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Microengineering methods for cell-based microarrays and high-throughput drug-screening applications

Feng Xu[1], JinHui Wu[1], ShuQi Wang[1], Naside Gozde Durmus[2], Umut Atakan Gurkan[1] and Utkan Demirci[1,3,4]

[1] Department of Medicine, Demirci Bio-Acoustic-MEMS in Medicine (BAMM) Laboratory, Center for Biomedical Engineering, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[2] School of Engineering and Division of Biology and Medicine, Brown University, Providence, RI, USA
[3] Harvard-MIT Health Sciences and Technology, Cambridge, MA, USA

E-mail: udemirci@rics.bwh.harvard.edu

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Abstract

Screening for effective therapeutic agents from millions of drug candidates is costly, time consuming, and often faces concerns due to the extensive use of animals. To improve cost effectiveness, and to minimize animal testing in pharmaceutical research, *in vitro* monolayer cell microarrays with multiwell plate assays have been developed. Integration of cell microarrays with microfluidic systems has facilitated automated and controlled component loading, significantly reducing the consumption of the candidate compounds and the target cells. Even though these methods significantly increased the throughput compared to conventional *in vitro* testing systems and *in vivo* animal models, the cost associated with these platforms remains prohibitively high. Besides, there is a need for three-dimensional (3D) cell-based drug-screening models which can mimic the *in vivo* microenvironment and the functionality of the native tissues. Here, we present the state-of-the-art microengineering approaches that can be used to develop 3D cell-based drug-screening assays. We highlight the 3D *in vitro* cell culture systems with live cell-based arrays, microfluidic cell culture systems, and their application to high-throughput drug screening. We conclude that among the emerging microengineering approaches, bioprinting holds great potential to provide repeatable 3D cell-based constructs with high temporal, spatial control and versatility.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Advances in combinatorial chemistry have allowed the emergence of chemical libraries consisting of millions of compounds [1]. The drug development process involves testing the metabolic function and toxicity of these compounds to determine therapeutic efficacy and risk potentials [2, 3]. Although animal models are commonly used for drug development and pharmacokinetic studies, animal use in research is generally associated with significantly high cost, time and labor-intensive processes, and faces ethical concerns [4–7]. Cells patterned in an array format (i.e. cell microarray) hold great potential in screening drug candidates for efficacy and toxicity at high throughput [8]. Recent studies have demonstrated that *in vitro* cell microarrays can prove to be effective in drug-screening applications (e.g., libraries from SPECS and Enamine) with reduced cost and time by significantly reducing the need for animal testing studies [8–11]. These microarrays enable cell analysis such as drug treatment response, cell–cell and cell–extracellular matrix

[4] Author to whom any correspondence should be addressed.

(ECM) interactions in a high-throughput manner (hundreds to thousands of samples on a single glass slide) [8, 11–18].

Three-dimensional (3D) cell microarrays provide an alternative to conventional two-dimensional (2D) multiwell-plate-based assays. 3D cell culture mimicking native ECM enables researchers to define structure-function relationships and to model cellular events and disease progression [19–23]. For example, tumor cells cultured on 2D and 3D show different cell morphology [24], metabolic characteristics (e.g., increased glycolysis of osteosarcoma cells in 3D culture, differences in the lactate and alanine levels) [22, 25], and drug response [26]. Several methods have been developed to form 3D cell constructs such as spontaneous cell aggregation, liquid overlay cultures, rotation and spinner flask spheroid cultures, microcarrier beads, rotary cell culture systems and scaffold-based cultures [27]. Recently, spheroid-based drug-screening methods have emerged [28]. However, it is challenging to form 3D cell microarrays using these methods. In contrast, emerging microengineering technologies enable versatile fabrication of 3D cell-based microarrays including soft lithography, surface patterning, microfluidic-based manipulation and cell printing.

In vivo, cells are in a microenvironment that usually consists of multiple cell types precisely organized in 3D [29]. For instance, tumors are complex tissues composed of, in the case of carcinomas, both cancer cells and stromal cells such as fibroblasts and endothelial cells [30, 31, 32]. These stromal cells are a key determinant in the malignant progression of cancer (e.g., angiogenesis [30], metastasis [33], invasiveness [34]) and represent an important target for cancer therapies [35]. However, the specific contributions of these stromal cells to tumor progression are poorly defined and many of the underlying mechanisms remain poorly exploited [36, 37]. The spatial position of cells is also important for their functionality which is regulated by the cell's genetic coding and its communication with neighboring cells [38]. A method that precisely positions cells forming 3D co-culture models at large number in a repeatable manner (i.e. a co-culture array) is helpful to understand the interaction between different cell types such as to understand cancer pathogenesis and to improve current therapies. In spite of the importance of cell co-culture and advances in surface patterning and microfluidic techniques, a controlled arrangement of multiple cell types in an array format is challenging.

In this paper, we report the state-of-the-art advances in microengineering methods to fabricate cell microarrays and describe existing methods used to introduce drugs to cell microarrays for drug-screening applications. Among these emerging fabrication methods, cell printing holds great potential to provide highly repeatable 3D tissue constructs, since it can control the cell positions temporally and spatially.

