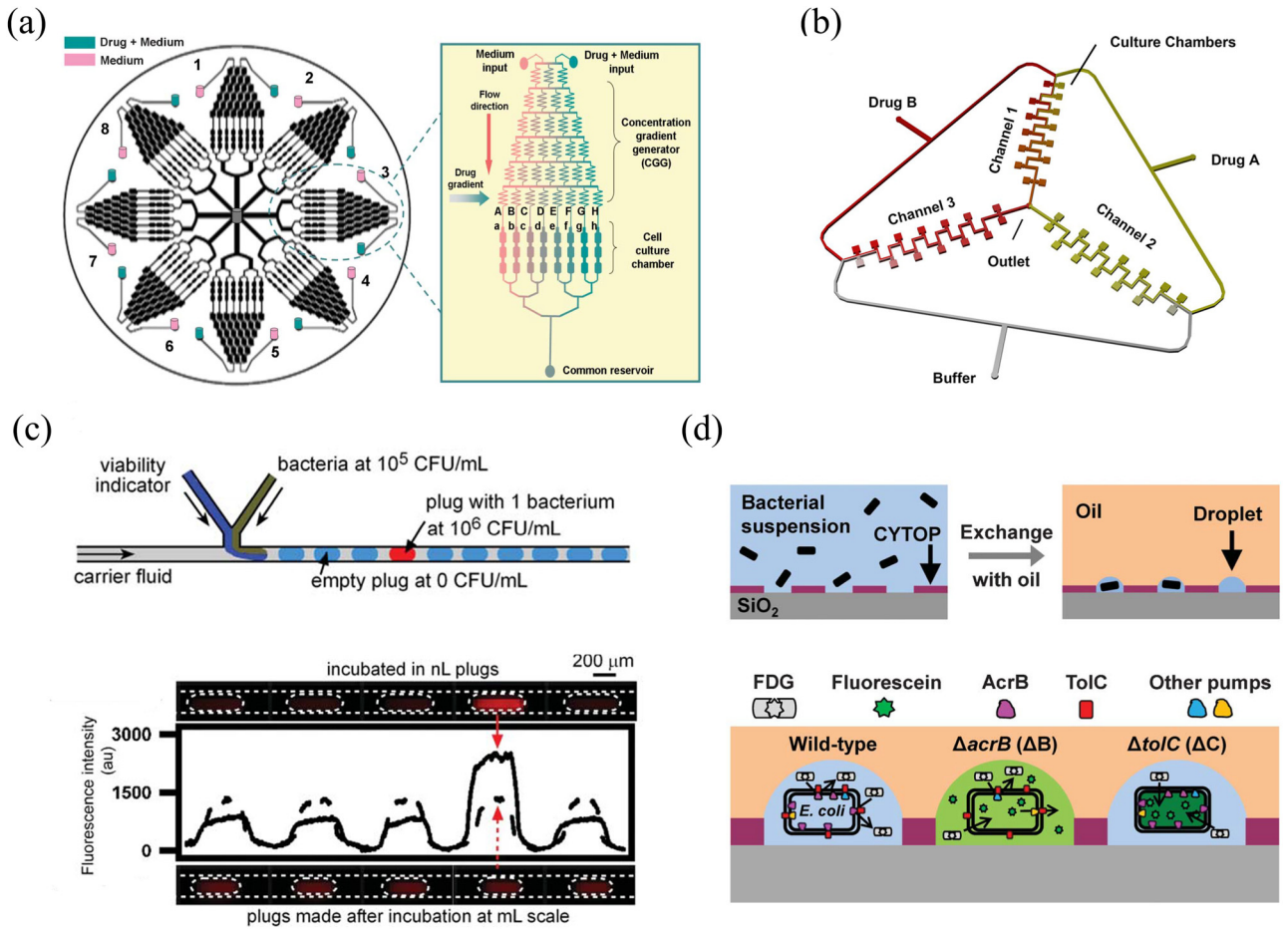

FIG. 2. Schematic of concentration gradient generator- and droplet-based drug screening microfluidic platforms. (a) High-content drug screening microfluidics. This chip consists of eight uniform structure units. Reproduced with permission from Ye *et al.*, Lab Chip **7**, 1696–1704 (2007). Copyright 2007 Royal Society of Chemistry. (b) Schematic of the technique for microfluidic-based drug concentration gradient generator. Reproduced with permission from Sun *et al.*, Microfluidics Nanofluidics **21**, 125 (2017). Copyright 2017 Springer Nature. (c) Schematic of the bacteria susceptibility test using the T-junction droplet generation structure and fluorescent readout of viability indicator in the droplet drug screening system. Reproduced with permission from Boedicker *et al.*, Lab Chip **8**, 1265–1272 (2008). Copyright 2008 Royal Society of Chemistry. (d) Schematic of the droplet generation using the hydrophilic-in-hydrophobic micropattern. Reproduced with permission from Iino *et al.*, Lab Chip **12**, 3923–3929 (2012). Copyright 2012 Royal Society of Chemistry.

different channels is less than 6%, which demonstrates the feasibility of the device to conduct high content screening.

