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On-chip anticancer drug screening – Recent progress in microfluidic platforms to address challenges in chemotherapy

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ABSTRACT

There is an increasing need for advanced and inexpensive preclinical models to accelerate the development of anticancer drugs. While costly animal models fail to predict human clinical outcomes, *in vitro* models such as microfluidic chips ('tumor-on-chip') are showing tremendous promise at predicting and providing meaningful preclinical drug screening outcomes. Research on 'tumor-on-chips' has grown enormously worldwide and is being widely accepted by pharmaceutical companies as a drug development tool. In light of this shift in philosophy, it is important to review the recent literature on microfluidic devices to determine how rapidly the technology has progressed as a promising model for drug screening and aiding cancer therapy. We review the past five years of successful developments and capabilities in microdevice technology (cancer models) for use in anticancer drug screening. Microfluidic devices that are being designed to address current challenges in chemotherapy, such as drug resistance, combinatorial drug therapy, personalized medicine, and cancer metastasis are also reviewed in detail. We provide a perspective on how personalized 'tumor-on-chip', as well as high-throughput microfluidic platforms based on patient-specific tumor cells, can potentially replace the more expensive and 'non-human' animal models in preclinical anticancer drug development.

1. Introduction

Cancer is the second primary cause of death worldwide leading with 1 in 6 people eventually dying from the disease (World Health Organization, 2018). In the United States alone, cancer is the primary cause of mortality in the elderly (age ≥ 80) after cardiac related deaths, and is the leading cause of death for people aged 40–79 (Cronin et al., 2018; Siegel et al., 2017). During the last century, the pivotal focus in cancer research has been to try to understand its multifactorial causes, especially the molecular biology aspects of cancer development. This has led to a vast number of exciting advances and has opened up new pathways for treating cancer (Hanahan and Weinberg, 2000; Weinstein and Case, 2008). One of the most effective ways of cancer treatment is chemotherapy (DeVita and Chu, 2008). History has shown that scientists and clinicians have always aspired to have a drug available to cure all types of cancer, but unfortunately the mechanisms of cancer development are not generic in nature: cancer is now famously known as "*the emperor of all maladies*" (Mukherjee, 2010). Every type of cancer and subsequent treatment regimen is peculiar and unique (Hanahan and Weinberg, 2011). In most cases, a combination of drugs is required to treat every type of cancer (Al-Lazikani et al., 2012; DeVita and Chu, 2008). Treatment options can also vary amongst patients having the same type of cancer due to their different genetic makeup that determines their subsequent cancer biology and associated drug sensitivity (Collins, 2010). Due to this inherent variability, there is an emerging shift towards personalized cancer treatment (Chin et al., 2011; Ocaña and Pandiella, 2010). The conventional drug development process involves pre-clinical trials, which includes drug screening in *in vitro* platforms and animal models, to check for drug specificity and toxicity needed for the development of new drugs (DiMasi, 2002; Nigam et al., 2018; Ocana et al., 2011). However, both are largely time-consuming, have limitations of their own, and are immensely costly with individual patient-specific trials being financially unfeasible (Sharpless and DePinho, 2006; Swinney and Anthony, 2011). Hence, in

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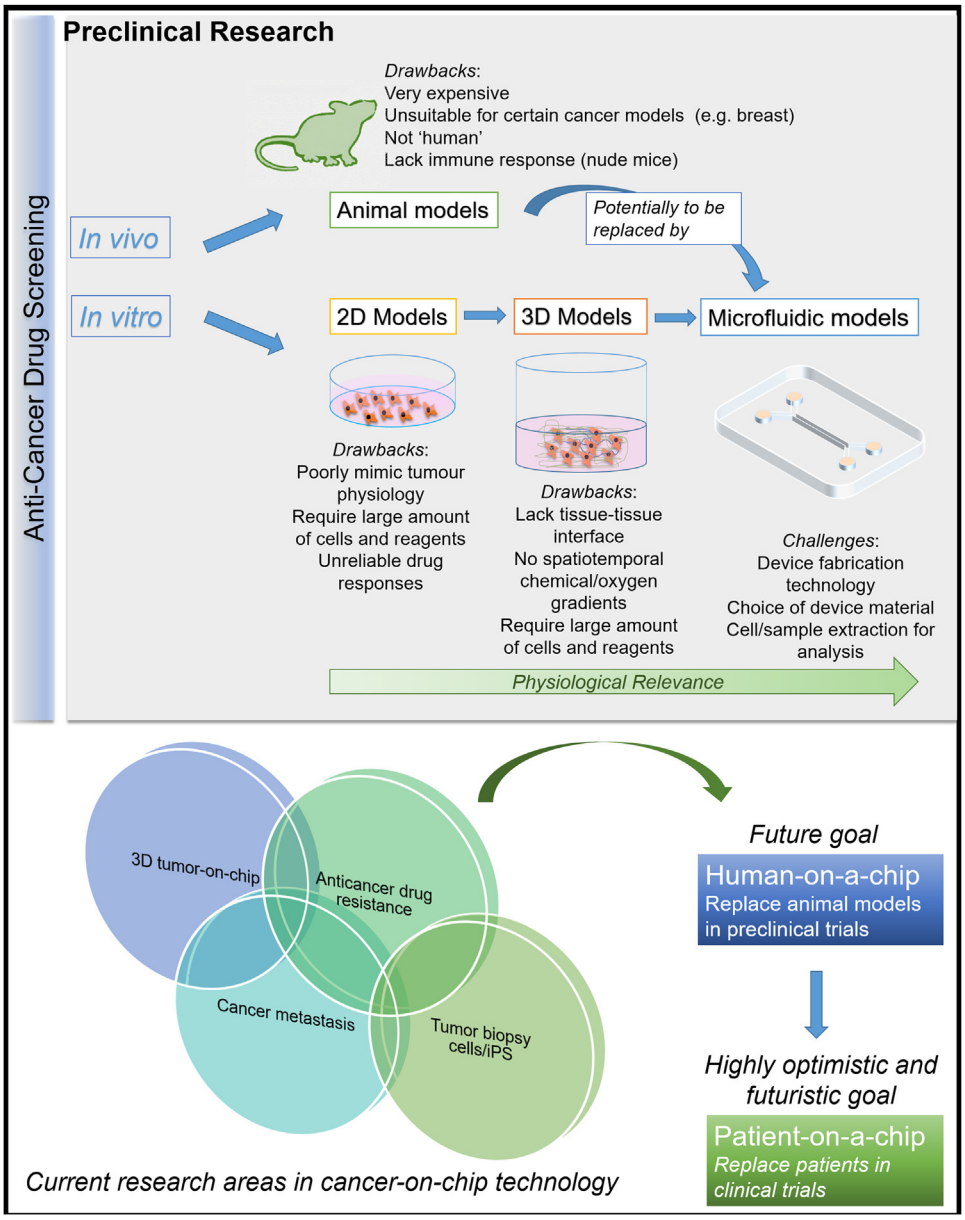

Fig. 1. Schematic diagram showing preclinical models for anticancer drug screening and the current research and development areas in the field of 'tumor-on-chip' for anticancer drug screening applications. Microfluidic tumor models are more biologically relevant, and in spite of the challenges involved, offer great growth and opportunities in the field of modelling responses to cancer drugs/therapy as compared to the conventional 2D and 3D models. The tumor-on-chip models definitely hold the promise to replace animal models in preclinical trials. Animal models lack the native human tissue-microenvironment and very often cannot correctly predict future drug responses in clinical trials.

order to move in the direction of personalized cancer treatment, which can significantly reduce the possibility and hence the cost associated with failing clinical oncology trials, the development of better and inexpensive *in vitro* tumor models is highly desirable (Fig. 1) (Hait, 2010; Hatzis et al., 2014; Hutchinson and Kirk, 2011).