2. 3D cell culture versus 2D cell culture

It has been shown that when cells are cultured in 2D monolayers, significant perturbations in gene expression are observed compared to cells in native tissues and in 3D culture conditions [39]. Furthermore, 3D cellular constructs can mimic the native tissue microenvironment and hence better emulate the drug responses observed in animal models compared to 2D monolayer cell cultures [24, 40, 41]. *In vivo*, cells are imbedded in 3D ECM with ligands such as collagens and laminins that allow cell–cell communication between neighboring cells [42, 43]. Furthermore, expression of genes responsible for angiogenesis, chemokine generation, cell migration and adhesion differs in 3D and 2D cultures [25, 27]. For example, β1-integrin and epidermal growth factor receptor (EGRF) in malignant human breast epithelial cells are over-expressed when cultured in a 3D matrix, but not in 2D monolayers [27]. In addition, tyrosine phosphylation, which plays a role in signaling of focal adhesion kinase (FAK), is down regulated in 3D culture [44]. Additionally, cancer cells show different responses to anti-cancer agents in 3D culture. Mouse mammary tumor cells have greater drug resistance to melphalan and 5-fluorouracil in a 3D collagen matrix as compared to 2D controls [45]. Anti-mitotic drugs (doxorubicin and 5-fluorouracil) become effective after 24 h of treatment in 2D cell culture (SA87, NCI-H460 and H460M tumor cell lines), whereas they cannot show efficacy until 1 week later in hyaluronic-acid-based (HA-based) 3D culture [46]. Furthermore, co-culture of endothelial, stromal, and/or epithelial cells has been achieved within 3D systems, which allows one to study the side effects of a drug on neighboring stromal cells [25]. So far, accumulative evidence demonstrates that *in vitro* 3D culture can better recapitulate *in vivo* cellular response to drug treatment than 2D culture, and has potential to be a superior platform for drug development. Based on these observations, it can be suggested that cellular responses to drug candidates observed in 2D may not be applicable to *in vivo* response. Therefore, there is a need for *in vitro* 3D cell culture models which would bridge the 2D monolayer cell culture systems and the animal models [18, 19, 41, 47–49].

3. Microengineering methods to fabricate cell microarrays

In this section, we describe the existing methods which have been developed to fabricate cell microarrays, including microwell-based methods, surface patterning methods, microfluidic methods, and cell printing (table 1).

3.1. Microwell-based method to fabricate cell microarrays

With advances in microengineering such as microfabrication and soft lithography, a high-density array of wells with microscale well sizes (e.g., tens to hundreds of micrometers) can be fabricated. When these wells are loaded with cells via cell seeding due to gravity, cells immobilized inside these microwells form cell microarrays.

Soft lithography has gained popularity in common laboratory settings because of low cost, and compatibility with a broad range of materials. In principle, multiple steps are involved to create cell arrays using microwells, including pattern design, fabrication of a photomask and a master, fabrication of polydimethylsiloxane (PDMS) stamps, fabrication of microwells, and loading cells of interest in

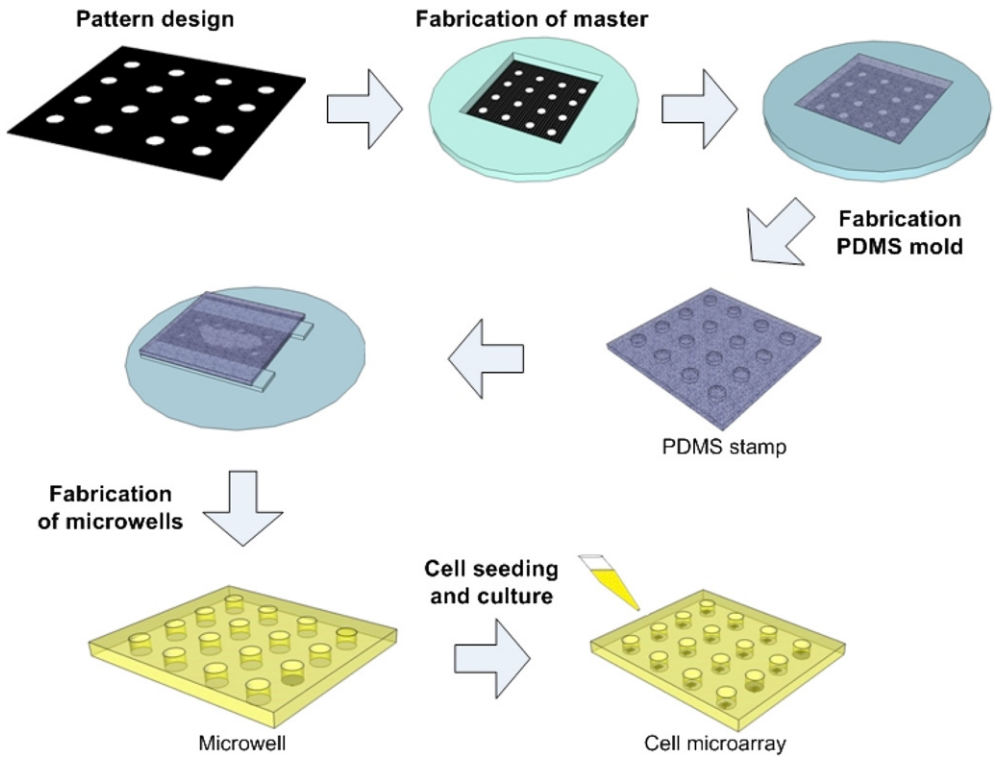

Figure 1. Schematic illustrations of fabricating cell microarrays using soft lithography. A mask pattern is designed and a photomask is fabricated based on the design. The mask is then used to fabricate the master on a silicon wafer via lithography. The silicon wafer master can be used repeatedly as a mold for casting PDMS stamps. The PDMS stamp containing protruding columns is pressed onto another hydrogel solution (e.g., PEG monomer solution) on a glass slide. The microwell array is formed by UV cross-linking of PEG and removing the PDMS stamp from the formed microwells. Cells are seeded to microwells to form cell microarrays. Reprinted from [50] © 2008, with permission from Elsevier.

Table 1. Comparison of different methods for fabricating a cell microarray.