Sun *et al.*[51] developed a low cost, convenient, and accurate optimal combinatorial drug screening microfluidic platform [Fig. 2(b)]. This chip contains cell culture with a microcolumn and three laminar diffusion channels. This combination generates a gradient concentration of two clinically relevant drugs in 90 μl/h optimal velocity, identifying the best combination of two drugs and to aid the effective screening of antitumor drug combinations. Seidi *et al.*[52] proposed a method for rapidly generating a 6-hydroxydopamine concentration gradient in a microfluidic device. The cells are cultured in a microfluidic channel and repeatedly moved back and forth to the solution to produce a neurotoxic concentration gradient. Although this method enables the analysis of a continuous concentration process, unexpected concentration diffusions can occur, causing a difference between the actual and desired concentration.

The advantage of this technology is the activity, and the toxicity of drugs with different concentrations can be tested using a highly integrated microfluidic device. This ability makes it especially suitable for high content screening and high-throughput screening. The disadvantage is that external instruments are required to assist with sample injection and mixing. Also, the mixing process is based on a diffusion mechanism highly related to external conditions, such as pressure or temperature. Although diffusion can be controlled in microfluidic devices, the mixing process could be unstable. One important characteristic of concentration gradient generator-based drug screening system is that the result is based on an average

performance of all the cells in one chamber, which can be used to analyze the response of organoids or tissues to different drugs. The result is more accurate with cell-cell interaction or the presence of the extracellular matrix.

2. Droplet-based microfluidic devices

Droplet formation is an important field in microfluidics, which provides a pathway for performing drug screening.[53] According to the reviews of Zhu and Wang,[54] a variety of device geometries can be used to generate droplets, which range from nanoliters to femtoliters.[55] These droplets can be easily mixed,[56] combined,[57] captured,[58] transported to off-chip incubation[59] or sorted,[60] facilitating reaction and analysis.[30] Boedicker *et al.*[61] developed a microfluidic device that is capable of generating monodispersed aqueous droplets through a classic droplet generation structure [Fig. 2(c)] and tested the susceptibility of *Staphylococcus aureus* toward various antibiotics. In their work, every droplet encapsulated a bacterial viability indicator and drug trials of different components. The fluorescence intensity in a droplet changes when the *S. aureus* is sensitive to its drug. The readout of the droplet-based drug screening method is better than the concentration gradient generator-based screening method in that it only needs to measure the fluorescence intensity to quantify the results [Fig. 2(c)]. Shih *et al.*[62] developed a novel droplet digital microfluidic device which combines microfluidic technology based on droplets in the channel and digital microfluidics. The system also includes other functions, such as the automatic analysis of data. The purpose of this system is to screen the influence of ionic liquids during cell growth and ethanol production.

The traditional T-junction structure is an efficient way to generate monodispersed droplets. However, the size and number of droplets are limited. Iino *et al.*[63] designed a hydrophilic-in-hydrophobic micropattern in a microfluidic device [Fig. 2(d)]. A monodispersed femtoliter droplet array can be generated by injecting an aqueous sample into the chip then wiping away the excessive aqueous phase with fluorinated oil. The biological assay is similar to the previous method.

The advantages of droplet-based drug screening assay include low reagent consumption, good repeatability, fast response time, rapid mixing, precise control of cell concentration, as well as quantifiable readouts with viability indicators. These advantages facilitate the measurement of target's susceptibility to drugs. Nonetheless, droplets would only encapsulate a single or only a few cells, which lack cell-cell interaction. In addition, the process requires external professional pumps, and the formation of stable droplets requires additional handing of oils and surfactants. Therefore, this method requires considerable cost.

3. Slip-driven microfluidic devices

Most conventional microfluidic devices require external pumps to conduct multistep movement. Slip-driven microfluidic devices can manipulate fluid by simple relative movement of two different plates.[64] First, the upper plate and the bottom plate are aligned to form a continuous channel. After the samples are injected into the chip, a slipping movement is introduced and the continuous fluid is separated into many parts.[65,66] This technology does not require external pumps yet still enables multistep movement.

Shen *et al.*[67] developed a SlipChip-based chemotactic screening method and tested the chemotactic migration behavior of bacteria and cells within a given time and the chemoeffector of a certain concentration [Figs. 3(a) and 3(b)]. The chemoeffector, cells, and buffers are injected into three separated continuous channels formed by chained microwells. After a simple slipping movement, the continuous channels are broken up to form a series of isolated reaction chambers that consist of all three parts [Fig. 3(c)]. After the chemotaxis experiment, the experimenter can slip the material back to their original positions and collect the chemotactic cells or bacteria.