Understanding the mechanisms of cancer development inside the human body is extremely difficult, as well as developing effective and reliable cancer drug treatments, especially if cancer physiology cannot be replicated using *in vitro* models. Modelling cancer began from growing cancer cells in two-dimensional tissue culture flasks and dishes, however, over time three-dimensional systems that better mimic the in *vivo* tissue/tumor environment became more widely used (Bhatia and Ingber, 2014; Lovitt et al., 2013). To get even more significant results that better mimic the physiological environment of cancer, there is a need to develop systems that offer more flexibility. This requires design of devices to control the tumor environment specific to a particular cancer type and enable organ-level cell interactions with dynamic biochemical and oxygen gradients (Huh et al., 2011). Microscale systems offer the capabilities to mimic the conditions found in the cancer microenvironment, such as a three-dimensional cell culture with multiple cell types, spatial-temporal nutrient diffusion, closer cell-cell placement, intra- and inter-cellular interactions, a native tumor microenvironment, generation of hypoxic conditions, and dynamic fluid flows (Huh et al., 2012, 2011). A single drug or combination of drugs needs to be tested for a period greater than a week on the same microdevice in order to be clinically relevant. Microfluidic devices contain micro-tissues that facilitate performing a number of tests to determine the types of tissue generated. The tests include live/dead assays, real time cell imaging, immunofluorescence assays, and quantitative PCR. These devices can also be designed to test for cancer cell-cell interactions including those with other cell types such as stem cells, and even different tissue types (Shin et al., 2012). The possibilities are endless and these models can assist in creating a more realistic picture of cancer cell biology including better *in vitro* studies of cell response to several drug types.

Here, we review recent microfluidic device developments for anticancer drug screening applications. Firstly, the types of 'tumor-on-chip' models based on the cell-culture type (two-dimensional or three dimensional) are discussed including the design and fabrication methods employed with emphasis on the drug screening results

Table 1
List of recently developed microfluidic tumor models (2D, 3D hydrogel, and 3D spheroids) to address one or more chemotherapy challenges for various cancer types.

Cancer Organ	Microfluidic Device Type	Co-Culture	Aim of the Study	References
Brain	2D	✗	Drug resistance	(Weltin et al., 2014)
	3D spheroid	✗	Drug resistance	(W. Liu et al., 2015)
	3D hydrogel	✗	Personalized therapy	(Chang et al., 2014)
			Combinatorial drug testing	(Fan et al., 2016)
Bone Marrow	3D hydrogel	✓	Drug resistance	(Khin et al., 2014), (Bruce et al., 2015)
Breast	2D	✓	Cytotoxicity and anti-metastatic testing	(Mi et al., 2016)
		✗	Non-invasive drug response monitoring	(Zhang et al., 2018)
	Single cell droplet	✗	Comparison of drug effectiveness across 2D, 3D, chip platforms	(Wong et al., 2017)
	3D spheroids	✗	Drug resistance	(Kim et al., 2012), (Sarkar et al., 2015)
			Multi-platform drug screening	(Yu et al., 2015)
			High-throughput spheroid generation via droplet microfluidics	(Sabhachandani et al., 2016)
			Combinatorial drug testing	(Lin et al., 2017)
	3D hydrogel	✓	Drug resistance	(Hwang et al., 2013), (Ozcelikkale et al., 2017)
			Cancer metastasis to bone	(Jeon et al., 2015)
Colon	3D spheroids	✗	Drug comparison	(Chen et al., 2015)
	3D hydrogel	✓	Multi-culture drug resistance	(Jeong et al., 2016)
			Drug resistance, anti-angiogenic testing	(Sobrino et al., 2016), (Phan et al., 2017)
Liver	2D	✓	Multi-culture drug resistance	(Jie et al., 2018)
	3D spheroids	✗	Drug combination	(Chen et al., 2015), (Patra et al., 2016)
	3D hydrogel	✓	Cell migration	(Kalchman et al., 2013)
		✗	Drug comparison	(Lei et al., 2018)
Lung	2D	✗	Drug combination	(Sun et al., 2017)
		✗	Non-invasive drug response monitoring	(Zhang et al., 2018)
		✓	Drug comparison, drug resistance; breathing lung cancer-on-a-chip	(Hassell et al., 2017)
	3D spheroids	✓	Drug resistance, drug combination	(S.W. Lee et al., 2018)
	Multicellular droplet	✗	Drug combination	(Du et al., 2013)
	3D hydrogel	✗	Cancer invasion	(Wang et al., 2013)
		✓	Drug resistance	(Hao et al., 2013), (Xu et al., 2013), (Dereli-korkut et al., 2014), (Ying et al., 2015), (Yang et al., 2018)
			Personalized therapy; Drug resistance	(Ruppen et al., 2014), (Ruppen et al., 2015)
Nasopharynx	Single cell droplet	✗	Personalized therapy	(Wong et al., 2017)
Ovary	3D spheroids	✗	Drug resistance	(Das et al., 2013)
Pancreas	2D	✗	Comparison of drug effectiveness across 2D, 3D and chip	(Beer et al., 2017)
			Personalized therapy, functional drug combination	(Eduati et al., 2018)
	3D spheroids	✓	Cell migration, drug combination	(J.-H. Lee et al., 2018)
Skin	2D	✗	Drug resistance	(Patel et al., 2015)
Urinary	2D	✗	Drug combination	(An et al., 2014)
	3D hydrogel	✓	Drug combination	(P. Liu et al., 2015)
Vasculature	3D hydrogel	✗	Anti-angiogenic drug comparison, formation of vascularized micro-organs	(Sobrino et al., 2016)
			Microvasculature; Human induced pluripotent stem cells as source of endothelial cells	(Kurokawa et al., 2017)

achieved in contrast to conventional 2D and 3D models. A summary of the cell types used for cancer modelling, drug screening, and materials used for 3D cell culture can be found in Table 1 and Table 2, respectively. Studies comparing two or more different drug types using 'tumor-on-chip' models are also reviewed, and a discussion is made on how they are more physiologically relevant compared to conventional *in vitro* models. The main question raised is, are 'tumor-on-chip' technologies capable of addressing the current challenges in anticancer drug screening and therapy such as drug resistance, cancer metastasis and variability of drug response among patients? An attempt is made to answer this question by updating advancements in microfluidic tumor models that study and analyse resistance towards anticancer drugs. In this regard, the potential of combinational drug screening as compared to single drug screening in overcoming or delaying the drug resistance is also reviewed. Next, a description of studies that analyse the effects of anti-metastatic drugs and agents on different types of 'tumor-on-chip' models is provided. A review of the potential of microfluidic devices to screen the effect of anticancer drugs on primary patient cancer cells is also included. Lastly, a brief summary is made of the limitations of microfluidic technology, and the review concludes with discussion of the future of microfluidic *in vitro* preclinical models for anticancer drug screening.

Readers interested in detailed device design and fabrication of 'tumor-on-chip' models, which is not covered in this article in depth, can refer to several recent reviews (Kashaninejad et al., 2016; Ronaldson-Bouchard and Vunjak-Novakovic, 2018; Shang et al., 2019; Tsai et al., 2017). There are several other important applications of 'tumor-on-chip' technologies such as optimization of nanoparticle transport and delivery, single-cell transcription analysis, isolation of circulating tumor cells (CTCs), proteomic analysis, and analysis of cancer cell metabolism. However, these topics are beyond the scope of this review and more details can be obtained from these excellent review papers (Dong et al., 2013; Sun et al., 2018). Recent developments in three-dimensional cellular scaffolds for microfluidic devices with focus on scaffold materials for tissue engineering as well as drug assay applications have also been recently reviewed (Wu et al., 2017). Cancer metastasis poses a big hurdle in cancer treatment and this process is being mimicked, analyzed and understood with microfluidic platforms, which is also the subject of detailed reviews (Caballero et al., 2017; Lee et al., 2016; Ma et al., 2018; Sleeboom et al., 2018; Xu et al., 2018).

Table 2
Types of natural and synthetic hydrogels used in tumor-on-chip drug screening models (reviewed in this paper).