Fabrication methods	Throughput	Cell co-culture capability	Control over cell density	3D capability	Single-cell array capability	Relevant references
Microwell	Medium	Yes	Low	Yes	Yes	[13]
Surface patterning	Medium	Yes	Medium	Yes[a]	Yes	[58–61, 131, 132]
Microfluidics	Medium	Yes	Low	Yes	Yes	[71, 73, 74]
Cell printing	High	Yes	High	Yes	Yes	[80, 91, 98, 100, 133]

[a] Cell encapsulating hydrogels are patterned on surface to form a 3D cellular structure [131, 132].

microwells (figure 1). Generally, a silicon wafer is used as a substrate, on which a photosensitive, thin film (e.g., SU-8) is placed. Once the film is exposed to UV light through a designed photomask, the film becomes solidified with a permanent microstructure created on the silicon wafer. The silicon wafer mold can be used repeatedly as a microstructure master for casting PDMS stamps, which can be prepared by mixing PDMS prepolymer, thermally curing the polymer, and peeling the resulted flexible and transparent films. The cast PDMS stamp is then used to prepare microwell arrays on a glass slide. Using this method, Moeller *et al* generated a microwell array with a high resolution of 20 000 dpi, which enables the fabrication of microwell arrays with seeded cells at a greater density [50]. Due to the 3D microenvironment on microwell arrays, mouse embryonic cells aggregate within microwells and form homogeneously sized embryoid bodies (EBs) [51].

Although PDMS has been widely used in biomedical engineering, it is restricted by innate hydrophobicity, absorption of organic solvents and small molecules, and water evaporation [52]. Alternatively, polyethylene glycol (PEG) and agarose can be used to fabricate microwells instead of PDMS [51, 53–55]. For example, Karp *et al* showed that homogenous and controllable EBs were formed within microfabricated PEG microwells, which can be used for high-throughput screening of drug candidates [51].

The shape and dimension of microwell arrays can be defined according to photomask micropattern to control the size and shape of cell aggregates in the wells [56]. By varying the size, shape and depth of microwells, single-cell arrays can be fabricated to evaluate cellular behavior at a single-cell level, which may be absent in a cell aggregate [13]. High-throughput measurements of single-cell responses are thus essential for a variety of applications including drug screening, toxicology

and cell biology [57]. However, the microwell method based on soft lithography has limited flexibility in changing pattern design due to reliance on photomasks.

3.2. Surface patterning for cell microarrays

Surface patterning is commonly used to prepare cell microarrays, where material surface (generally a cell-resistant surface) is modified locally in an array pattern with cell adhesive biomolecules (e.g., collagen, laminin, fibronectin). When cells are seeded onto the surface, they will attach only to the patterned area modified with biomolecules of high affinity to cells forming cell microarrays [14].

Via the surface patterning method, Flaim *et al* prepared a cell microarray to study the effects of different combinatorial matrices of ECMs on the differentiation of mouse embryonic stem cells (ESCs) [58]. In this study, 32 different ECM combinations were spotted onto a polyacrylamide gel-coated glass slide using a standard DNA spotter (pin printing). Mouse ESCs can only attach to ECM-coated areas, resulting in an ECM-based cell microarray, which allows the investigation of cell–ECM interactions in a high-throughput manner. Similarly, Ceriotti *et al* microarrayed ECM proteins (e.g., fibronectin) on plasma-deposited polyethyleneoxide (PEO-like) film-coated glass slides [59]. In another study, Anderson *et al* developed a nanoliter scale platform synthesizing biomaterial libraries in an array format with the aid of a robotic liquid handling system [60]. With this method, 1700 cellular–material interactions were simultaneously investigated on a single glass slide.

Recently, Zawko *et al* developed an inexpensive, off-the-shelf surface patterning method (figure 2) to fabricate cell microarrays [61]. The method is based on micropatterning of 3D alginate grids on glass slides using a woven nylon mesh, eliminating the lithography step. The hydrogel grids were used to guide cell seeding on a glass slide to form cell microarrays at a density of 21 000 spots cm^{-2} (single cell array) or 6000 spots cm^{-2} (multi-cellular array).

3.3. Microfluidic methods

Microfluidics has emerged as a promising technology with widespread applications in engineering, biology and medicine [62, 63]. Microfluidics offers special advantages for manipulating cells since local cellular microenvironment can be controlled [64]. Cell microarrays containing multiple cell types have been fabricated using microfluidic methods [65–68].

Meyvantsson *et al* developed compartmentalized microfluidic cell arrays with a high density (up to 768 micro-chambers in a 128 × 86 mm^2 area) [69]. In this array, cells in a 2D or 3D microenvironment were cultured via droplet-based passive pumping with maintained basic microfluidic operations including routing, compartmentalization and laminar flow. The use of external tubing and valves to control the liquid flow was avoided because of direct access to individual elements via holes in the microfluidic channel surface. This design offers the advantage of reduced device volume and minimal dead volumes. In another study, Wang *et al* developed a microfluidic cell array with individually addressable chambers controlled by pneumatic valves for cell culture and cell-reagent response [66]. In this cell array, different types of cells can be directed into designated chambers for culture and observation. Mirsaidov *et al* fabricated a 3D co-culture cell microarray by integrating microfluidics and time-shared holographic optical trapping (figure 3) [65]. In this method, *E. coli* were manipulated using 3D arrays of optical traps, and then conveyed to an assembly area using a microfluidic network (figure 3(*a*)). In the assembly area, the cells were encapsulated and assembled in a small volume (30 × 30 × 45 μm^3) of PEG (figure 3(*b*)). This step was repeated to form cell microarrays (figure 3(*c*)). However, the optical trapping force is dependent on laser power which may affect cell viability [65], and limits the maximum area of the array (350 × 350 μm^2). In addition, Wu *et al* formed a microfluidic platform allowing self-assembly of spheroids of tumor cells and characterized the dynamics of spheroid formation [70]. In this study, U-shape traps, which have inner volume of 35 × 70 × 50 μm^3, were designed and integrated in the microfluidic array device. It was observed that MCF-7 breast cancer cells formed spheroids (7500 spheroids per cm^2) with a narrow size distribution (10 ± 1 cells per spheroid). The perfusion of cell media allows for prolonged cell culture period, which can be potentially used to evaluate anti-cancer drugs in a high-throughput manner [70].

