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Review

Anti-Cancer Drug Screening with Microfluidic Technology

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Abstract: The up-and-coming microfluidic technology is the most promising platform for designing anti-cancer drugs and new point-of-care diagnostics. Compared to conventional drug screening methods based on Petri dishes and animal studies, drug delivery in microfluidic systems has many advantages. For instance, these platforms offer high throughput drug screening, require a small amount of samples, provide an in vivo-like microenvironment for cells, and eliminate ethical issues associated with animal studies. Multiple cell cultures in microfluidic chips could better mimic the 3D tumor environment using low reagents consumption. The clinical experiments have shown that combinatorial drug treatments have a better therapeutic effect than monodrug therapy. So many attempts were performed in this field in the last decade. This review highlights the applications of microfluidic chips in anti-cancer drug screening and systematically categorizes these systems as a function of sample size and combination of drug screening. Finally, it provides a perspective on the future of the clinical applications of microfluidic systems for anti-cancer drug development.

Keywords: drug screening; monodrug or combinatorial drug screening; anti-cancer

1. Introduction

Microfluidic technology has various potential applications in cancer research, including drug screening, drug discovery, immunotherapy, and clinical oncology [1,2]. In this review, we focus on the recent advances in cancer drug screening using microfluidic technology.

The 2D cell culture cannot mimic the native 3D physiological environment and cellular response in vivo [3]. On the other side, animal models could not detect the side effects of drugs well because of the biological differences between humans and animals. There is evidence that in vitro 3D tumor models can improve the preclinical predictions for cancer therapies due to their physiological relevance [4]. Hence, 3D cell tumor models are increasingly interesting to study the cell response to various stimuli. However, there are concerns with the reproducibility of the results achieved by 3D models due to large variabilities among these models [5]. So, these models still do not meet the required standards for preclinical trials. The 3D tumor models can be classified into scaffold-free systems, scaffold-based systems, hydrogel-based models, bioreactor-based models, microcarrier-based models, cancer-on-a-chip, and bioprinted models [6-8]. Organoids and patient-derived tumor xenograft are advanced strategies more desired for mimicking 3D

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tumor architecture and the in vivo microenvironment, respectively. Despite the great efforts in drug screening on 3D models, enormous challenges still exist for personalized drug screening for clinical trials [9]. The establishment of standardized methodologies can bring these 3D models to preclinical tests [6,10]. More details on the tumor microenvironment in terms of its physical, chemical and biological composition can be obtained from the excellent review paper [10]

Different microfluidics technologies in cell-based high-throughput drug screening, including perfusion flow, droplet and microarray modes are reviewed by [11]. Microfluidic concentration gradient generators, cell co-culture models, organ-on-chips and combinations of microfluidic with other high-throughput systems can be found in the detailed review by [12].

Combinatorial drug treatment has shown its superiority over monodrug therapy for many diseases, especially for cancers, due to intra-tumor heterogeneity [13,14]. Although different anti-cancer drugs have been developed for cancer, mono-drug treatments typically fail in the war against cancer [15]. Compared to mono-drug, drug combinations could simultaneously inhibit multiple excessive pathways of tumor cells and have better efficacy [16,17]. In this context, microfluidics could overcome the expensive and time-consuming process of combinatorial drug screening with high throughput screening (HTS) of more compound candidates [18]. Microfluidics is a promising science and technology that can be used in various industrial [19] as well as biological and biomedical applications, including cell culture [20], cell separation [21], cryopreservation [22] and drug delivery [23].

The recent progress in microfluidic platforms for anti-cancer drug screening is well-reviewed [24] in which current microfluidic devices are categorized as 2D, 3D, and droplet-based. Besides, new researches to address drug resistance, combinatorial drug therapy, cancer metastasis, and cancer heterogeneity is also discussed. Valente et al. also reviewed anti-cancer drug development with the application of microfluidic technology with a focus on the modeling of the tumor microenvironment, High-throughput assays, and microfluidic-integrated biosensors [25].

Since the sample size is different for each type of cancer, the developed microfluidic systems should be tuned based on the sample size [26]. For example, liquid, tissue or surgical biopsy can be used to capture the circulating tumor cells (CTCs) [27,28], a small piece of cancer tissue or a large sample of cancer tissue, respectively. This review guides the reader to the appropriate microfluidic drug screening chips developed for their available sample size. Since different modes of drug testing (mono or combinatorial) could be desired, this study classifies the presented microfluidics into three categories based on sample size and combinatorial or mono-drug for drug screening application. These categories include monodrug screening on a microscale sample, monodrug screening on a macroscale sample, and combinatorial drug screening on a microscale sample.

The main goal of the first class (mono drug screening on a microscale sample) is to examine the effect of a single drug on a microscale sample. For instance, in biological applications, micro samples (i.e., single cells or multicellular aggregates) are exposed to specific drug doses. As such, this class is not suitable to evaluate cancer tissues for ex vivo applications. The second class (mono drug screening on a macroscale sample) is designed to eliminate the limitation of sample size associated with the first class. So, the drug gradient is generated in a macroscale sample (e.g., microdissected tissues). However, these two classes of microfluidics cannot evaluate the combination therapy. Accordingly, the third type of microfluidic platform has been designed to test combinations of anti-cancer drugs on microscale samples. However, there is a lack of combinatorial drug screening on macroscale samples. Furthermore, all mentioned classes can only generate and test countable concentrations due to the discrete concentration gradient generator.

Therefore, the novel class of microfluidic systems to simultaneously deliver continuous gradients of two drugs on a macroscale sample to address the aforementioned limitations is highly demanding. In the following sections, the pros and cons of each of these classes will be thoroughly discussed in more detail.