Hydrogel Material	Cross-linking strategies	Advantages	Disadvantages	Reference for Microfluidic Tumor Models
Collagen type – 1	pH, temperature	• Majority of tumor microenvironment is composed of collagen type – 1 • Multiple cell-adhesion sites • Easily remodeled by the cultured cells	• Batch-to-batch variability • Weak mechanical properties • Narrow range of stiffness • Expensive • Highly sensitive to temperature	(Khin et al., 2014), (Bruce et al., 2015), (Hwang et al., 2013), (Ozcelikkale et al., 2017), (Jeong et al., 2016), (Kalchman et al., 2013), (Hao et al., 2013), (J.-H. Lee et al., 2018)
Fibrin	Thrombin and calcium ions (enzymatic activity)	• Nano- and micro- architecture mimics native extracellular matrix • Broader range of stiffness (~1–30 kPa)	• Batch-to-batch variability • Short-term cell culture	(Jeon et al., 2015), (Sobrino et al., 2016)
Matrigel or Basement Membrane Matrix	Temperature (protein precipitation)	• The "golden standard" for tumor spheroids, especially, for primary cancer cells	• Batch-to-batch variability • Murine-derived	(Hwang et al., 2013), (Shin et al., 2013), (Xu et al., 2013), (Wang et al., 2013), (Ying et al., 2015), (P. Liu et al., 2015)
Sodium alginate	Calcium Chloride	• Low cost • Blank cellular matrix highly suitable for custom modifications for cell adhesions • A good addition to composite hydrogels	• Require chemical modification for addition of cell adhesion sites (RGD peptides)	(Lin et al., 2017), (Yu et al., 2015), (Sabhachandani et al., 2016)
Agarose	Chain entanglements	• Suitable medium for impedance measurement • Quicker gelation	• Require chemical modification for addition of cell adhesion sites (RGD peptides)	(Lei et al., 2018)
PuraMatrix®	Salt-based gelation	• Synthetic • Devoid of animal-derived factors which might influence cancer cell response	• Highly sensitive to even low amount of salts • Extra care during handling	(Dereli-korkut et al., 2014)
Polylactic-co-glycolic) acid (PLGA)	Electrospun nanofibers	• Synthetic polymer • Easy to manipulate for porosity and degradation	• Hydrophobic • Degradation products	(Yang et al., 2018)

2. Current cancer microfluidic devices

The microfluidic devices were first introduced in the 1990s for use in cell culture and analysis and the field has subsequently undergone a huge transformation since then. The type of cell culture inside the channels determines whether devices are categorized as 2D or 3D (Fig. 2). 2D microfluidic chips are designed to allow culture of cells or formation of a monolayer of cells on one surface of the channel. While 2D devices can be simpler, as described in more detail later, they can be modified according to the type of the organ where the tumor originates from. For instance, endothelial cells grown into a monolayer would mimic the native environment more than if they are encapsulated and cultured inside a hydrogel. 2D devices can also be multichannel with one channel on top of the other with a porous membrane separating the two (Fig. 3). This design offers the capability to have multiple cell types cultured on each side of the membrane, hence allowing to model complex organs/tumors in one chip.

3D devices require a compartmentalized design that for example can house a 3D hydrogel, with or without encapsulated cells depending upon the organ/tumor one needs to model. The most famous design is based on the capillary-burst valve model, which basically contains three adjacent channels along with an array of posts between them. The posts provide enough surface tension to hold the hydrogel/cells in the central channel. The nutrients are supplied using media flow in the adjacent channels (Fig. 2) and this design can be easily modified to have more than three channels. Microfluidic devices are also designed to aid in the formation of 3D tumor spheroids as well as being used for drug screening analysis at the single cell level, and these systems are described in detail in the following sections.

2.1. Two dimensional cancer microfluidic models for drug screening

Two dimensional (2D) cancer models used for drug screening offer a simpler cell culture platform and are relatively easier to design and fabricate. The 2D models consist of a cell monolayer which comes in direct contact with growth medium supplied with or without drugs and provides high surface area to volume ratios (Fig. 2). Despite 2D models not having the three-dimensional stromal characteristics present in the native tumor biology, they can incorporate other parameters that are otherwise difficult to include in 3D models. Examples of these parameters include: concentration gradient generators for media/drugs (An et al., 2014), physiometric devices such as biosensors for measuring parameters such as pH and presence of other cell metabolites (Weltin et al., 2014), drug combination testing capabilities (Jie et al., 2018), and devices for incorporating mechanical movements (Hassell et al., 2017). One such device developed was a first-of-its-kind transparent borosilicate glass chip microfluidic chip incorporating sensors for pH, glucose, lactate and oxygen to study adherent cancer cells in culture (Weltin et al., 2014). Stop/flow pump cycles have been applied for dynamic perfusion of cell culture media (with or without cytochalasin B, a biomolecule that inhibits actin filament formation) over a 2D monolayer of human glioblastoma multiform brain cancer cells. The device is designed with the cell culture regions being physically separated from the sensors. This integrated system is highly sensitive and measures slight variations in cell metabolism in response to the chemicals added to the cell culture medium (Weltin et al., 2014). Although the study did not test any anticancer drugs, the results generated by screening cytochalasin B (CB) validated that the system has the capability to screen anticancer drugs at very low doses.

2D devices can also help investigate different types of novel preliminary cell analysis tools that can later be adapted and modified for complex 3D microfluidic platforms. In a recent study, a new way to analyse cell responses to anticancer drugs at the single cell level was demonstrated by Zhang et al. (2018). Raman spectroscopy, a non-invasive technique, was used to differentiate between doxorubicin treated and untreated cells on an MgF_2-based PDMS (polydimethylsiloxane)