Microfluidic technologies have also been used to fabricate single-cell microarrays [71–73]. Kaneko *et al* developed a cell microarray loaded with single cardiomyocytes, which were interconnected via microfluidic channels [72]. With this cell microarray, it is found that cell–cell communication affects cell response to drug treatment. Recently, Xu *et al* designed a microfluidic single-cell microarray for testing drug response of individual cells [73]. The array consisted of 8 parallel channels with 15 cell-docking units in each channel. This design enabled simultaneous monitoring of the cellular responses exposed to various drug candidates (e.g., specific activators and inhibitors of the Ca^{2+} release-activated Ca^{2+} channels) in multiple microchannels. Moreover, combinations of hydrogels and microfluidics have been used to fabricate 3D cell microarrays, which may provide new methods for drug screening in a physiologically relevant environment. For example, Tan and Takeuchi developed a bead-based dynamic 3D cell microarray by introducing cell encapsulating alginate beads into a microfluidic system, and arraying the beads using a fluidic trap [74].

3.4. Cell printing

Cell printing is an emerging technique [75] and has been used to fabricate 2D or 3D cell microarrays. Cell printing is different from other cell microarray approaches described above: (i) cell printing is automated through computer-control enabling high-throughput manufacturing of cell arrays with high spatial resolution and control [76, 77], e.g., the dimensions of the array and spot-to-spot distances can be altered; (ii) cell printing can place different types of cells onto intended positions (spatial control) by switching multiple

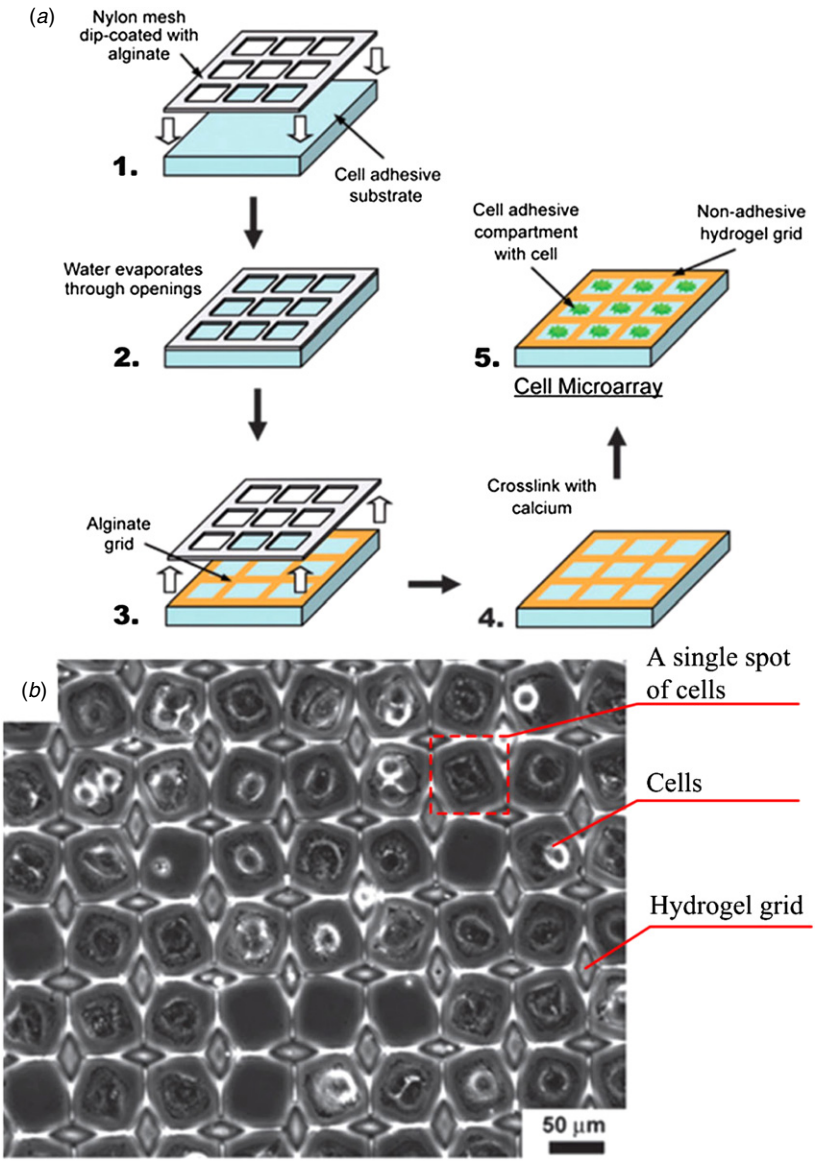

Figure 2. Fabrication of a cell microarray using surface patterning [61]. (*a*) Alginate dip-coated in a nylon mesh is stamped on a cell adhesive substrate (e.g., glass). Alginate is crosslinked after water evaporation with a solution of calcium chloride forming hydrogel spots. The cell microarray is achieved by seeding cells within the hydrogel compartments. (*b*) A fibroblast array with density of 21 000 cm^{-2} was achieved using this method (24 h in culture). Figures reprinted from [61]; reproduced by permission from the Royal Society of Chemistry.

ejecting nozzles temporally [78]; (iii) 3D cell models can also be fabricated using cell printing [79–81]; (iv) cell printing has been shown to produce repeatable and uniform 3D cell aggregates and constructs [78, 81]. Current cell printing and deposition techniques include inkjet printing [82, 83], laser printing [76, 84, 85], bio-electrosprays (BES) [86], and cell spotting [87, 88]. However, there are challenges with existing cell printing technologies such as low cell viability, loss of cellular functionality and clogging of ejectors. Recently, several improved droplet generation methods were introduced [89–93]. Acoustic cell printing technologies have been developed to deposit cells and polymers that are sensitive to heat, pressure and shear [91, 94–98]. Alternatively, valve-based printing has been used to print cell-encapsulating hydrogels [100], single cells for RNA analysis [102] and blood cells for blood cryopreservation [103, 104]. These technologies have advantages over existing printing technologies in terms of higher cell viability and functionality [98, 105].