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2. Monodrug screening on a microscale sample

Based on the microfluidic design, different techniques are employed for monodrug screening. Among them, polymer-based and paper-based microfluidic platforms have been well studied. Polymer-based devices are mainly built from polydimethylsiloxane (PDMS) using soft lithography techniques. Some PDMS-based microchips take advantage of concentration gradient generators (CGGs) [23,29,30], while others set drug dosage manually on a microwell platform or automatically by diffusivity of the reagents. Moreover, they can culture cells 2D or 3D. Hong et al. developed a paper-based microchip that uses a CGG to set different drug dosages on 3D cell culture arrays [31].

Some of these microfluidic platforms for monodrug screening on a microscale sample are shown in Figure 1. The microwell platform shown in Figure 1a uses a 2D cell culture, and the nutrients/drugs diffuse from microchannels to microwells [32]. In contrast, in Figure 1b, alginate 3D cell culture is injected by micropillar chip onto microwell platform [33]. A microarray spotter dispensed the drug solutions onto the microwell chip. Figure 1c is a two-layer PDMS-based microfluidic device with an integrated cell culture membrane that can be used for both drug delivery and mechanotransduction [23]. It should be noted that the size of the membrane can be tailored based on the sample size. Figure 1d shows automatically generation of drug dosages based on the diffusion process onto a 3D biopsy derived spheroids microwell platform [34]. Although 2D cell cultures are still used for most research, the 3D cell culture industry is more appropriate, especially in cancer research. In the following, we classified various monodrug testing on microscale samples based on cell-culture type (two dimensional or three dimensional)

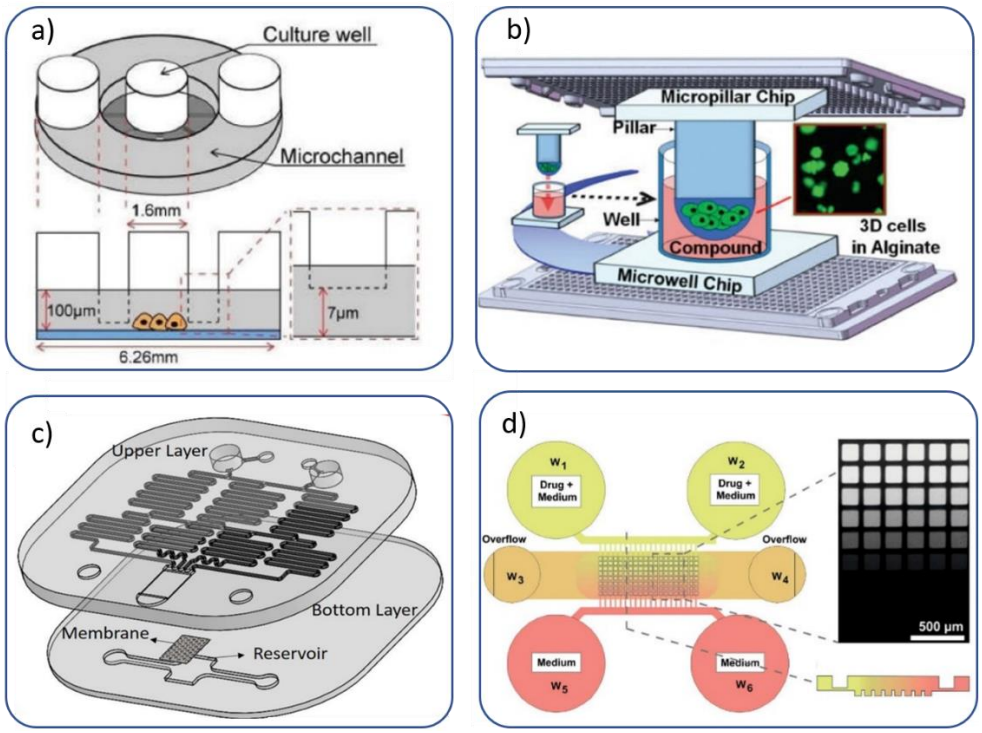

Figure 1. Monodrug screening on a microscale sample; a) A microwell platform to trap cells in 96-well plates. Reproduced from Ref. [32] under an open access Creative Common CC BY license. b) Micropillar-Microwell platform for alginate 3D cell culture (Since the cell-containing micropillar chips lay onto the medium-containing microwell chips, replacing the growth media in this platform is easier than conventional hydrogel 3D cell cultures). Reproduced with permission from Ref. [33]. c) An integrated microfluidic concentration gradient generator for mechanical stimulation and drug delivery. The device consists of two layers of PDMS with an integrated membrane sandwiched between the two layers. The device can generate four different concentrations of a drug and, at the same time, it can impose the adjustable shear stress and osmotic pressure gradients on the cultured cells. Reproduced from Ref. [23] under an open access Creative Common CC BY license. d) A

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diffusion-based gradient generation micro-well platform for biopsy-derived spheroids. Reproduced from Ref. [34] under an open access Creative Common CC BY license.