The advantage of utilizing SlipChip technology to conduct drug screening is that the cell of interest can be recollected easily for further analysis after the experiment. Also, no additional equipment is required in the experiment, and all operation can be performed with a pipette and the SlipChip itself. The disadvantage of this technology is that the environment inside the glass SlipChip is not suitable for long-term culture, which limits its application in drug screening.

4. Other devices

In general, microfluidics has been used to generate various analytical devices, including concentration gradient-based microfluidic devices, droplet-based devices, and SlipChip. Other devices, such as printer-based chip, digital microfluidics,[68] paper-based microfluidics,[69] microfluidic electrochemical assays,[70] electrowetting-based chip, and 3D printed microfluidics,[71] have the potential to be applied to drug assays as well. Ding *et al.*[72] developed a low cost, high efficiency, and high-throughput microfluidic print to a screen system, which combines printing technology with a screening method in order to screen an effective combination of agents. In this platform, the authors used plug and play microfluidic cartridges to study microfluidic impact printing [Fig. 3(d)]. The droplet volume is controllable and its position precise. The platform has significant advantages, including automated combinational printing, high-throughput parallel drug screening, low cost, and simplification of the experimental process.

At present, paper-based microfluidic devices with low cost and good flexibility are also widely used. It is a device composed of paper fibers prepared by photolithography or plasma treatment, which can guide drug microfluidics through imbibition, where drug diluents are set up on the microfluidic device. By designing a microspore on the paper matrix, the paper fibers produce drug diffusion models of different concentrations. Then, the paper matrix is placed on the measuring electrode and the impedance is measured to analyze the drug effect.[73]

Continuous flow microfluidics and droplet microfluidics are promising drug screening methods. Compared to well plates, they allow biochemical reactions in very low volumes, thereby reducing reagent-related costs while further increasing throughput. Droplets have high-throughput screening capabilities, low reagent consumption, and postdroplet processing. Continuous flow microfluidics can be read quickly and characterized by a variety of different detection methods. However, it is not easy to perform multistep liquid

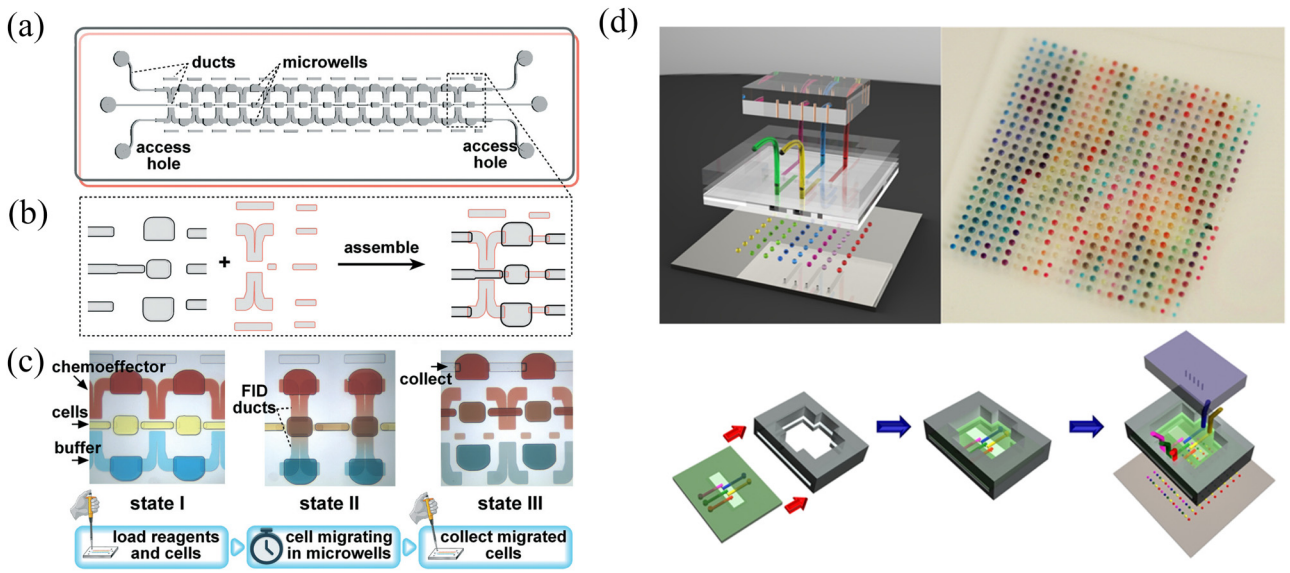

FIG. 3. The operation of slip-driven and printer-based drug screening microfluidic devices. (a) Schematic of the slip-driven device. (b) Zoom-in view of the assembly detail of the top and bottom plate for the slip-driven device. (c) Illustration of sample loading, cell migration, and in cell recollection for the slip-driven device. Reproduced with permission from Shen *et al.*, Lab Chip **14**, 3074–3080 (2014). Copyright 2014 Royal Society of Chemistry. (d) Illustration, prototypes, and plug-and-play assembly of the printer-based drug screening microfluidic device. Reproduced with permission from Ding *et al.*, Anal. Chem. **87**, 20, 10166–10171 (2015). Copyright 2015 American Chemical Society.