Hart *et al* [87] used a robotic microarray spotting device (pin printing) to print cells onto streptavidin-coated slides in an array format. With this method, high density has been achieved with ~4700 discrete HeLa cells printed on a single slide using an 8-ejector printer. Recently, cell printing has been used to directly deposit cell encapsulating scaffolding materials (e.g., ECM materials such as collagen, alginate, elastin, and agarose) onto glass surfaces at high throughput to 3D cell microarrays (e.g., figure 4) [100, 106, 107]. The scaffolding materials can support cells mechanically and allow for perfusion of nutrients, thus enabling long-term cell culture [108–110]. For example, cells can be encapsulated in nanodrops of collagen or alginates, which are mounted onto a functional glass slide

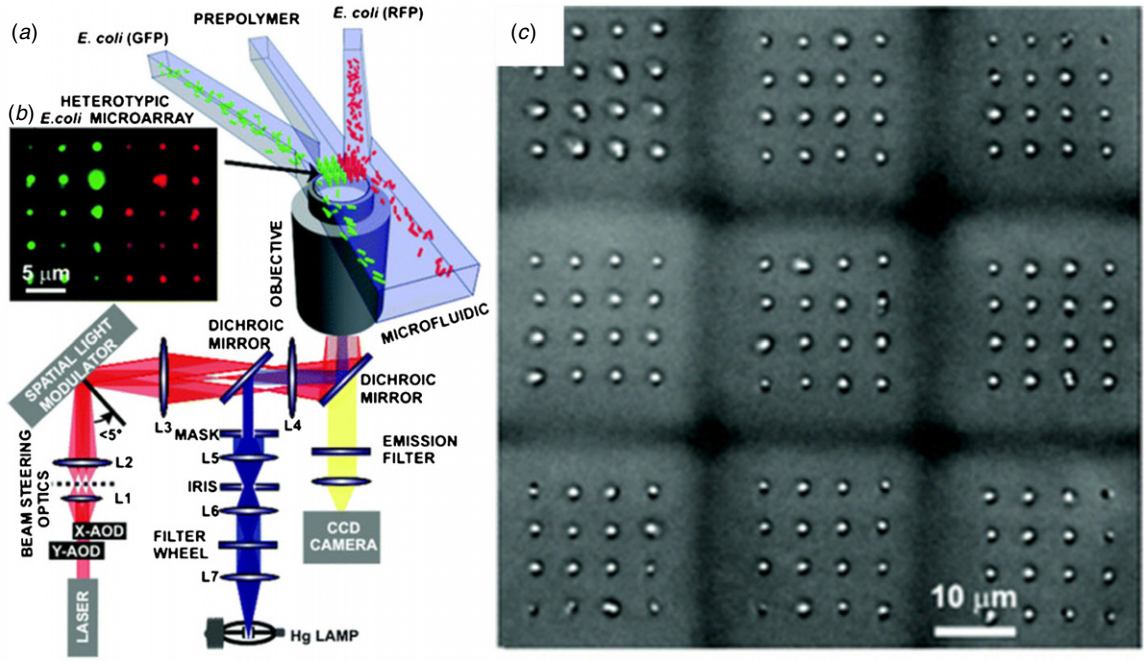

Figure 3. Fabrication of a cell microarray using microfluidic methods [65]. (*a*) Time-multiplexed, 3D arrays of optical traps were used to manipulate cells. The optical traps were created using infrared light (red path) from a Ti:sapphire laser beam. A microfluidic network is used to deliver the multiple types of cells mixed with hydrogel precursor to the assembly area: two types of cells (*E. coli* RFP, *E. coli* GFP) flow in different channels with a third cell-free channel in middle. In the assembly area, the cells are encapsulated within the hydrogel through photo crosslinking forming cell array ((*b*) 2D 5 × 6 microarray). (*c*) Nine homogeneous 4 × 4 microarrays of G1 *E. coli* forming a 3 × 3 microarray. Figures reprinted from [65]; reproduced by permission from the Royal Society of Chemistry.

Table 2. Comparison of different methods to add drugs to cell-based assays.

Fabrication methods	Throughput	Cross-contamination	3D drug loading capability	Relevant references
Drug patterning	Medium	Yes	Yes	[112]
Stamping method	Medium	Yes	Yes	[88, 113]
Microfluidic drug loading	Low	Yes	No	[117]
Aerosol spray	High	No	No	[115]

by a robotic system to form 3D cell microarrays [60, 88, 111]. The same bioprinting platform can then be used to deliver the drug candidates into the cell arrays in a controllable and high throughput manner. Therefore, bioprinting technology offers a versatile method for formation of the 3D cell arrays followed by compound delivery and testing.

4. Methods for adding drugs into cell-based assays

Controlled delivery of drug candidates into cell microarrays is the key to successful drug screening (i.e., decreased failure rate) at high throughput. A direct way is to load drug candidates to each spot using a robotic system (e.g., Perkin Robot Loading System). However, loading thousands of chemicals to cell microarrays usually takes hours and even days, which significantly affects the viability of cells during the loading process and the reproducibility of cell-to-drug responses. In addition, it is essential to introduce chemical or genomic stimuli to each cell spot and avoid cross-contamination between thousands of spots on the cell microarray. To address these technical difficulties, various methods have been developed to efficiently deliver drugs to cell microarrays, including drug patterning [112], stamping [113], microfluidic drug loading [114] and aerosol sprays [115, 116]. These methods differ in throughput, compatibility with co-culture arrays, and control over the cell density (table 2).