2.1. Two-dimensional cell cultures

Ye *et al*. described a microfluidic device consisted of multiple drug gradient generators to measure different cellular parameters in anti-cancer drug-induced apoptosis of human liver carcinoma HepG2 cells [35]. This platform consists of eight uniform structure units, and each unit has an upstream CGG and downstream parallel cell culture chambers. Their microscale cell culture arrays allow high throughput screening in response to different doses of drugs with discrete concentration gradients. They also evaluated other cellular responses such as nuclear size and membrane permeability thoroughly. Wlodkowic *et al*. reported a PDMS-based cell array consisted of 440 micromechanical traps to screen anti-cancer drugs in real-time [36]. In this kinetic on-chip assay, researchers investigated the staurosporine-induced tumor cell death over human promyelocytic leukaemia (HL60) and histiocytic leukaemia (U937) cell lines. Utilizing this platform realizes high throughput screening and automated single-cell microarray cytometry with a smaller number of cells and eliminated the need for single-pass flow cytometer measurements.

Li *et al*. developed a microfluidic chip to evaluate the cytotoxicity of two oxygen-dependent anti-cancer drugs (TPZ and Cisplatin) to A549 cells [37]. They tested a single drug dose under different oxygen tensions.

Wong *et al*. exploited a droplet-based microfluidic device for drug screening on cancer cell lines and cells separated from human primary tumor [38]. All samples were tested on-chip against four anti-cancer drugs, namely Bortezomib, Epirubicin, Cisplatin, and Vorinostat, for 5 assay conditions based on sample input size and throughput requirement. This platform provides a rapid and low-input sample drug screening of potentially all types of cancer.

Microwell chips are widely used to trap cells or tissue slices for drug screening. Ma *et al*. described a micro-gap plate with 96 units arranged in the standard format of 96 well plate to test the response of breast cancer cell lines (MCF7 and MDA-MB-231) and patient primary samples to two anti-cancer drugs, namely Cisplatin and Docetaxel [32]. Their presented design consisted of culture wells, micro-gaps and microchannels. The small height of micro-gaps helped to cell conservation during solution exchange.

Wang *et al*. engineered a multifunctional microfluidic device to estimate the activity-toxicity in a cell model simultaneously [39]. The optimum drug combination with maximum efficacy and minimal toxicity was predicted by adjusting different concentration ratios of three anti-cancer drugs, including Dinatin, Diosmetin, and Cisplatin, on HEK293 cells.

Mitxelena-Iribarren *et al*. developed a microfluidic platform for nanoparticle-based chemotherapeutic drug screening [40]. They used integrated microstructures in the cell chamber to enhance fluid mixing and drug-cell interaction. Several methotrexate-based treatments were tested over an osteosarcoma cell monolayer. This platform provides a powerful tool for simultaneous screening of up to five different drugs and shows that nanoparticles are a promising target therapy for cancer therapy.

2.2. Three-dimensional cell cultures

Yu *et al*. developed a droplet-based microfluidic system for anti-cancer drug screening on alginate beads with entrapped breast tumor cells [41]. Multicellular aggregates create a three-dimensional environment to encapsulate cells for on-chip incubation. The authors tested the drug response of spheroids originated from LCC6/Her-2 breast tumor cells at four concentrations of doxorubicin. Utilizing this platform with the precise positioning of beads, they showed that multicellular resistance is higher than standard monolayer culture.

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Dereli-Korkut *et al*. reported a 3D microenvironment with microcirculation consisting of three PDMS layers to monitor the dynamic responses of potential or clinical anti-cancer drugs (Tarceva, Staurosporine, and TNF-α with cycloheximide) [42]. The bottom layer has microchambers with cancer cells (human ductal breast epithelial tumor cell line (T47D), human non-small cell lung cancer cell line (PC9), and adult human dermal blood microvascular endothelial cells (HMVEC)) encapsulated in a hydrogel, the middle layer is a permeable membrane and the upper layer has microchannels with seeded endothelial cells. The advantage of this platform over previous microfluidics without 3D extracellular matrix is that the cells are not in direct contact with the flow, and diffusion is the primary transport mechanism.

Lee *et al*. manufactured a 3D cell culture micropillar/microwell chip made of poly (styrene-co-maleic anhydride) which is ideally suited for drug testing on hydrogels of primary cancer cells [33]. The therapeutic efficacy of 24 anti-cancer drugs on a U251 brain cancer cell line and three primary brain cancer cells was measured and well correlated with in vivo previous results of the mouse xenograft model with the three primary brain cancer cells.

Ruppen *et al.* developed a microfluidic chip to form homogeneous spheroids from either cell lines or human primary cells by sedimentational trap in the microwells [43]. Chemosensitivity tests with Cisplatin were performed on primary spheroids cultured as monoculture with epithelial cells and co-culture using epithelial cells and pericytes. The comparison of chemoresistance showed that pericytes protect lung cancer epithelial cells against the chemotherapeutical drug. Thus, this microfluidic device can be used for chemosensitivity tests for personalized medicine.

Chen *et al*. developed a 3D cell culture microwell chip for anti-cancer drug screening [44]. They used several human cancer cell lines (colon cancer cells HCT116, breast cancer cells T47D and hepatocellular carcinoma cells HepG2) to form multicellular tumor spheroids for mimicking the ex vivo tumor micro-tissue model for drug screening research. After spheroids formation, two anti-cancer drugs (doxorubicin and paclitaxel) with desired gradient concentrations were injected into the microchannels. They have not reported the specifications of their applied concentration gradient generation. However, their results show that only three drug dosage is tested.

Liu *et al*. developed a pneumatic microfluidics for high throughput anti-cancer drug screening [45]. The advantage of this recyclable microfluidics over conventional 3D tumor culture system, is cell trapping and producing similarly-sized 3D tumor spheroids from human glioma U251 cells. This single-cell array provides the analyze of different response dynamics and apoptotic signals during chemotherapy with two clinically used anti-cancer drugs (Vincristine and bleomycin).