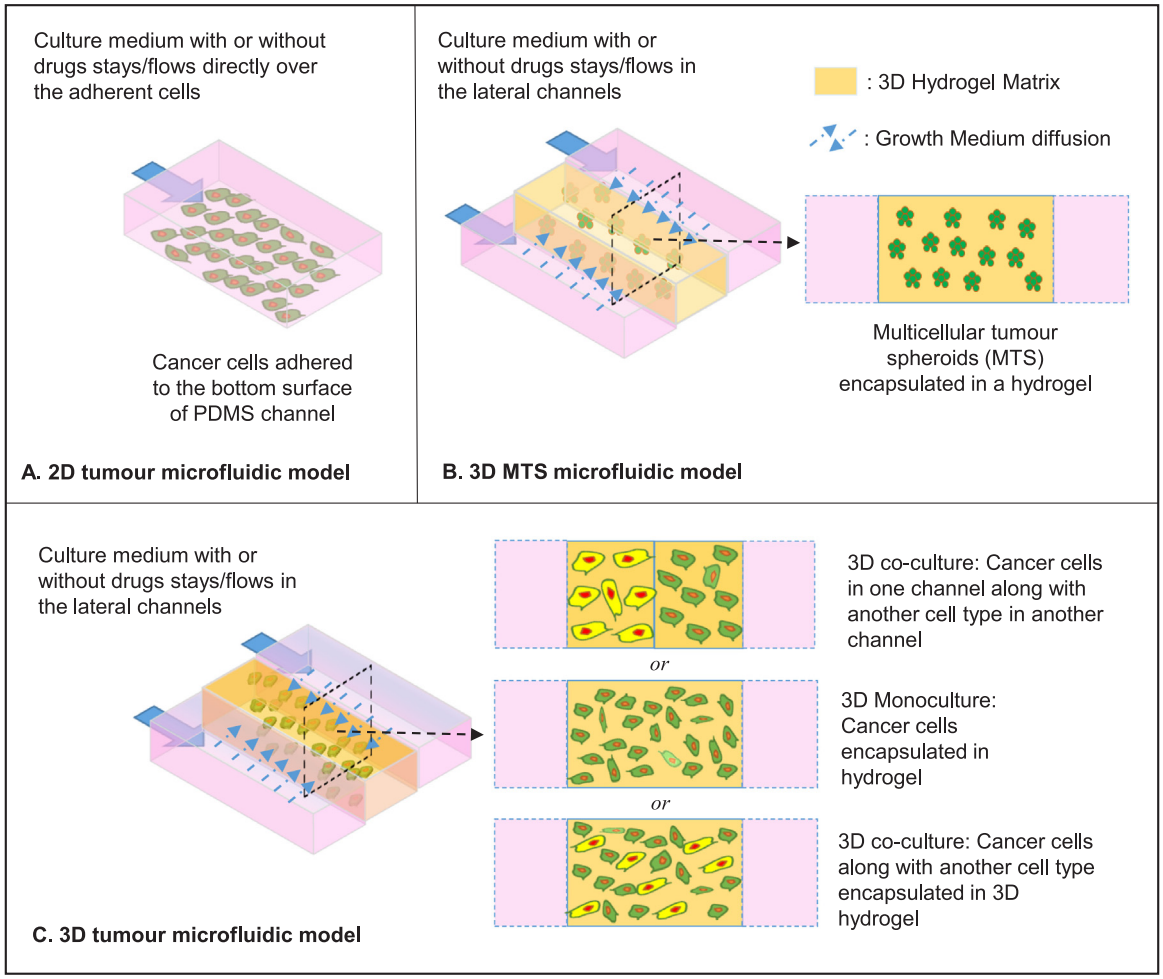

Fig. 2. Schematics showing typical cell-drug interface in the microfluidic models. **A.** 2D tumor model on a PDMS microfluidic device shows a monolayer of cancer cells adhered to bottom surface (PDMS/glass). The monolayer could be of single cell type or multiple cell types in adjacent channels depending on the device design. **B.** A microfluidic device for the culture of multicellular tumor spheroids (MTS), which can be encapsulated in a variety of hydrogels. The central channel contains the hydrogel contained with the help of PDMS pillars (not shown here) and the lateral channels flanking it contain the medium, which provides nutrition to the cells. The medium can be either static or dynamic (perfused using a peristaltic or syringe pump). **C.** A typical microfluidic device for 3D cell culture and the design is similar to the one described in B. The number of channels can be increased depending on the culture (monoculture or co-culture).

device. The PDMS channels were enclosed at the top and bottom by MgF_2 glass plates that acted as optical windows to enable Raman spectroscopy (in the range 600 cm^{-1} to 1800 cm^{-1}) with minimal background noise. Over a 24 h period of drug exposure, three types of cancer cell lines were examined for biochemical changes. The spectral analysis showed clear distinctions between the drug-treated group and the control group for all the three cancer cell types (Zhang et al., 2018). The study, however, did not elaborate on how the device could be adapted to explore the same in cells cultured in 3D.

In another study, 2D co-culture models were developed to create cancer models that depicted three levels of cancer severity by co-culturing the breast cancer cell line MDA-MB-231 with human mammary epithelial cells (Mi et al., 2016). The applications of this anti-metastatic drug screening device is discussed in detail in Section 3.2. Two other recently reported 2D cancer models employed combinatorial drug screening on a monoculture of lung cancer cells (Sun et al., 2017) and a co-culture of liver and intestine cells (Jie et al., 2018), and both studies are discussed for their drug screening applications in the Section 3.1.2. In another study, pancreatic ductal adenocarcinoma (PDAC) human cell lines (PANC1, BxPC3 and MiaPaCa2) were cultured on a collagen-coated chip made of cyclic olefin polymer using perfusion and dielectrophoresis. All three cell types showed different biomolecular characteristics on the chip as compared to 2D and 3D spheroid cultures. A significantly higher amount of the drug cisplatin was required to reduce cell viability in the microfluidic chip compared with 2D or 3D cultures of the same cell type (Beer et al., 2017). The reason behind this could be that the cells in the chip exhibited different growth characteristics since they are present inside the ECM that mimics the complexity of native tissue, which is known to enhance drug resistance.

The famous “lung-on-a-chip” device (Huh et al., 2010) was modified to generate an orthotopic breathing lung cancer model using human cancer cells cultured with airway epithelial cells in one channel. A second channel beneath was cultured with endothelial cells and both cultures were separated from each other by a PET (polyethylene terephthalate) porous membrane or a PDMS membrane (Fig. 3A (i) and (ii)) (Hassell et al., 2017). The device is also equipped with two side channels on each side of the two main cell chambers connected to a vacuum for suction to create mechanical movements, such as breathing in this case. They compared two EGFR-targeted drugs, namely erlotinib and rociletinib, on a conventional well-plate format and found that rociletinib was significantly more effective than erlotinib (Fig. 3A (iii)). Drug resistance to the more effective drug rociletinib was found to increase significantly using the physiologically-breathing chips compared to both static chips and the static plate culture (Fig. 3A (iv)) (Hassell et al., 2017). However, both stromal and immune cells, which play a remarkable role in cancer drug resistance, were missing from this