4.1. Drug patterning

Drug patterning utilizes a printing robot to array chemicals on a substrate. When cells are seeded on the top of this chemical loaded substrate, only cells on each arrayed dot are affected and then form affected-cell array. Since drug patterning does not need cell printing, the method is easy to be utilized in high-throughput drug screening. For example, Bailey *et al* developed a drug patterning method to screen for small molecular compounds using mammalian cells at high throughput [112]. In this system, small molecular compounds were encapsulated in a scaffold made of poly-(D), (L)-lactide/glycolide copolymer (PLGA). On a Ni-chelated slide, small molecular compounds were spotted (pin printing). Cells were then seeded on top of these spots to form a monolayer of cells. Since compounds encapsulated in the PLGA matrix can slowly diffuse to attached cells, the dose-response can be plotted as a function of distance to the spot

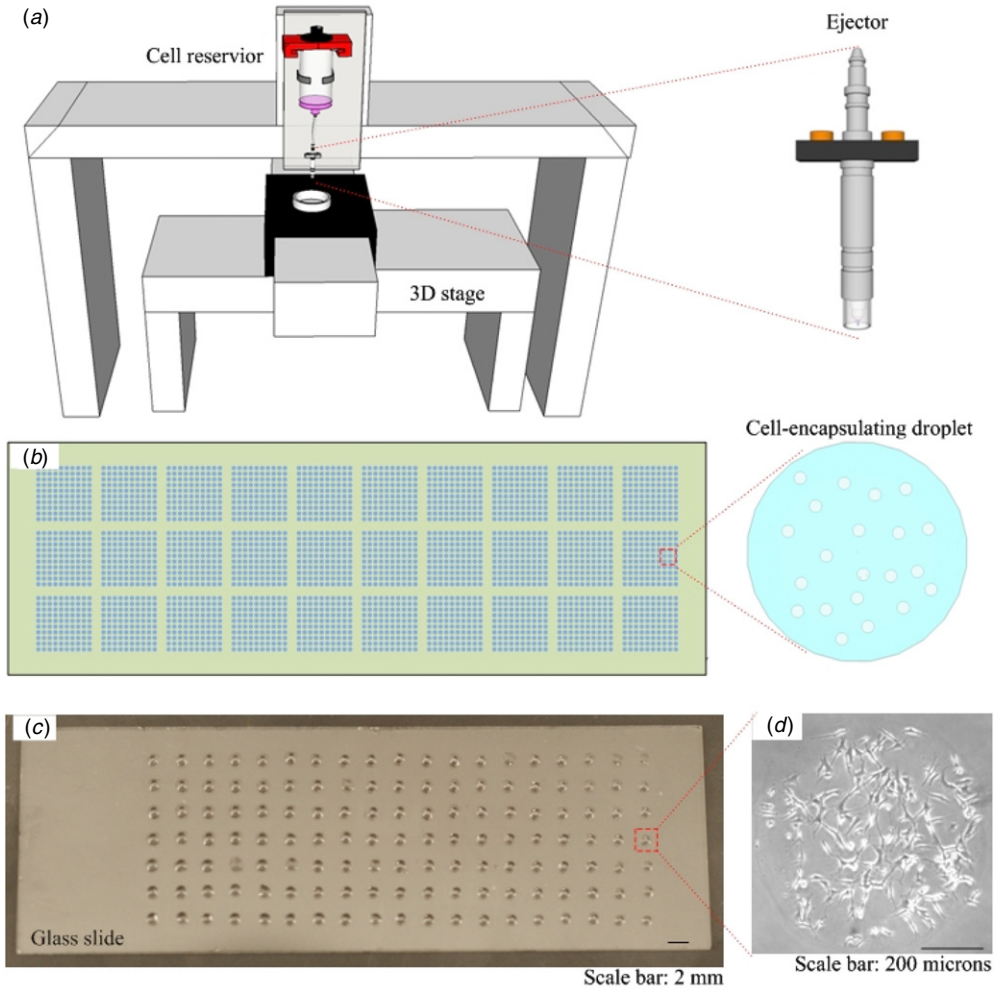

Figure 4. Fabrication of a cell microarray using the cell printing method. (*a*) Schematic of a printing system. A valve-based ejector is connected with a 3D stage which offers ejection of cell encapsulating droplets (e.g., hydrogels) high spatial resolution. (*b*) The droplets can be patterned in an array format on a substrate (e.g., Petri dish, glass slides). (*c*) A sample of high-density cell microarrays.

center. It was observed that the reduced expression of tuberous sclerosis complex gene 2 (TSC2), which was achieved by transient RNAi, was highly correlated to the resistance of cells to a compound, mactecin II. Combined with imaging-based readouts, the drug patterning method consumed small amounts of test compounds and few cells compared to microplate-based screening methods. However, the diffusion of gradually released drugs requires large spot-to-spot distance to avoid crosstalk between neighboring spots, thus limiting the cell densities.

4.2. Stamping method

The stamping method involves two chips, one is a cell chip on which cells are arrayed, and the other is a drug chip. When high-throughput screening is initiated, chemicals are spotted on a drug chip and then stamped onto a cell chip. The stamping method makes it possible that thousands of screening experiments are performed on a single glass slide. For example, Lee *et al* developed a simple cell array-based stamping method to evaluate the drug metabolic process, which is mainly mediated by enzyme P450 in liver cells, at high throughput [88, 113]. This stamping method is consisted of three major steps, including fabrication of cell arrays, drug loading (stamping) and data analysis (figure 5). Initially, an array chip (metachip) containing human P450 and prodrugs was prepared. The selected prodrugs can be cyclophosphamide, tegafur and acetaminophen, which were the substrate of P450. When spotted on the array (pin printing), prodrugs were digested by P450, generated metabolites, mimicking the metabolism of prodrugs *in vivo*. Meanwhile, 3D cell aggregate arrays were prepared by spotting collagen solution containing MCF-7 breast cancer cells onto collagen-modified slides. Upon stamping, metabolites of prodrugs from the chip can diffuse into 3D cell aggregate array and affect cell proliferation. Monitoring of biological events on the cell array allows evaluating the bioactivity/toxicity of prodrug metabolites. Wu *et al* developed a stamping method suitable for screening drug–drug interactions in cell-based assays [55]. This stamping method includes a drug combination chip and cell chip. Drug combinations were printed on a PDMS post-array and stamped to the cell-seeded microwells. In this way, drug combination effects were evaluated in the sealed chamber, and three chemicals were found to have the