Hong *et al*. described a paper-based microfluidic with Hela cells, a human epithelial cell line derived from cervical carcinoma cultured with collagen hydrogel as a 3D scaffold and incubated in the device reservoirs [31]. They studied the cell response to a discrete concentration gradient of doxorubicin as a model anti-cancer drug. This hybrid microchip combining paper-based microfluidic and cell culture reservoirs is more cost-effective than traditional cell-based bioassays using multi-well plates, which is a promising platform for high throughput drug screening.

Integration of microfluidics and microelectrodes in a 3D tumour microenvironment is a powerful platform to accurately and rapidly monitor the response of cancer cells to different drugs. Pandya *et al*. engineered a microfluidic platform integrated with the microsensors to measure the electrical response of cancer cells seeded in a 3D gel matrix to a chemotherapeutic drug [46]. This device can delineate three cancer cells, including drug-susceptible, drug-tolerant, and drug-resistant, in less than 12 h. They tested five different drug concentrations (Carboplatin for B16-F10 and 4T1 cancer cell lines and Paclitaxel for prostate cancer cell lines).

Shirure *et al*. developed a microfluidic platform mimicking drug transport to the tumor cell lines and patient-derived breast cancer organoids through the vascular network [47]. Utilizing this tumor on-a-chip model, different features of tumor progression

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including cell proliferation, angiogenesis, cell migration, and tumor cell intravasation can be studied. Furthermore, chemo- and anti-angiogenic drugs can be tested on individual tumors for precision medicine. The device has three parallel tissue chambers which are separated by a porous membrane. The central tissue chamber is for the microvasculature and the two side chambers are for loading tumor samples. The fluid flow through the vasculature chamber due to the pressure difference between two fluidic lines.

Mulholland *et al*. also presented a diffusion-based microfluidic device to screen primary human prostate cancer cells, grown in 3D as a heterogeneous culture from biopsy-derived tissue. As proof-of-concept screening, a panel of prostate anti-cancer drugs (Cisplatin, Docetaxel and Enzalutamide) with eight different dosages were tested on spheroids derived from two patients biopsies [34].

Khoo *et al*. described a microfluidic device for the expansion of patient-derived circulating tumor cells (CTC) without pre-enrichment procedures [48]. In this platform, CTCs are co-cultured with immune cells on inverted dome-shaped elliptical microwells that provide the formation of CTC clusters within 2 weeks. The addition of inlets in the tree-like gradient generator facilitated the efficient screening of drugs as a combinatorial treatment [49].

3. Monodrug screening on a macroscale sample

Chang *et al*. demonstrated a microfluidic device to study the response of the live slice cultures to a range of drug doses in parallel [50]. They confirm the viability of both the organotypic and GBM xenograft mouse brain slice cultures prior to starting chemotoxicity tests. This PDMS device includes 80 inlet wells connected to downstream microchannels act as concentration source or sink. Sources deliver the medium while sinks prevent lateral spread between channels. The delivery channels act as a source, and their adjacent channels act as concentration sinks that prevent lateral spread between delivery channels. So, a prescribed concentration profile is transported to the tissue slice through the PTFE porous membrane. Later, Rodrigues *et al*. manufactured a thermoplastic version of this platform made in PMMA which features less drug absorption. This platform consists of a 40-well plate with an integrated channel network layer for multiplexed drug testing onto GBM xenografts and patient-derived colorectal cancer tumor slices [51].

Astolfi *et al*. described micro-dissected tumor (MDT) tissues on-chip for personalized therapy [52]. The MDTs were trapped by sedimentation into their respective wells in which they are stable and shielded from excessive shear stress. The advantage of their system is long term survival without the need for continuous perfusion due to the small size of MDTs (below 420 micron). A great superiority of this technique to spheroids on-chip is that it can be used for all types of solid tumors with no dependency on the ability of cells to self-aggregate.

Recently, a PMMA microfluidic chip to trap cuboidal-shaped microdissected tissues or "cuboids" is engineered [53]. The similarly sized cuboids (400 μm×400 μm×400 μm) derived from normal mouse liver and human glioma xenograft tumors (U87) were used and cultured within a collagen hydrogel layer on top of the microwell, with an air interface above and culture medium below. For proof-of-concept, cisplatin drug was tested in their eight-well microfluidic device with three different doses.

4. Combinatorial drug screening on a microscale sample

Drug combinations are interesting in cancer treatment for better therapeutic efficacy as compared to a single drug. Different strategies are used for implementing combinatorial drug screening. Concentration gradient generators and valve-based techniques are presented in Figure 2a,b.