treatment in droplets, which makes it difficult to carry out long-term cell culture and limits detection methods. In addition, more surfactants and oil are needed, which means increased cost. Continuous flow microfluidics has low throughput and its sample size is not isolated. In addition, the microfluidic chips must be fabricated. Lithography is the most commonly used preparation method but it has a low throughput and high cost. In the future, 3D printing technology may become an ideal solution. Droplet microfluidics can provide precise drug guidance in drug screening, but multistep and multiconcentration liquid treatment is also difficult to achieve. Collaborations with other microfluidic platforms, such as digital microfluidics, have broad prospects. In drug screening technology, microfluidics is playing an ever-broader role.

IV. APPLICATIONS OF CELL-ON-CHIP DRUG SCREENING SYSTEM

The cell-on-chip drug screening microfluidic system aims to stimulate cells, which are cultured on chips and have different functions, using drugs and have the cells cooperate with automated detection devices to collect data of cell responses. This system achieves the goal of screening different drug ingredients. The basic premise is to fully simulate the microenvironment that is close to what cells interact with in real tissues. In this way, researchers can evaluate new drugs before performing tests with *in vivo* models, which provides an efficient way to screen drugs.

A. Drug activity screening

Researchers have focused on the screening of active pharmaceutical ingredients using cell chips.[74] Wu *et al.*[75] designed a microarray "sandwich" high-throughput screening chip, which delivered compounds or drugs to isolated cell cultures by "sandwiching" the column containing the drugs with the micropores cultivated with the cells [Fig. 4(a)]. By detecting the cell (MCF-7 human breast cancer cell) viability using fluorescence after drug exposure, 9-methoxy-camptothecin, a potential antitumor drug, was screened out. This benchtop cell-based assay provides a fast, low-cost way for screening active pharmaceutical ingredients. Fukuda and Nakazawa[76] presented a spheroid microarray chip (SM chip) that immobilized liver cancer cells and can be used for screening appropriate drug concentrations. They used an alkoxyresorufin O-dealkylase assay to analyze the P450 enzyme activities and evaluate drug metabolism. Since the spheroid culture approach best maintains cells' specific functions, the SM chip has a much higher efficiency while detecting the P450 enzyme and therefore could serve as a novel culture platform for drug screening.

Tran *et al.*[77] reported *in vitro* cells screening with microfluidic chips for the treatment of osteoporosis. The chip was designed to provide the drug concentration of each chamber. The chip includes a network of microchannels used to transport and mix chemical samples, which were designed to provide the drug concentration of each chamber. Kwon *et al.*[78] expanded the cell array culture chip and designed a type of the cell array screening chip with 2100 culture chambers. According to the principle of chemical-induced death of the breast cancer cell MCF-7, antitumor drugs were screened out.

B. Optimal combinatorial drug screening

In clinical treatments, the combination of two or more drugs may improve the effectiveness of combating certain diseases.