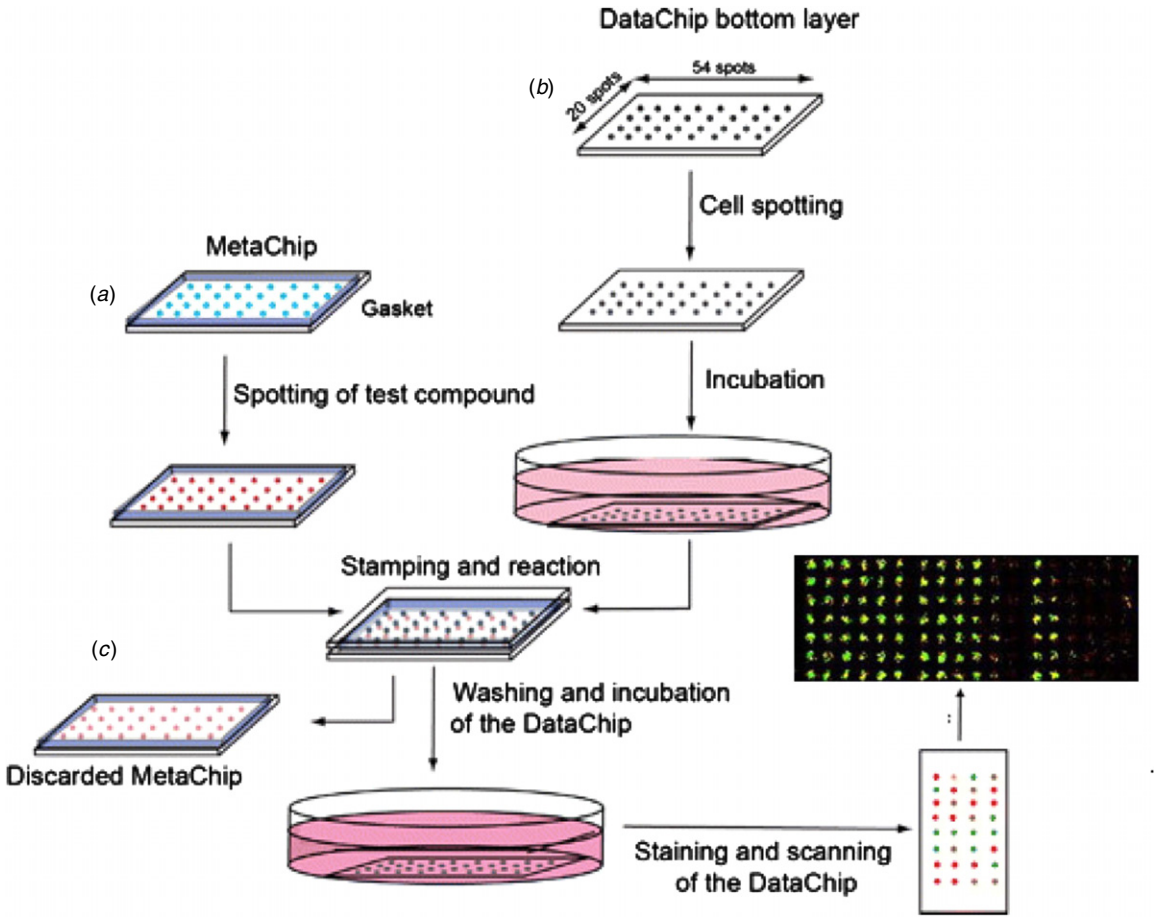

Figure 5. Schematic illustration of drug loading using the stamping method [88]. The stamping method consists of three main steps to achieve precise drug loading. (*a*) Compounds of interest are spotted on an array chip (Metachip). (*b*) Cells are grown on a PDMS base, defined as a cell array (DataChip). (*c*) The array chip and DataChip are stamped together to allow for perfusion. The toxicity of compounds on cells is evaluated using live/dead staining on the cell array. Each cell spot has a diameter of 600 μm. Reproduced by permission from PNAS [88]. © 2008 National Academy of Sciences, USA.

drug–drug interactions with verapamil. The stamping method offers opportunities for rapid and inexpensive combinatorial drug screening to the common research lab. This cell array-based stamping method is simple and rapid, significantly reducing the complexity of drug loading to cell array and thus improving the throughput [88].

4.3. Microfluidic drug loading

Methods based on microfluidics have also been developed to deliver drugs onto high-throughput drug-screening platforms. For instance, Hung *et al* fabricated a PDMS microchamber containing 10 × 10 arrays as a drug-screening platform on which long-term cell culture is enabled [117]. The microchamber is surrounded with microchannels to exchange medium and load reagents for biochemical assays. HeLa cells are introduced into the microchamber by a syringe and continuous perfusion of the medium through these microfluidic channels enables long-term cell culture at 37 °C. However, this method has some drawbacks such as inhomogeneous cell distribution in the 10 × 10 arrays and challenges in further miniaturization of the device. In another study, Upadhyaya *et al* developed a microfluidic device to control drug supply in a cell-based microarray for high-throughput screening [114]. This device consists of three layers, an agar gel to support adherent cell culture, a micropatterned nanoporous membrane layer and a PDMS layer containing two microfluidic channels (figure 6). Compounds of interest can be loaded into the microchannel and then spatially distributed via an electrical field into the agar gel through the nanoporous membrane. By controlling the electric field across the nanoporous membrane, microscale drug spots with the diameter as small as 200 μm can be obtained with inter-spot distances ranging from 0.4 to 1 mm. In both studies, microfluidics drug delivery has been demonstrated, with potential to enable long-term evaluation of drug-cell interactions at high throughput [114, 117].