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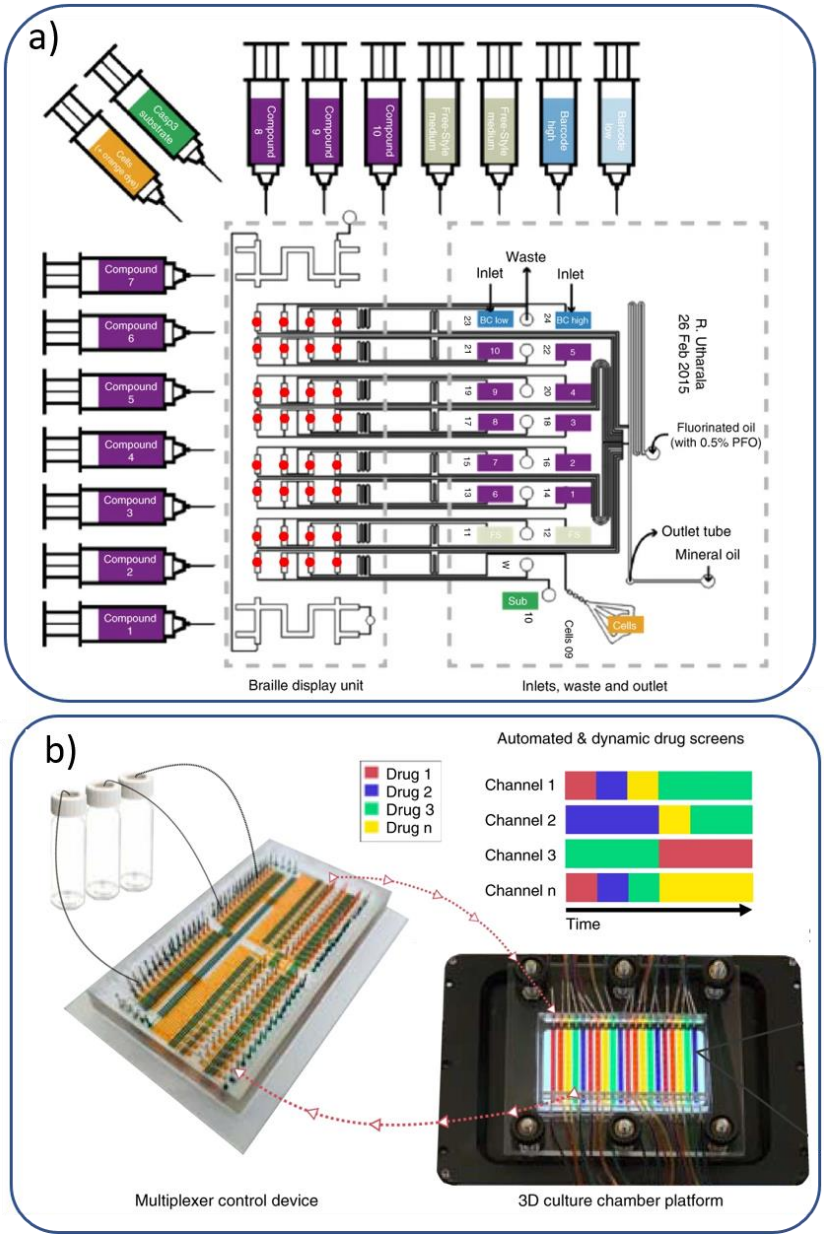

Figure 2. Combinatorial drug screening using; a) a plug-based microfluidic device using braille valves designed on pancreatic cancer biopsies. Reproduced with permission from Ref. [54] under an open access Creative Common CC BY license, b) A programmable membrane-valve-based microfluidic chip with PDMS-based 3D culture chamber. Reproduced with permission from Ref. [55] under an open access Creative Common CC BY license.

4.1. 2D cell cultures

In this way, a radial microfluidic concentration gradient generator of two model anticancer drugs is developed [56]. They tested 65 combinatorial compounds over human uterine cervix cancer (HeLa) cells This compact platform with fewer inlets and splitting- and mixing steps and the large range of predictable concentration gradient is suitable for high throughput drug screening.

Another combinatorial drug screening microchip generates up to 100 combinatorial concentrations from two input drugs [57]. However, this system has also not been applied for screening studies with cells. Similarly, Kim *et al*. described a programmable microfluidic cell array for combinatorial drug screening on PC3 prostate cancer cells either sequentially or simultaneously [58]. In their device 64 pair-wise concentration combinations of

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either Doxorubicin or Mitoxantrone with TRAIL (TNF-alpha Related Apoptosis Inducing Ligand) are tested on cells cultured in 64 independent cell culture chambers. This microfluidic device uses two concentration gradient generators to deliver different concentrations of two drugs to an 8×8 chamber array with two micropumps for the sequential drug treatment. The advantage of this system is no need for complex multi-layer structures or continuous medium perfusion. However, it should be programmed to individually control the opening and closing of the array of valves, which is a formidable task. This platform is also tested for Curcumin/TRAIL combinational chemotherapy in human prostate cancer PC3 cells [59].

Eduati *et al*. presented a plug-based microfluidic device for combinatorial drug screening of multiple drugs on pancreatic cancer biopsies [54]. They use braille valves design to generate up to 1140 plugs per biopsy by 10 different drugs and their pairwise combinations. This is an effective tool for rapid and personalized drug screening on small biopsy samples.

Ding *et al*. presented a print-to-screen (P2S) platform for high throughput screening combinatorial chemotherapy [60]. They tested 165 different drug combinations (2 or 3 drugs) of 10 clinically used anti-cancer drugs against ovarian cancer cells (SKOV-3) for proof-of-concept study. The essential part of their presented P2S platform is the multichannel microfluidic cartridge with the duty of printing a combinatorial droplet array. The cartridge compromises a pluggable microfluidic chip and an adapter to align the microfluidic chip with the dot-matrix pinhead. The assembly of the cartridge components is in a plug-and-play manner, and the adapter can be modified for other modular designs. Triplicate sets of all drug combinations generated in a 23×23 array format was spotted onto a gel array on PDMS substrate. Then, a sheet of the cell embedded gel was laid on the top of the printed drugs array. Their results showed that the highly cytotoxic compounds over the studied cells were combinations of three drugs.