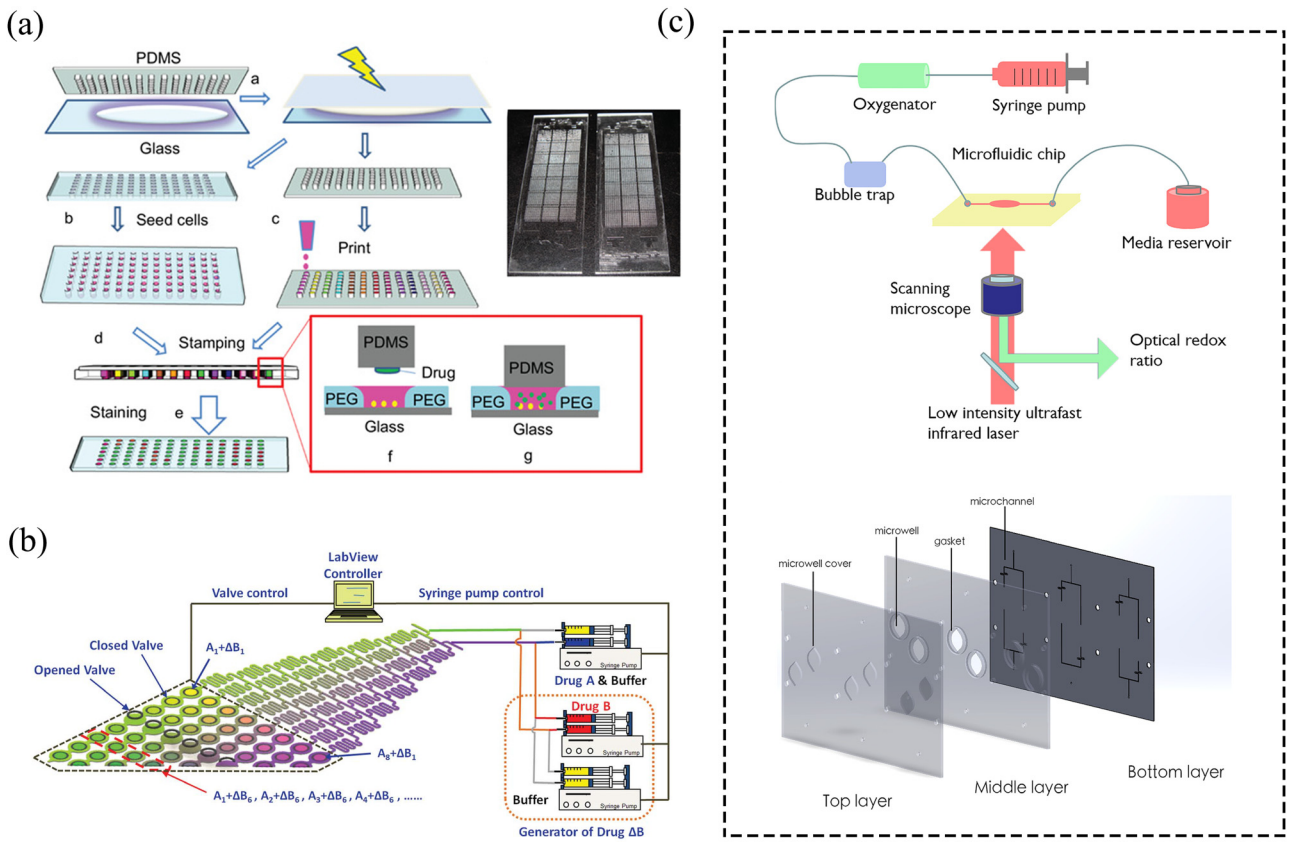

FIG. 4. Applications of the cell-on-chip drug screening system. (a) Schematic of a microarray sandwich system for the high throughput drug activity screening. Reproduced with permission from Wu *et al.*, Biomaterials **32**, 3, 841–848 (2011). Copyright 2010 Elsevier. (b) Schematic of the programmable cell-on-chip drug combination screening system. Reproduced with permission from Kim *et al.*, Lab Chip **12**, 1813–1822 (2012). Copyright 2012 Royal Society of Chemistry. (c) Schematics of the microfluidic system and the exploded view of the chip for drug toxicity testing. Reproduced with permission from Yu *et al.*, Biomicrofluidics, **11** (3), 034108 (2017). Copyright 2017 AIP Publishing LLC.

Therefore, it is important to research high-efficiency optimal combinatorial drug screening with different drug concentrations. Du *et al.*[79] performed a cell-on-chip drug combination screening system based on the sequential operation droplet array technique. They used an oil-covered droplet array chip as the cell culture and analysis platform and applied the system to combinatorial drug screening for A549 nonsmall lung cancer cells. They tested combinations including kinase inhibitor flavopiridol and 5-fluorouracil and paclitaxel (two commonly used anticancer drugs). Finally, a combination of 200 nM flavopiridol and 100 μM 5-fluorouracil resulted in the highest inhibition efficiency. A KAIST research team[80] developed a microfluidic-based drug screening chip to identify combinatory antibiotic interactions. It could automatically generate 121 pairwise concentrations between two antibiotics and allow various analyses for all the drug pairs in only 7 h. They observed the inhibition of bacterial growth by the orthogonal antibiotic gradients over 6 h and classified different interaction types of antibiotic pairs.

Kim *et al.*[25] developed a programmable microfluidic device for combinatorial drug screening [Fig. 4(b)]. This device integrated a pneumatic layer which acts as a valve. By controlling the air pressure in the pneumatic layer, the experimenter can decide whether to let the drug solution access the cell chamber, enabling the microfluidic chip to generate 64 different concentration gradients or an 8×8 combinatorial when mixing drugs. Lin *et al.*[81] reported an open microfluidic tissue array chip for discovering the orthogonal drug combination test of 3 factors and 3 levels. The results show that this microfluidic chip can execute multidrug combination tests with highly simulated tissue. Therefore, it has the potential to be a powerful tool for guiding individualized precise treatment of tumors.

C. Drug toxicity test

Cytotoxicity screening has played a crucial role in determining the safety and efficacy of new drugs. Wang *et al.*[82] designed a multilayered screening chip to culture and label mammalian cells. The microchannels for cell culturing are orthogonal to channels for toxin exposure. They chose three different types of cells (BALB/3T3, HeLa, and bovine endothelial cells) to screen five toxic substances and tested the effect on cell morphology and viability. This chip enabled

a high-density parallel drug toxicity screening method. Toh *et al.*[83] reported a 3D HepaTox Chip for *in vitro* drug toxicity testing based on three-dimensional hepatocyte culture. The chip based on multi-channels ensures the 3D microenvironment of hepatocyte synthesis and metabolism and can simultaneously release linear concentrations of drugs in different channels to stimulate the cells and moreover to predict *in vivo* hepatotoxicity.