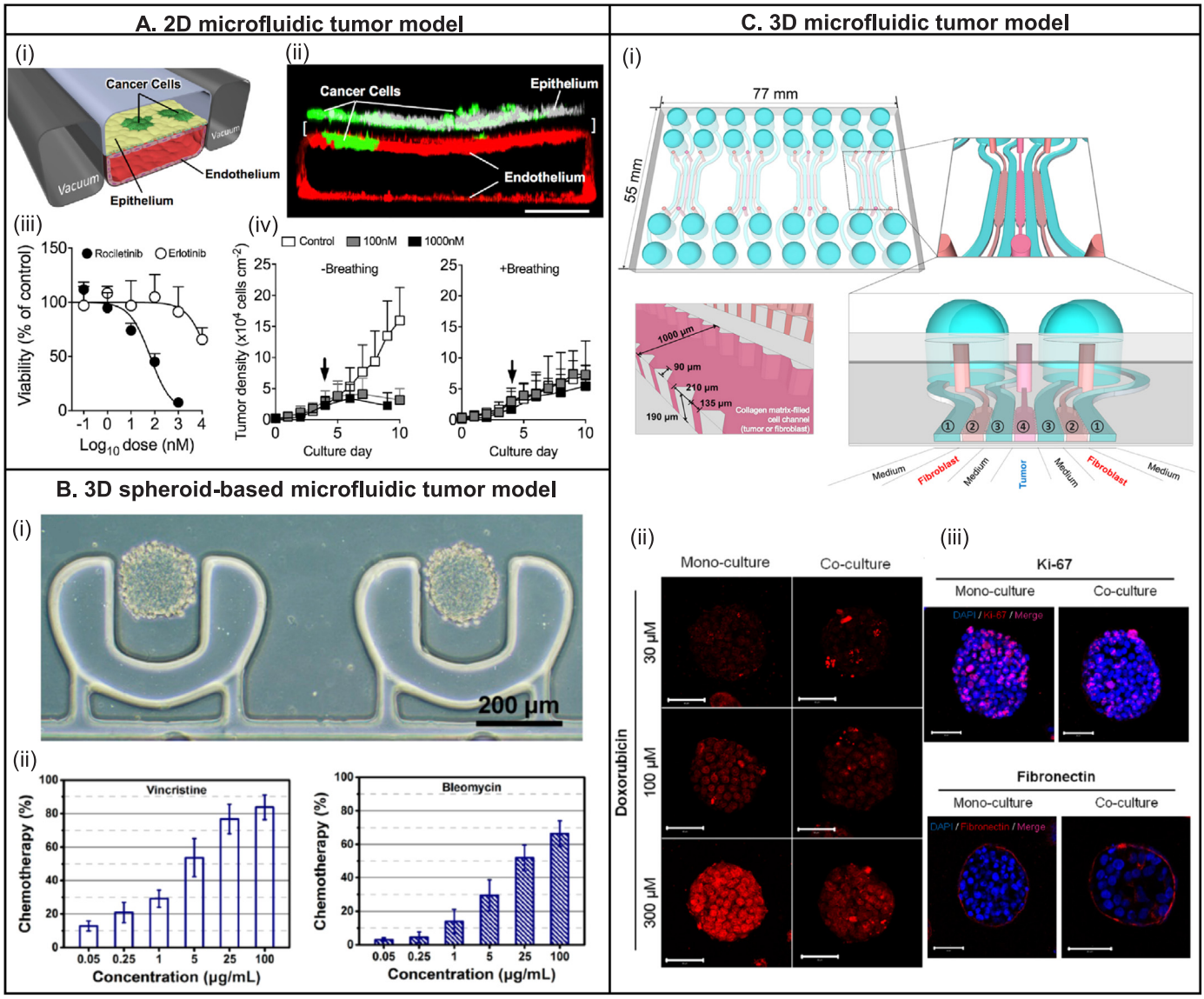

Fig. 3. Examples of the different types of microfluidic tumor models utilized for anti-cancer drugs screening. **A.** (i) Schematic picture showing the two-channel model of lung cancer cells co-cultured with epithelial and endothelial cells. (ii) Fluorescence image of the cross-section of the device showing the upper channel containing lung cancer cells (in green) and epithelial cells (in white), and endothelial cell monolayer (in red) in the lower channel (Scale bar, 200 µm). (iii) The drug rociletinib is more effective against the lung cancer cells than the drug erlotinib. (iv) The mechanical movements on the chip mimicking a breathing lung lead to a significant increase in drug resistance to rociletinib as compared to the non-breathing chips (arrow indicates time of drug addition). *Reproduced with permission from* Hassell et al. (2017). **B.** (i) Optical image of the tumor spheroids (human glioblastoma cells) after 10 days in culture. (ii) Dose-dependent time response and chemotherapy efficacy of the drugs vincristine and bleomycin. *Reprinted with permission from* W. Liu et al. (2015). *Copyright 2015, American Chemical Society.* **C.** (i) Schematic diagram showing design of a 3D tumor model device containing seven channels for culturing human colorectal cancer cells encapsulated in a hydrogel, a monolayer of fibroblasts, and perfusion of growth medium. (ii) Reduced sensitivity to the drug doxorubicin observed in the tumor spheroids (in co-culture with fibroblasts) as compared to the monoculture. (iii) Fibroblast-led production of Ki-67 and fibronectin in the ECM of the tumor spheroids (Scale bar: 50 µm). *Reproduced from* Jeong et al. (2016) *under the Creative Commons Attribution 4.0 International Public License* (https://creativecommons.org/licenses/by/4.0/).

model. Another orthotopic microfluidic device with a similar design approach but for breast cancer modelling was also tested for toxicity and efficacy of anticancer drug paclitaxel (Choi et al., 2015).

2.1.1. Opinion

Significant progress has been made with simple cell culture on 2D chips. The initial studies involving screening of drugs against a cancer cell monolayer grown on one surface of the chip have now transitioned to highly complex multi-channel orthotopic tumor models containing different cell types cultured together, which can yield more meaningful cancer drug responses. Addition of biosensors to monitor biophysical and cellular responses against cancer drugs in real-time is a huge advantage of the 2D tumor models and needs further exploration. Major challenges to overcome are integration of the multiple sensors in the microfluidic channels without affecting long-term cell culture, technically robust electronic circuitry that is compatible with the microfluidic chips and last but not the least, a compact, automated and scalable system with high-throughput capabilities.

2.2. Three dimensional (3D) cancer microfluidic models for drug screening

2.2.1. 3D cancer microfluidic model: tumor spheroids

Tumor spheroids or multi-cellular tumor spheroids (MTS) are self-assembled aggregates of cancer cells with diameters ranging from 100 to 1000 µm (van den Brand et al., 2017). Microscale systems allow formation of homogenous spheroids in a high-throughput fashion as

opposed to the conventional spheroid forming methods such as hanging drop methods, and use of low-adhesion 96-well plates (Hirschhaeuser et al., 2010; Nath and Devi, 2016). MTS within microfluidic systems are subjected to a dynamic perfusion environment instead of just relying on diffusion, and are highly efficient anticancer drug screening platforms for drug comparisons (Das et al., 2013; Patra et al., 2016). In one such device, quick formation of uniformly sized breast cancer (MCF-7) spheroids was achieved using a small number of cells. The drug resistance was found to be higher in the 3D microdevice when compared to a conventional 2D plate. This may be due to the inaccessible nature of 3D tumor spheroids, similar to *in vivo* tumors, compared to the 2D culture plate. Also, out of the three drugs tested, mitomycin-C was found to be much more toxic to the cells, followed by 5-fluorouracil and doxorubicin (Kim et al., 2012). This highlights the possibility of erroneous conclusions drawn from drug screening results obtained from 2D *in vitro* tumor models.

In another spheroid-based study, PDMS microwell arrays were used to form uniform spheroids of breast cancer cells that were screened against two anticancer drugs. Doxorubicin reduced the viability of breast cancer cell (T47D) spheroids in a dose-dependent manner, however, paclitaxel, completely disintegrated the spheroids at the same dosage. (Chen et al., 2015). The same device was also used to study testing of doxorubicin and paclitaxel on spheroids of two other cell lines HCT116 (human colon carcinoma) and HepG2 (hepatocellular carcinoma), but the effects of both drugs were drastically different for these cancer cell types. (Chen et al., 2015). As an alternative way to forming MTS, pneumatic microfluidics were utilized for uniform cell trapping and 3D tumor spheroid formation in a reusable microdevice (Fig. 3B (i)). 3D tumors were found to be more resistant to cancer drugs compared to those in 2D, which was further confirmed with an increase in mitochondrial depolarization and caspase-3 activity (both indicating cell apoptosis or death) after treatment with vincristine and bleomycin (Fig. 3B (ii)) (W. Liu et al., 2015). Section 2.3 discusses the generation of spheroids in a high-throughput manner using droplet microfluidics for anticancer drug screening.

2.2.1.1. Opinion. Even though conventionally generated tumor spheroids have cell-cell and cell-ECM interactions and can develop a necrotic core similar to native tumors, they still do not replicate the complexity of native 3D tissue architecture and mechanical forces (fluid flow, shear stress, etc). Whereas, spheroids inside a microfluidic platform can at least have those dynamic forces acting on them if kept under medium perfusion. The other factor that can be incorporated into the design is to add the spheroids to a hydrogel before placing them inside a microfluidic device, but that puts a limit on the size of the spheroids. Smaller spheroids cannot develop a necrotic core, but can be a means to study drug resistance. Despite this, spheroids lack one essential element, an internal vasculature providing essential nutrients and growth factors.

2.2.2. 3D cancer microfluidic models: cells in 3D hydrogel matrices

Cancer cells reside in a 3D environment inside the human body. To enhance the biological relevance of tumor models, hydrogels can be employed to strategically encapsulate cancer cells or cancer spheroids (MTS) to create better 3D microenvironments. These 3D models are designed to incorporate vasculature, dynamic forces, multiple cells types, and ECM in the same manner as present in native tissues. Various types of natural and synthetic hydrogels are used as matrices in microfluidic devices to contain cells in 3D for anticancer drug screening (Lei et al., 2018), reconstructing cancer models, or tumor diagnosis (Park et al., 2017; Sepantafar et al., 2017). Each hydrogel has unique properties with its own advantages and disadvantages, and the choice of the hydrogel depends on the type of study and the design of the microfluidic device (Song et al., 2014). Collagen type 1 and matrigel are the most commonly used hydrogels to culture cancer cells or MTS in microfluidic chips (Table 2). Such microfluidic chips with cells captured in 3D hydrogels are now well known as 'tumor-on-chip' devices (Fig. 3C (i)).

These devices, which house tumor models in an organ-specific environment, can enhance our understanding of cancer drug resistance, cancer metastasis, responses to combinatorial drugs, and help design patient-specific therapies using a patient's tumor cells. These application-specific devices with respect to their anticancer drug screening potential are reviewed in extensive detail in Section 3.