Li *et al*. fabricated a plug-and-play, drug-on-pillar platform for combination drug screening using a microfluidic pneumatic drug printing platform [61]. They nano formulated chemotherapeutic drugs, which is more stable for storage, shipping, and subsequent rehydration [62]. MDA-MB-231 triple-negative human breast cancer cells were cultured in a 1536-well plate. Then a 48×32 printed drug array was laid on top of the cell culture plate. Utilizing this device, a 14×15 array containing 189 pairwise drug combinations for seven drugs with three concentrations and 21 single-drug groups, eventually, 1260 drug spots with six repeats in one 1536-well plate were tested. This system provides a new approach for efficient and convenient combination drug screening.

Shen *et al*. evaluated the mono-drug and combinatorial drug treatments of tumor cell lines (MCF-7 and HepG2) with two anti-cancer drugs (Doxorubicin and Cisplatin) [63]. They constructed a three-set of drug gradient with a unique microchannel structure in a PDMS-based microfluidic device. Since this gradient generator is flow rate-independent, this device is insensitive to operational conditions and is ideal for evaluating personalized medicine of tumor cells.

4.2. 3D cell cultures

Bai *et al*. developed a PDMS-based microfluidic device by co-culturing carcinoma cell with human umbilical vein endothelial cells (HUVECs) for anti-invasive and anti-metastatic combinatorial drug screening [64]. Fan *et al*. engineered a hydrogel-based microfluidic chip for Glioblastoma multiforme (GBM) brain cancer [65]. They cultured U87 cells to form spheroids in microwells and performed combinatorial treatment of Pitavastatin and Irinotecanin. They found that dual drug administration is more effective than those of individual drugs. This is a powerful platform for high-throughput screening and prolonged drug release and can be used in the future with small biopsy-derived tissue samples. Fabrication of photo-polymerizable poly(ethylene) glycol diacrylate (PEGDA)-based microfluidics is easier than polydimethylsiloxane (PDMS). Moreover, PEGDA blocks protein absorption and better mimics the 3D tumor microenvironment

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Zhang *et al*. developed a high-throughput pairwise drug-combination screening device for arbitrary number of drugs [66]. In this system, a logarithmic concentration gradient is generated between each drug pairs. Furthermore, for inlets arrangement a sudoku puzzle is solved to guarantee the adjacent drug pairs cover all the possible combinations. As a proof-of-concept, 7-drug combinatorial treatments were tested over 1032 cancer spheroids of pancreatic cancer patient-derived Xenograft cell lines on this three-layer chip. The advantages of this study are for precision medicine with high-throughput screening due to low sample consumption and fast analysis.

Khoo *et al*. recently evaluated the effect of anti-inflammatory combinatorial treatment of doxorubicin and aspirin on cell lines and patient-derived CTC clusters of breast cancer [67]. This microfluidic device could be a valuable clinical decision-support tool for tumor relapse prevention

Chang *et al*. developed an automated microfluidic platform based on Kim *et al*. platform [58] using pneumatic valves. The combinatorial treatment of doxorubicin and paclitaxel on a thin-gel 3D cell-culture from MDA-MB-231 and MCF-7 breast cancer cell lines is tested [68]. They assembled two air-controlled channel layers, two drug channel layers and two porous membrane layers top and bottom of a cell culture layer. So, the cell culture chamber layer is sandwiched between two layers of porous PDMS membranes.

Schuster *et al*. [55] developed an automated microfluidic platform for dynamic and combinatorial drug screening of tumor organoids. They cultured organoids from different samples, including MDA-MB-231 cell lines, patient-derived pancreatic tumor, and human-derived colon tissue samples. Utilizing this device, 200 different conditions (i.e., 20 different subsets of 10 individual chambers) can be tested. They evaluated individual, combinatorial, and sequential drug screens of chemotherapeutic drugs and found that the temporally modified treatments are more effective than static ones in vitro.

Table 1 summarizes the presented drug screening platforms for cancer therapy.

Table 1. Summary of studied anti-cancer drug screening platforms

Monodrug screening on a microscale sample			
Sample type	**tested drugs**	**number of dosages tested**	**Ref**
carcinoma (HepG2) cell line	daunorubicin, idarubicin, cisplatin, carboplatin, mitomycin, bleomycin and actinomycin D	8	[35]
human promyelocytic leukaemia (HL60) and histiocytic leukaemia (U937) cell lines	staurosporine	1	[36]
A549 cell line	Tirapazamine and cisplatin	1	[37]
suspended (Jurkat E6.1) and adherent (MDA-MB-231) cancer cell lines	Bortezomib, Epirubicin, Cisplatin and Vorinostat	7	[38]

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breast cancer cell lines (MCF7 and MDA-MB-231) and patient primary samples	Cisplatin and Docetaxel	4	[32]
HEK293 cells	Dinatin, Diosmetin, and Cisplatin	9	[39]
U-2 OS osteosarcoma cell line	methotrexate-based treatments (free MTX, MTX loaded Lecithin-PVA nanoparticles, MTX loaded Lecithin-Tween 80 nanoparticles)	2	[40]
3D spheroids from LCC6/Her-2 breast tumor cells	Doxorubicin	4	[41]
3D hydrogel from human ductal breast epithelial tumor cell line (T47D), human non-small cell lung cancer cell line (PC9), and adult human dermal blood microvascular endothelial cells (HMVEC))	Tarceva, staurosporine, and TNF-α with cycloheximide	1	[42]
3D hydrogel from human glioma U251 cell line and three primary GBM cells	24 different anti-cancer drugs	6	[33]
homogeneous 3D spheroids from either mesothelioma cell line (H2052) or epithelial adenocarcinoma primary cells	Cisplatin	mono-culture:10 co-culture (with primary pericytes):6	[43]
spheroids from carcinoma (HepG2) cell line	doxorubicin and paclitaxel	3	[44]