Yang *et al.*[84] developed an integrated microfluidic array system to evaluate the toxic and teratogenic potentials of drugs. The system includes a concentration gradient generator and numerous micro-structures for embryo culture (egg culture and egg positioning). Yu *et al.*[85] used two-photon metabolic imaging to detect drug toxicity using a cell-on-chip drug screening system [Fig. 4(c)]. They found that IC_{50} values measured by this metabolic imaging method are more sensitive than traditional methods, allowing real-time detection of drug toxicity and side effects on cells.

Cell-on-chip drug screening systems are being rapidly developed, and its advantages include miniaturization, integration, and automation, which can save time, energy, and money. However, it is not without problems. There is a difference between the cells in the chip and the cells in the human body. The cells in the human body have complicated 3D shapes and structures, so the translatablity of the *in vitro* results to *in vivo* scenarios is yet to be confirmed. Cell drug screening experiments can only reflect the activity of drugs at the cellular level. Additionally, reduced sample size from the microliter range to the nanoliter range results in fewer cells, in which the interaction among cells may be different from that in the body.[86] The human body has many cells and organs that work together. These cells and organs create an intricate web of physiological reactions, metabolic pathways, and paracrine effects. Therefore, research studies are taking efforts to design tissue-on-chip and even organ-on-chip that replicate this environment. The future goal is to mimic the *in vivo* environment on a chip, thus reducing the times of animal trials. Another aspect of cell-on-chip development is to realize personalized treatment.[87] Due to miniaturization, integration, and automation, it can offer drug screening and other outcomes individually, quickly, and automatically. Eventually, these microfluidic devices can quickly and efficiently realize drug screening and benefit humankind.[26]

V. ORGAN-ON-CHIPS FOR DRUG SCREENING

Organ-on-chip is a system that is based on biological micro-electromechanical systems or microfluidics to simulate the micro-structure and physiological functions of specific human organs.[88,89] The disease model associated with specific organs is built on the chip, which can be used to test the pharmacological activity or biological toxicity of drugs and select the most suitable drugs,[90,91] that is, organ-on-chip drug screening system.[92] Different organs have different biological microenvironment and microstructure. Since the concept of organ-on-chip has been proposed, various types of organ-based device have emerged, such as liver-on-chip,[93] lung-on-chip,[94] and heart-on-chip[95] systems.

A. Liver-on-chip

Liver is an important drug metabolism organ in the human body, so the chip system of liver plays a very important role in understanding the metabolism and toxicity of drug treatment. At present, research studies use liver chips to carry out drug screening.[96,97] The preparation and principle of the liver chip[98] is shown in Fig. 5(a). The hepatic spheroids are embedded in the hydrogel scaffold, which are bioprinted into the microfluidic device. The bioreactor chip is then assembled, and the cell culture medium is infused in the chip. In the hepatotoxicity test, a medium containing acetaminophen was used as the model drug to observe the decrease of cell metabolic activity and the decrease of biomarker contents, which successfully indicates that this model could screen the hepatotoxicity of drugs with high throughput.

B. Lung-on-chip

Lung is a human respiratory organ, and it plays an important role in the evaluation of aerogel drugs. The toxic side effects of these drugs may cause a variety of injuries to lungs, such as pulmonary interstitial changes, pulmonary edema, and pulmonary hemorrhage. So, a lung-on-chip system is necessary in drug screening processes. Lung-on-chip platform is more dependent on a bilayer structure. Huh *et al.*[99] reconstructed the alveolar-capillary interface by a parallel microchannel in a microfluid device [Fig. 5(b)]. The upper channel of this platform is alveolar and is exposed to air, whereas the lower channel is a fluid-filled microvascular system; these two channels are separated by a porous membrane. A vacuum cycle is used to simulate the human breathing process. The authors injected interleukin-2 (IL-2) into the microvascular channel and found that IL-2 infiltrates into the alveoli channel, which is the same process as IL-2 causes pulmonary edema. Mechanical stress, which is used to simulate breath, can exacerbate the leakage caused by IL-2. Feasible treatment options can be found by testing this disease model with drugs and checking if the drugs applied are able to inhibit the leakage caused by IL-2.

C. Heart-on-chip

Heart promotes blood flow through myocardial contractions. Agarwal *et al.*[100] developed a Muscular Thin Film structure based on anisotropic cardiac cells and elastomeric substrates [Fig. 5(c)]. This structure is then used to build multiple heart disease models, so that drug evaluations can be completed based on them. First, to induce myocardial cells spontaneously from anisotropic tissue, they created surface patterns on the PDMS layer and modified it with closely spaced lines of fibronectin. A 2 Hz bipolar square pulse acting on a platinum electrode is used to induce membrane depolarization and contraction of myocardial cells. The relationship between myocardial contractility and cellular structure was studied, so the device can reproduce contraction characteristics of cardiomyocyte tissue. By measuring the amount of stress generated by the cardiomyocytes after drugs, the efficacy or toxicity of drugs can be evaluated.