2.3. Droplet microfluidics: generation of spheroids on chip and drug screening on single cells

Droplet microfluidic systems are mainly designed to generate droplets at a quicker rate using confinement and capillary forces and can be engineered to contain single or multiple cancer cells within each droplet (Mashaghi et al., 2016). The major advantage of droplet microfluidic platforms are their ability to be used as a high-throughput platform, utilizing less number of cells per device as well as per assay, which is highly advantageous and suitable given the low number of cells available from patient tumor samples (Kang et al., 2014). Droplet microfluidics can also be used to generate multi-cellular tumor spheroids (MTS) in a high-throughput manner for drug screening on-chip (Du et al., 2013). One such study fabricated a one-layer based PDMS device to generate a core-shell alginate hydrogel encapsulating spheroids of breast MCF-7 cancer cells (Yu et al., 2015). However, screening with two drugs (tamoxifen and docetaxel) was performed in conventional culture flasks, and the screening results showed that 3D spheroids have lower drug sensitivity than the 2D cells. Research groups have used two varieties of breast cancer cells - drug sensitive and drug resistant MCF-7 cells – to screen anticancer drugs on microfluidic devices (Sabhachandani et al., 2016; Sarkar et al., 2015). They used a microfluidic platform to generate 1000 individual 3D alginate co-culture spheroids and tested doxorubicin and paclitaxel in a sequential treatment on the same platform. As compared to a single drug treatment, a 12% reduction in cell viability was observed after the combination drug treatment (Sabhachandani et al., 2016). These studies have great potential as many patients with drug resistance are treated with drug combinations by a 'hit and trial' method after surgery. More details on drug combination therapies are discussed in the Section 3.1.2.

Droplet microfluidics also enables easy and quick analysis of drug responses on single cancer cells. Single cell analysis is necessary to understand the mechanism behind chemotherapy failures such as acquired drug resistance due to intratumoral heterogeneity (Schmidt and Efferth, 2016). Epigenetic modifications, DNA damage repair, reduced apoptotic induction and improved stress adaptation can easily be analyzed at the single cell level (Liang and Fu, 2017). In this regard, one study encapsulated one cancer cell per droplet (MCF-7 drug sensitive and drug resistant breast cancer cells) as well as multiple cells in droplets and tested them against doxorubicin (DOX) (Fig. 4B (i), (ii)). Low viability was seen in the drug-sensitive variety but not in the drug-resistant variety (Fig. 4B (iii)). Drug-resistant cells also showed variable responses depending upon their retention capacity for extracellular materials. Cells with a lower efflux capability showed the least viability. Additionally, cell fusion in multicellular droplets helped both types of cells to survive higher DOX dosages (Sarkar et al., 2015). Droplet microfluidic platforms also offer rapid screening to compare a variety of anticancer drugs. In one such study, Wong group, tested two anticancer drugs (cisplatin and epirubicin) on a rapid-screening (~ 24 h) microfluidic platform with MDA-MB-237 breast cancer cells (Fig. 4A (i),(ii)) (Wong et al., 2017). The IC50 values (the drug concentration value at which 50% of cell death is achieved) were significantly higher in the microfluidic platform as compared to the conventional well plates. Cisplatin was the most effective drug on chip, while epirubicin efficacy was highest and consistent in all three platforms (Fig. 4A (iii)) (Wong et al., 2017). This shows that cancer cells respond differently to the therapies inside a microfluidic device as compared to the other *in vitro*

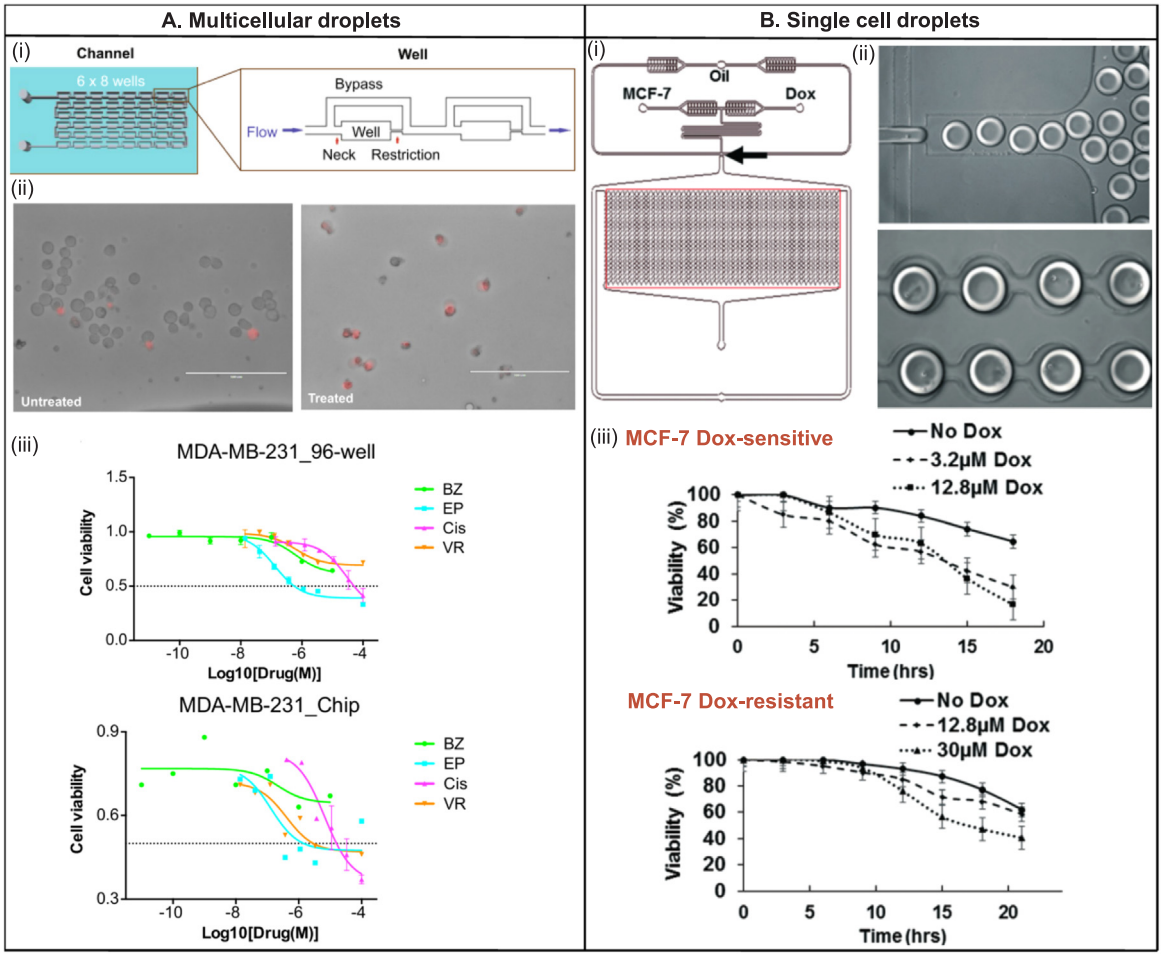

Fig. 4. Droplet microfluidics for high-throughput drug screening. **A.** (i) Microfluidic device for multicellular droplet generation of cancer cells. The design shows an array of 6 × 8 wells with each well containing two separate channels: one channel consists of a neck at the entrance, a well for droplet generation, and a restriction neck to prevent droplet escape, and another bypass channel which connects to the next well. (ii) Merged bright field and fluorescent images show deal cells (stained with ethidium homodimer 1) before and after drug treatment (Scale bar: 100 μm). (iii) Dose-dependent drug response of breast cancer cells of four different drugs (BZ: bortezomib, EP: epirubicin, Cis: cisplatin, VR: vorinostat) in a conventional well plate and on a microfluidic chip. *Reproduced from* Wong et al. (2017) *under the Creative Commons Attribution 4.0 International Public License* (https://creativecommons.org/licenses/by/4.0/). **B.** (i) The arrow points to the junction for droplet generation and the area below it shows the droplet docking array. (ii) Droplet generation (top) and droplets inside the docking array (bottom). (iii) Percentage viability of dox-sensitive and dox-resistant breast cancer cells (MCF-7) treated with 12.8 μM or 3.2 μM dox and 12.8 μM or 30 μM dox, respectively, as compared to the control (no dox) cells. *Reprinted with permission from* Sarkar et al. (2015). *Copyright (2015) Royal Society of Chemistry.*

models. Most importantly, they show that cells can survive higher drug concentrations, which is a significant finding because the conventional *in vitro* models show the opposite.