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similarly-sized 3D tumor spheroids from human glioma U251 cells	Vincristine and bleomycin	6	[45]
3D hydrogel from human epithelial cell line derived from cervical carcinoma (Hela)	Doxorubicin	5	[31]
3D gel matrix from B16-F10 mouse melanoma, 4T1 mouse breast cancer and DU 145 human prostate cancer cell lines	Carboplatin and Paclitaxel	5	[46]
cell lines and patient-derived breast cancer organoids	Bevacizumab, TGFβ, paclitaxel	4	[47]
3D spheroids from primary human prostate cancer cells	enzalutamide, docetaxel or cisplatin	8	[34]
Monodrug screening on a macroscale sample			
Sample type	**tested drugs**	**number of dosages tested**	**Ref**
both the organotypic and GBM xenograft mouse brain slice cultures	staurosporine	7	[50]
GBM xenografts and patient-derived colorectal cancer tumor slices.	17-AAG, Bortezomib, Cisplatin, DMSO, Mocetinostat, MLN 2238 and Parthenolide	1	[51]
micro-dissected tumor (MDT) tissues (four mouse xenografts derived from human cancer cell lines, three from ovarian and prostate cancer patients, and one from a patient with benign prostatic hyperplasia)	Carboplatin	1	[52]

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cell lines and patient-derived breast cancer organoids	Bevacizumab, TGFβ, paclitaxel	4	[47]
uniformly-sized cuboids of normal mouse liver and human glioma xenograft tumors (U87)	cisplatin	3	[53]
Combinatorial drug screening on a microscale sample			
Sample type	**tested drugs**	**number of combinations tested**	**Ref**
human uterine cervix cancer (HeLa) cells	5-fluorouracil and Cyclophosphamide	65	[56]
PC3 prostate cancer cells (PC3)	Doxorubicin or Mitoxantrone with TRAIL (TNF-alpha Related Apoptosis Inducing Ligand) either sequentially or simultaneously	64	[58]
human prostate cancer PC3 cells	Curcumin and TRAIL	64	[59]
two genetically different pancreatic cancer cell lines, xenograft mouse models and human solid tumour-derived cells	10 drugs: ACHP, AZD6244, Cyt387 GDC0941, Gefitinib MK-2206, PHT-427, Gemcitabine, Oxaliplatin, TNFα	56	[69]
ovarian cancer cells (SKOV-3)	Methotrexate, Adriamycin Hydrochloride, BCNU, Tamoxifen, Thalidomide, Celecoxib, Actinomycin D,	165 (2 or 3 drugs) Triplicate	[60]

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	Streptonigrin, Mitomycin, and Ellipticine		
MDA-MB-231 triple negative human breast cancer cells	doxorubicin, nilotinib, olaparib, capsaicin, tamoxi-fen, cisplatin, and tretinoin	210 (1 or 2 drugs) sextuple	[61]
tumor cell lines (MCF-7 and HepG2)	Doxorubicin and Cisplatin	3	[63]
3D hydrogel aggregates of carcinoma cell (A549 and T24) co-cultured with human umbilical vein endothelial mon-olayer cells (HUVECs)	MK-2206, AZD-0530, A83–01 and CI-1033	12	[64]
3D spheroids from Glioblastoma multi-forme (GBM) brain cancer (U87) cells	Pitavastatin and Iri-notecanin.	8	[65]
spheroids of pancreatic cancer cell lines (MIA PaCa-2) and patient-derived Xen-ograft cells	7 drugs: Cisplatin, Docet-axel, Doxorubicin, Gemcita-bine, Irinotecan, Oxaliplatin, 5-FU	28	[66].
cell lines and patient-derived CTC clus-ters of breast cancer	doxorubicin and aspirin	8	[67]
thin-gel 3D cell-culture from MDA-MB-231 and MCF-7 breast cancer cell lines	doxorubicin and paclitaxel	64	[68]
3D cell structures from a cancer cell line (MDA-MB-231) grown into aggregates, pancreatic tumor organoids from patient-derived samples, and colon organoids from	Gemcitabine, Paclitaxel, Fluorouracil (5-FU), Docet-axel, CPT11, Oxliplatin, Cis-platin	5 combinatorial for 3 pa-tients	[55]

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human-derived normal colon tissue samples			

5. Limitations of current microfluidic platforms for drug screening

Although microfluidic devices can be considered as a next generation platform for anti-cancer drug screening and personalized medicine, these devices have a number of bench-to-bedside translational challenges that need to be addressed. Broadly speaking, several preclinical aspects such as repeatability, safety, formulation, dose, the timing of drug delivery and most importantly, pharmacokinetics must be satisfied before the results of an in vitro drug delivery platform can move on to the human trials [70]. In terms of safety, while most microfluidic materials are biocompatible, there are several challenges associated with choosing the right microfluidic material. In other words, microfabrication technology hugely affects the selection of a microfluidic material. For instance, PDMS is the material of choice for the soft lithography process. While PDMS is biocompatible, transparent and oxygen permeable, leaching of uncross-linked oligomers from PDMS may adversely affect the inherent cell's behavior [71]. Moreover, due to the scalability issues associated with PDMS casting, PDMS-based microfluidic devices are not suitable for large-scale commercialized applications. Accordingly, shifting from PDMS to thermoplastic materials, especially cyclic olefin copolymer, and adopting the related microfabrication processes in academia can rectify this issue [72]. With the advent of 3D bioprinting technologies, biofabricating a viable sample at the organ level has been feasible [73]. These platforms can rectify cell viability for extended duration by including vascular networks on the bioprinted organ to facilitate exchanging the required oxygen and nutrients and removing the biological wastes. There is still huge room for incorporating bioprinted vascularized organs for drug screening applications that need to be explored.