D. Bone-on-chip

Bones play an important role in the human body. Specifically for bone marrow, it has a significant impact in the immune system due to its high-density hematopoietic activity. Torisawa *et al.*[101] developed a bone marrow on chip devices by combining tissue

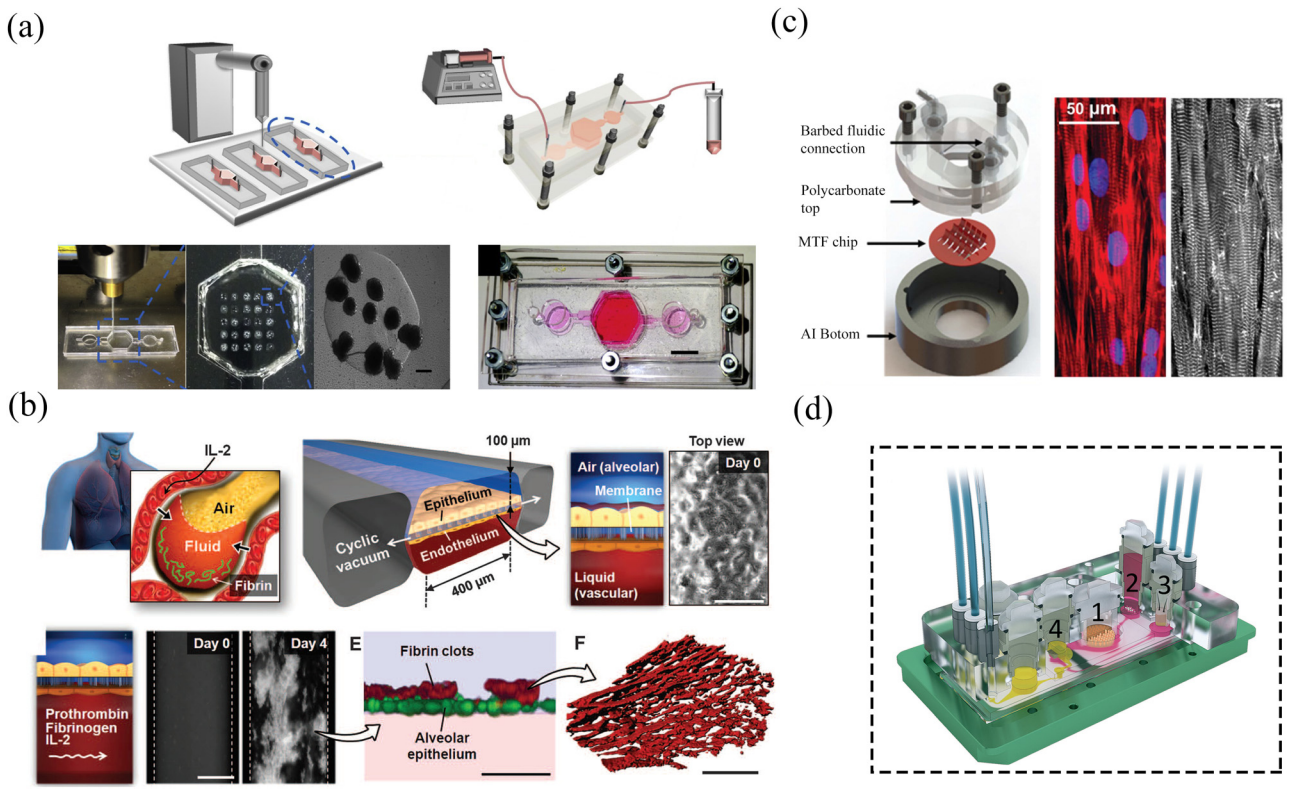

FIG. 5. Applications of the organ-on-chip drug screening system. (a) Schematic of a liver-on-chip system to screen the hepatotoxicity of drugs with high throughput. Reproduced with permission from Knowlton and S. Tasoglu, Trends Biotechnol. **34**, 9, 681–682 (2016). Copyright 2016 Elsevier. (b) Schematic of a lung-on-chip microdevice to test drug toxicity. Reproduced with permission from Huh *et al.*, Sci. Transl. Med. **4**, 159, 159ra147 (2012). Copyright 2012 The American Association for the Advancement of Science. (c) Schematics of the microfluidic heart-on-chip for higher throughput pharmacological studies. Reproduced with permission from Agarwal *et al.*, Lab Chip **13**, 3599–3608 (2013). Copyright 2013 Royal Society of Chemistry. (d). Schematics of a four-organ-chip for interconnected long-term coculture of human intestine, liver, skin, and kidney equivalents. The numbers represent the four tissue culture areas of intestinal (1), liver (2), skin (3), and kidney (4) tissues. Maschmeyer *et al.*, Lab Chip **15**(12), 2688–2699 (2015). Copyright 2015 Author(s), licensed under a Creative Commons Attribution (CC BY) License.

engineering and microfluidic approaches. They generated fully functional engineered bone marrow, replicating the hematopoietic niche physiology *in vitro*. This system involves not only an *in vitro* hematopoietic model but also the analysis of bone marrow responses to toxic agents.