2.3.1. Opinion

All the studies reviewed above show that droplet microfluidics provides a highly suitable and efficient anticancer drug screening platform when the circumstances necessitate low drug volumes, high-throughput data generation, and low cell numbers. For instance, when only a low number of cancer cells can be obtained from tumor biopsies. The main challenge to overcome would be to reduce or eliminate the cell processing steps before they can be applied to the droplet microfluidic device, to make it an absolute automated high-throughput platform. Other future considerations would be to incorporate sensing technologies for analyzing each droplet and making droplet devices portable and a 'point-of-care' option. Single-cell analysis is immensely important for understanding cancer heterogeneity in a precise manner and droplet microfluidics offers a powerful tool to do exactly that.

3. Drug screening on chip: targeting current challenges in chemotherapy

The major challenges that presently face cancer therapy are: cancer drug resistance, combinatorial drug treatment optimization, cancer metastases, and designing personalized cancer therapies (Hutchinson, 2014; Zugazagoitia et al., 2016). The following sections describe how microfluidic tumor models can closely mimic natural cancer biology for drug screening applications, testing primary patient cells, observing and understanding cancer metastasis, and allow simultaneously testing of single or combinations of drugs. These sections are not mutually exclusive and some of the microfluidic models have tried to address one or more challenges in the same assay (Table 1). The anticancer drugs tested in the microfluidic platforms over the past five years are summarized in Table 3. The table also includes drug classification, the type of agent or mechanism and the target of the drug.

3.1. Cancer drug resistance

Drug resistance in cancer cells is caused by a number of factors including wild evolution in cancer cells (both genetic and epigenetic

Table 3
List and classification* of anticancer drugs screened in the tumor-on-chip models reviewed in this paper.

Name of the Drug	Mechanism of Action	Target	Reference for the Microfluidic Model
I. Chemotherapeutic (cytotoxic) drugs			
Irinotecan	Topoimerase I inhibitor	Topoimerase I	(Fan et al., 2016)
Doxorubicin	Topoimerase II inhibitor	Topoimerase II	(Shin et al., 2013), (Chen et al., 2015), (Sarkar et al., 2015), (P. Liu et al., 2015), (Sabhachandani et al., 2016), (Ozcelikkale et al., 2017), (Lin et al., 2017), (Zhang et al., 2018), (Jeong et al., 2016), (Lei et al., 2018), (Zhang et al., 2018)
Etoposide (VP − 16)			(Lei et al., 2018), (Hao et al., 2013)
Epirubicin			(Wong et al., 2017)
Genistein	Isoflavonoid	Topoimerase II, MMPs, and tyrosine kinase	(Jie et al., 2018)
Paclitaxel (Taxol)	Microtubule poison	Tubulin	(Chen et al., 2015), (Mi et al., 2016), (Sabhachandani et al., 2016), (Lin et al., 2017), (Jeong et al., 2016), (Xu et al., 2013), (Du et al., 2013), (Das et al., 2013), (Ying et al., 2015), (Sun et al., 2017), (J.-H. Lee et al., 2018), (S.W. Lee et al., 2018)
Docetaxel			(Yu et al., 2015)
Vincristine (VCR)			(W. Liu et al., 2015), (P. Liu et al., 2015)
Gemcitabine	Antimetabolite	DNA synthesis	(Xu et al., 2013), (P. Liu et al., 2015), (J.-H. Lee et al., 2018), (Eduati et al., 2018), (S.W. Lee et al., 2018)
Methotrexate			(P. Liu et al., 2015)
Bleomycin			(W. Liu et al., 2015)
Cytarabine (Ara-C)			(Bruce et al., 2015)
5-Fluorouracil			(Kim et al., 2012), (Du et al., 2013)
Mitomycin C	Antitumor antibiotic	DNA	(Kim et al., 2012)
Cisplatin	Platinum compound		(Wong et al., 2017), (Lin et al., 2017), (Ruppen et al., 2015), (Patra et al., 2016), (Sun et al., 2017), (Beer et al., 2017)
Oxaliplatin			(J.-H. Lee et al., 2018), (Eduati et al., 2018)
Carboplatin			(Das et al., 2013)
Cytochalasin B	Actin poison	Actin, GLUT inhibitor	(Weltin et al., 2014)
Temozolomide	Alkylating	DNA (guanine)	(Chang et al., 2014)
Dacarbazine			(Jie et al., 2018)
Melphalan	Nitrogen mustard alkylating agent		(Khin et al., 2014)
Pitavastatin	Statin compound	Antiproliferative	(Fan et al., 2016)
Vorinostat	Histone deacetylase (HDAC) inhibitor	HDAC enzyme group (class I and class II)	(Wong et al., 2017)
Resveratrol	Proapoptotic, antiproliferation, and anti-inflammation	Under-research (Aluyen et al., 2012), (Varoni et al., 2016), (Elshaer et al., 2018)	(Patra et al., 2016)
Tirapazamine	Hypoxia-induced selective toxicity; Experimental drug (not FDA approved)	DNA	
Staurosporine	Apoptotic: Experimental drug (not FDA approved)	Caspases	(Dereli-korkut et al., 2014)
II. Specifically-targeted anticancer agents/drugs			
Gefinitib	Inhibits phosphorylation	EGFR	(Xu et al., 2013), (Yang et al., 2018), (Eduati et al., 2018)
Erlonitib	Reversible tyrosine kinase inhibitor		(Dereli-korkut et al., 2014), (Hassell et al., 2017)
Rociletinib	Irreversible tyrosine kinase inhibitor		(Hassell et al., 2017)
Alvocidib (Flavopiridol)	Synthetic flavonoid: Experimental drug (not FDA approved)	Cyclin-dependent kinase 9 (CDK9)	(Du et al., 2013)
Sunitinib	Multikinase inhibitor	VEGFR, PDGFR, KIT (CD117), RET, CSF − 1R, and flt3.	(Kurokawa et al., 2017)
Sorafenib		VEGFR, PDGFR, serine/threonine kinase Raf	(Sobrino et al., 2016)
Linifanib		VEGFR, PDGFR, Tie2	
Cabozantinib			
Pazopanib	Receptor tyrosine kinase inhibitor	VEGFR	
Apatinib			
Axitinib			
Vandetanib			
Vemurafenib	Competent kinase inhibitor	BRAF kinase	(Patel et al., 2015)
Bortezomib	Proteasome inhibitor	Proteasome	(Khin et al., 2014), (Wong et al., 2017)
Tamoxifen	Anti-estrogen (inhibiting agent in mammary tissues)	Estrogen receptor	(Hwang et al., 2013), (Yu et al., 2015), (Mi et al., 2016)

* The information on the classification of drugs was obtained from (Holohan et al., 2013) and The DrugBank database (http://www.drugbank.ca/) (Wishart et al., 2018).

changes) (Shaffer et al., 2017), the tumor microenvironment (Polyak and Kalluri, 2010), and signals from other cell types in the vicinity (coordinated cellular behavior) (Gottesman, 2002; Maier et al., 2005). Anticancer drug resistance can develop in both chemotherapy as well as targeted molecular therapies. The molecular mechanisms behind cancer drug resistance are now well known (Cree and Charlton, 2017), and this awareness necessitates that cancer biologists and oncologists stay one step ahead of the capability of cancer cells to evolve. Customizable preclinical cancer models, such as tumor-on-chip, can increase experiences with traditional anticancer drugs and enable design strategies to combat drug resistance build up (Patel et al., 2015; Rosa et al., 2014; Stock et al., 2016).