Another translational challenge of a microfluidic platform for drug screening is the level of user-friendliness and external accessories they require for operation. While using on-chip micropumps and microvalves have been developed for most lab-on-a-chip applications, using these integrated components for high-throughput drug screening is still unexplored. On the other hand, adding these integrated components may overcomplicate the platform and decrease its user-friendly level, thus inhibiting its acceptance in clinical settings.

Another important challenge of in-vitro microfluidic platforms is the accurate study of the absorption, distribution metabolism and excretion, commonly known as pharmacokinetics. To this aim, the effect of a drug on various organs, including lung, liver, kidney and gut, should be studied simultaneously. In the context of anti-cancer drug screening, multi-organs-on-a-chip platforms should be coupled with tumor-on-a-chip one to better study the pharmacokinetics and related side effects of the drug. As such, a combination of these platforms with concentration gradient generators for high-throughput multi-drug screening can open up a new avenue in this field.

In the context of personalized medicine, the microfluidic devices should be compatible with patient-derived samples. According to the limitation of current microfluidic platforms, there is a lack of combinatorial drug screening on macroscale samples, e.g., a patient's tissue sample obtained through surgical biopsy. A continuous gradient is generated on a macroscale sample in a few of the current microfluidic devices. In this way, the recently microfluidic device developed by Rismanian *et al*. [74] with the capability of delivering two different reagents simultaneously on a microdissected tissue sample would be a promising platform as shown in Figure 3. Utilizing the presented microfluidic device, continuous linear gradients of two reagents could be generated along with the two different directions on a millimeter-sized sample (sample length and width). So, all possible

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combinations of two drugs would be distributed on the sample surface. Future works could be drug screening on the cancer tissue samples loaded in this microfluidic device.

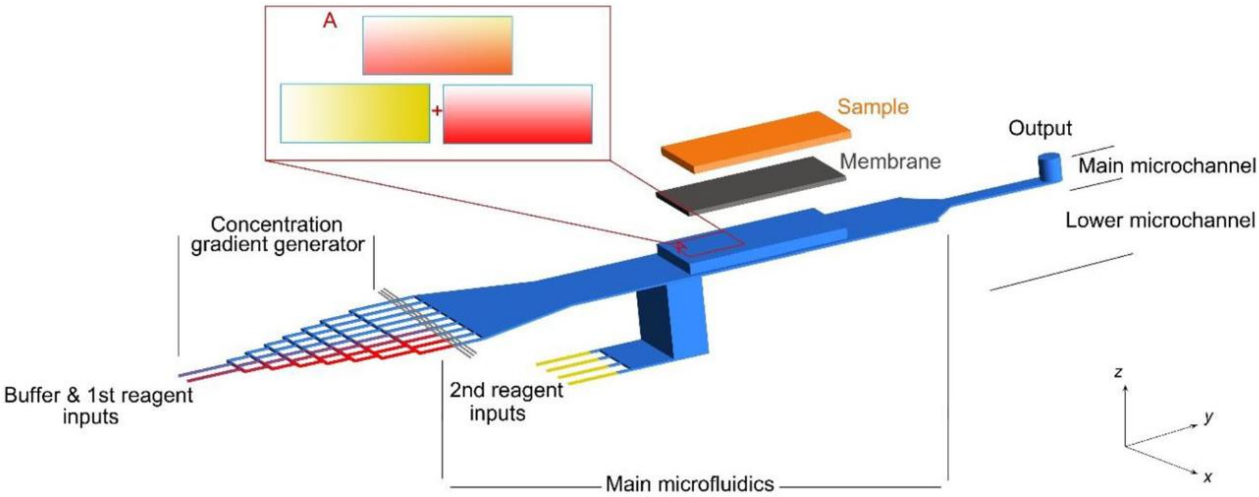

Figure 3. A microfluidic platform for delivering continuous concentration gradients of two different reagents on a tissue sample simultaneously. Reproduced with permission from Ref. [74]

6. Conclusion and future perspectives

The main objective of this review was to provide a guideline to select the appropriate microfluidic drug screening chips based on the available sample size. Accordingly, this study classified the current microfluidic platforms for drug screening into three categories based on sample size and the mode of drug screening (i.e., combinatorial or mono-drug). Specifically, the following microfluidic platforms for drug screening were critically overviewed: i) monodrug screening on a microscale sample, ii) monodrug screening on a macroscale sample, and iii) combinatorial drug screening on a microscale sample.

Moreover, we briefly discussed the limitations of current limitations of microfluidic platforms for anti-cancer drug screening and presented some possible solutions to tackle these problems. In particular, microfluidic material, microfabrication techniques and pharmacokinetics of drugs should be carefully considered in the research and development (R&D) phase before these platforms can be translated to real clinical settings. To this aim, more advanced multi-organs-on-a-chip platforms should be integrated with the tumor-on-a-chip system, while the user-friendly level and clinical acceptance of such complex platforms should be considered in the R&D phase. In addition, more microfluidic drug screening platforms study the continuous gradient of multi-drugs of a macroscale sample (i.e., patient's tissue) need to be developed. Finally, with the advent of 3D bioprinting technologies, all of the evaluated platforms can be 3D printed with a biocompatible material and can be further investigated for drug screening. Most importantly, 3D bioprinting technique can realize the drug screening on vascularized organs and totally revolutionize this field.

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