E. Gut-on-chip

Intestinal tract plays a vital role in the digestion process. Therefore, research on the absorption of new oral drugs through intestinal cells has become an important step in drug screening. Kim *et al.*[102] developed an *in vitro* living cell-based model of the intestine that simulates varieties of properties of the human gut. The model consisted of two overlapping cell culture chambers separated by a porous flexible membrane stretched by a negative pressure lined with Caco2 cells to model the intestinal barrier. Besides the barrier function of human intestine, this model also shows absorptive properties, which would be utilized for drug absorption studies.

F. Human-on-chip

When assessing drug efficacy, their effects on surrounding organs are as important as the effects on the target organ, especially when testing for drug toxicity. More importantly, certain organs are linked to each other. So, integrating multiple chips with different organ functions into one chip system (human-on-chip) is more effective for drug screening. Maschmeyer *et al.*[103] constructed a four-organ-chip for coculturing of intestine, liver, skin, and kidney [Fig. 5(d)]. The device has two microfluidic circulations, with micropumps controlling the direction of medium circulation. They built the intestine, liver, skin, and kidney model separately based on this multiorgan-chip system and connected them using microfluidic circulation. This experiment shows that the system can achieve long-term coculture of multiple organs. Therefore, by constructing individual organ chips that simulate various specific human organ functions and are effectively integrated on the same chip system, in theory, this would provide a pathway for research on relevant medical experiments, drug screening, and disease models at the organ level.

Organ-on-chip is an *in vitro* model that simulates normal or pathological human physiology in a microfluidic system with high measurement accessibility and control. It has significant advantages. First, like other microfluidic technologies, the organ-on-chip drug screening method is miniature, integrated, and low cost. Second, compared to the cell culturing and animal model, the organ-on-chip system can reconstruct the human microenvironment, so it is more representative of human reactions. And, it is much cheaper than animal models. Moreover, organ-on-chip systems can realize long-term coculturing multiple types of cells.

Despite current technological advances, there are also disadvantages. First, even the most advanced organ chips do not fully represent the functions of living organs. After all, no organ can exist independently out of the body. Second, the low culture volumes and cell numbers in organ-on-chip systems cause defects in detection sensitivity. Significant differences exist in different batches of devices simulating the environment of the same organ, so devices have large variabilities.[104] At present, due to low cost and the high degree of human body simulation, organ-on-chip systems have been used for simple drug screening experiments and are gradually entering the market. If the patient's own cells can be used to construct individualized devices, the development of personalized drugs can be promoted and the quality of drug screening can be improved.

VI. CONCLUSION

As presented, microfluidic devices possess certain characteristics that make them an interesting alternative in the field of drug screening and development. Several methods show promising results, demonstrating the advantages of microfluidic chip technology in cell operation analysis compared with conventional methods. Nevertheless, there are still shortcomings in operation technology and means used, such as cost and the requirement for many instruments and equipment. The implementation of cell culture environment still differs from the actual cell's microenvironment. Further development is needed to attain the full potential of microfluidic devices.

For instance, photolithography, time-consuming and costly, is widely used for chip synthesis but can be replaced with 3D printing. However, the biocompatibility of material used for 3D printing with cell and drugs must be improved. There is a need for total automation, which can reduce waste of samples during drug screening. Data analysis on a microfluidic platform is carried out by imaging software, making it tedious. Artificial intelligence and deep learning can be incorporated to make the devices more appealing. Paper microfluidics is cost-effective and can be used in resource-limited areas as well. However, interferences of paper substrates with autofluorescence (a common method for drug screening) are impeding factors. Currently, microfluidics devices rely on heavy instruments for data acquisition and analysis. To overcome this limitation, data acquisition and analysis can be integrated into portable devices or in the cloud. Single cell analysis has obtained desirable results in diagnosis and therapeutics. In addition, single cell drug screening carried out on digital microfluidics can be considered for improved outcomes in the future.

As a platform for drug screening still being experimented on, microfluidic chip technology is still in the early stages of research and development. Most microfluidic chips are in the laboratory demonstration stage and have not been commercialized or made accessible. Hence, microfluidic chip technology has a long way to go to replace traditional drug screening models and become the new drug screening authority. Each step on this path gets us closer to a reality in which each individual will be able to receive personalized treatment, thanks to cheap, efficient, and reliable microfluidic technology.

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