4.4. Aerosol spray

To load the protein solution on a chemical array simultaneously, aerosol spray is an efficient way. In this method, thousands of chemicals are first arrayed on a substrate and then the protein solution is sprayed. Then chemicals are reacted with the protein solution simultaneously and ultra-high-throughput screening can be performed. For example, protein microarrays are often used to evaluate the interactions

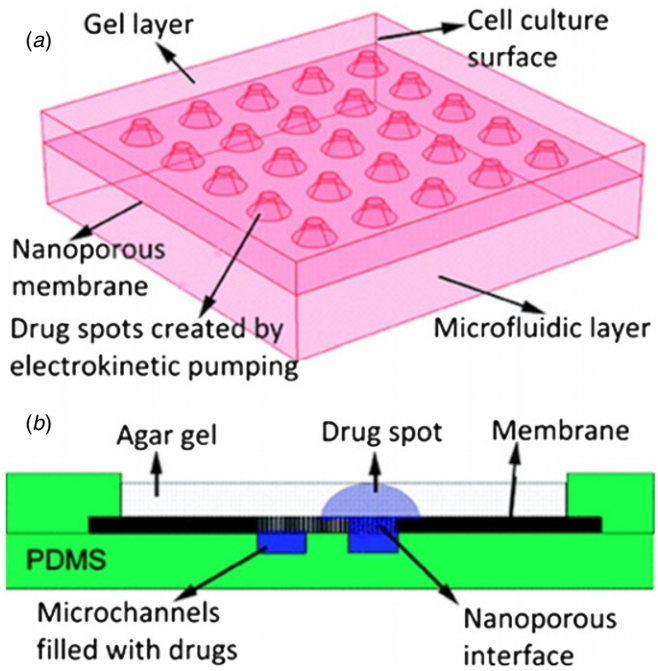

Figure 6. Schematic of the microfluidic device for high-throughput drug loading [114]. (*a*) This device consists of three layers, a gel layer to support adherent cell culture, a micropatterned nanoporous membrane and a microfluidic layer made by PDMS. (*b*) Compounds of interest can be loaded into the microchannel patterned on the PDMS layer and spatially located into the gel layer through the nanoporous membrane upon an electric field. Figures reprinted from [114]; reproduced by permission from the Royal Society of Chemistry.

between chemicals and enzymes. However, immobilization of chemicals onto glass slides in an array format is time consuming and often leads to protein degradation [118, 119]. Furthermore, it is challenging to rapidly deliver droplets to each spot without evaporation and causing cross-contamination. To address these challenges, Gosalia *et al* established a platform for enzymatic reactions in a nanoliter liquid phase [115]. In this method, a library of 352 compounds was microarrayed in glycerol droplets on ten glass slides at a density of 400 spots cm^{-2}. Biological samples such as caspases 2, 4 and 6, thrombin and chymotrypsin were aerosolized and sprayed onto the drug microarray using an ultrasonic nozzle. Enzymatic reactions were carried out by subsequent spraying the drug microarray with nanoliters of reagents, significantly reducing the consumption of materials and reagents. Similarly, Ma *et al* also used the spray strategy to achieve ultra-high-throughput drug screening [116]. Via this strategy, over 6000 homogeneous reactions per 1 × 3 $inch^2$ microarray were carried out, which significantly reduced the amount of reagents used (1 nl) by >10 000-fold compared to the 384-well plate assays (10 μl). This technique is compatible with many conventional well-based reactions and can be carried out using instruments available in industrial and academic institutions, such as liquid handlers, DNA microarrayers, chip scanners and data analysis software. Hence, this spray method can be simply implemented to achieve high-throughput drug screening without the need for sophisticated equipment.

5. Conclusions and future perspectives

Identifying millions of drug candidates for disease treatment is costly and time consuming with current drug-screening technologies such as the multiwell-plate-based screening method. Cell-based microarrays have recently been employed to address the challenges associated with conventional microwell-plate-based methods for high-throughput drug-screening applications. Cell microarrays have been broadly used as a biological tool to study target selection, drug candidate identification as well as preclinical test and drug dosage optimization [120, 121]. Current microarray fabrication methods include soft lithography, surface patterning, microfluidic methods and cell printing, which provide a platform to studying cell responses to different treatments (e.g., drug screening, cytotoxicity screening) in a high-throughput manner. These methods, which can increase the throughput with significantly reduced cost on amounts of expensive test reagents and materials (e.g., chemical compounds, cells), are needed [16]. However, as the number and types of cells to control increase, tracking these cells in microchannels with multiple valving steps requires a complex peripheral system before and after sorting, as the cells need to travel and be spatially patterned at a specific location as required by some of the existing applications. These emerging cell-based methods are broadly applicable and can be extended to applications such as assessing stem cell differentiation, characterizing interactions between cells and their microenvironment, and analyzing genomic functions by RNAi.

There are several challenges associated with cell microarrays as high-throughput drug-screening methods. One of the main challenges is efficient loading of drug candidates into the cell microarrays. Several methods have been developed to address this challenge, including drug patterning, stamping, microfluidic drug loading and aerosol spray methods. Although these methods enable the application of cell microarrays in high-throughput drug screening, there remain unaddressed challenges. For example, an ideal polymer is needed to maintain biological and chemical properties of various drug candidates in drug patterning methods. Also, in stamping and aerosol spray methods, tests are performed in the same fluid medium limiting the range of experimental conditions that can be attained (i.e., lack of compartmentalization) and cross-contamination between neighboring spots always exists which limits the cell density. Therefore, further advances are needed to develop efficient techniques to load drugs into microarrays without cross-contamination. Cell printing holds great potential to address this challenge and could be used for drug loading, as it has been utilized to load growth factors and other biomolecules with or onto cells [122–124]. Another challenge is to form 3D cell arrays. For instance, only limited human tumor cell lines (<100) can form and grow in 3D spheroid format *in vitro,* which is a typical native cancer structure [125]. Although cellular constructs in 3D can be formed with existing methods in an array format (e.g., microwells, microfluidics, printing), further studies are needed to verify that these constructs show

a similar, if not the same, response to drug treatment. For instance, the existing *in vitro* platforms of 3D cellular models are not relevant to most human cancers *in vivo* (*e.g.*, cancers of the blood) [126]. In addition, screening cell response to drugs at high-throughput (e.g., via imaging) and analysis of such a large amount of screening data are also challenging. This may become a major bottleneck for drug-screening applications [127]. With advances in microscopy and corresponding image analysis techniques [128–130], cell microarrays and emerging drug loading techniques, especially bioprinting, hold great potential to provide highly repeatable 3D cellular constructs that could be a powerful tool for studying cell-drug response in a high-throughput manner.

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