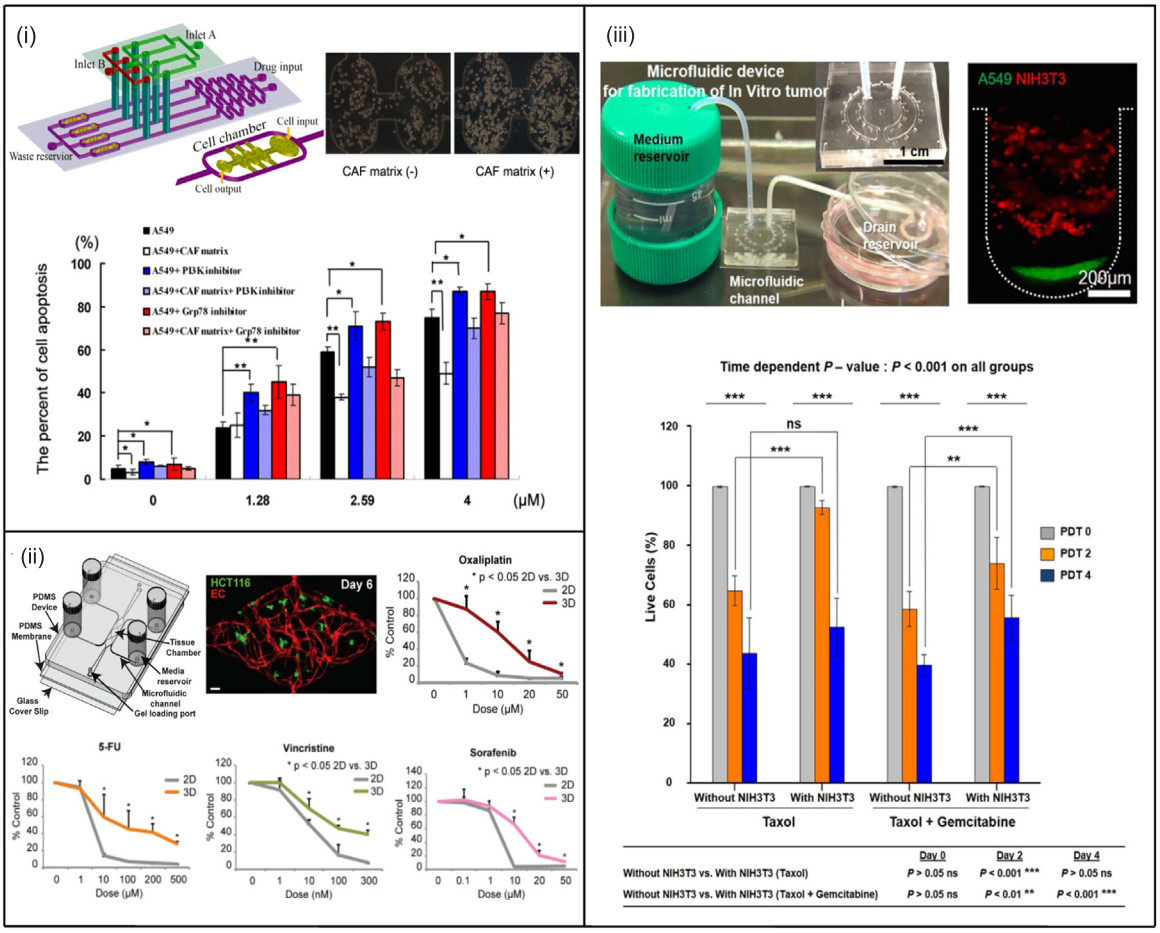

Fig. 5. Studying drug resistance on microfluidic models: **(i)** Top left: Schematics showing the microfluidic device with concentration gradient generators and 3D cell culture chambers. Top right: Lung cancer cells showing different morphology in the presence of cancer associated fibroblasts. Bottom: Percentage of cancer cells undergoing apoptosis subjected to six different conditions (with or without mixture of maintenance medium and CAF matrix in the presence or absence of the PI3K or GRP78 inhibitor) and treated with the anticancer drug paclitaxel (n = 3, *P < 0.05; **P < 0.01; cells were stained with Hochest33342 and PI). *Reproduced from Ying et al. (2015) under the Creative Commons Attribution 4.0 International Public License* (https://creativecommons.org/licenses/by/4.0/). **(ii)** Top left: 3D vascularized tumor model of human colorectal cancer cells (top center) on a microfluidic device. Top right and bottom: Dose-dependent drug response of four different drugs (cytotoxic as well as anti-angiogenic) and their comparison across two cell culture formats (2D plate and 3D chip). *Reproduced from Sobrino et al. (2016) under the Creative Commons Attribution 4.0 International Public License* (https://creativecommons.org/licenses/by/4.0/). **(iii)** Combinatorial drug screening: Top left: Microfluidic device with continuous flow perfusion arrangement. Top right: Fluorescently labelled lung cancer cells A549 (eventually forming multicellular spheroids) situated below the NH3T3fibroblasts in a chamber below a perfusion channel lined with endothelial cells (not labelled). Bottom: Combination of two drugs, namely, taxol (paclitaxel) and gemcitabine tested on the lung tumor spheroids with or without fibroblasts and the graph shows the percentage of viable cancer cells on day 0, 2 and 4. (**p < 0.01; ***p < 0.001; PDT: post-day of treatment) *Reproduced from* S.W. Lee et al. (2018); J.-H. Lee et al. (2018) *under the Creative Commons Attribution 4.0 International Public License* (https://creativecommons.org/licenses/by/4.0/).

3.1.1. Multi-culture tumor-on-chip models to study drug resistance

3.1.1.1. Role of cancer-associated fibroblasts. A tumor microenvironment contains cell types other than the cancer cells, which can directly or indirectly impact cancer progression and contribute towards drug resistance (LeBleu and Kalluri, 2018). Carcinoma-associated fibroblasts (CAFs) are myofibroblasts or activated fibroblasts associated with cancer tissue and promote cancer initiation, cancer progression and cancer metastasis (Kalluri and Zeisberg, 2006). CAFs are also responsible for enhancing anticancer drug resistance by secreting a growth factor namely, hepatocyte growth factor (HGF), which affects downstream signalling pathways, such as PI3/AKT mediated GRP78 (a glucose regulatory protein) upregulation, that enhances resistance towards various cytotoxic drugs (Deying et al., 2017). To extend cancer therapy as a personalized medicine, we need to gain more insights into the tumorigenic effects of these CAF cell populations and investigate direct drug therapies against their mechanism (P. Liu et al., 2015; Madar et al., 2013). A recent study by Ying *et al.* conducted on a 3D microfluidic device cultured lung cancer cells along with CAFs and monitored chemoresistance against paclitaxel (Fig. 5i). The apoptotic effects of paclitaxel were found to be significantly reduced due to the presence of cancer associated fibroblasts (CAFs) in the vicinity due to HGF-mediated GRP78 upregulation (Fig. 5i) (Ying et al., 2015). A similar trend was confirmed in another study where indirect fibroblast activation into myofibroblasts was induced by lung cancer cells. It was found that high expression level of GRP78 (a glucose regulatory protein) in myofibroblasts was responsible for drug resistance towards etoposide (VP-16). This drug resistance was overcome by inhibiting the GRP78 activity using epigallocatechin gallate (EGCG), an antioxidant found in tea (Hao et al., 2013).

In another preclinical microfluidic assay Khin et al., screened the drug melphalan in a co-culture of primary myeloma cells (derived from patients) and bone-marrow derived stromal cells. The resistance to melphalan was found to be significantly higher in the co-culture system as compared to the individual cells (Khin et al., 2014). Another study showed that the co-culture of the lung cancer cells with stromal cells led to a two-times decrease in the apoptotic rate of lung cancer cells in response to the drug paclitaxel as compared to the monoculture system

(Xu et al., 2013). In another 3D microfluidic study, tumor spheroids co-cultured with fibroblasts showed less sensitivity to both paclitaxel and doxorubicin due to the changes in fibronectin and Ki-67 contents in the extracellular matrix brought upon by the fibroblasts (Fig. 3C). It was also observed that paclitaxel was highly toxic to fibroblasts (Jeong et al., 2016). All the studies taken together show that stromal cells play a significant role in cancer progression, which traditional *in vitro* models do not consider. Since we are still far from a complete understanding of the role of these cells in varying the drug responses, CAFs need to be incorporated in future cancer models developed on microfluidic platforms.

3.1.1.2. Role of endothelial cells. Angiogenesis, which is a major cancer hallmark, leads to both cancer growth as well as metastasis in different cancer types, and is also a major target for cancer therapy (Hanahan and Weinberg, 2011). Endothelial cells play a key role in angiogenesis and, therefore, it is quite apparent that cancer cells should be studied together with endothelial cells. Several studies have incorporated endothelial cells into microfluidic tumor models (Dereli-korkut et al., 2014; Hassell et al., 2017; Jeon et al., 2015; Kalchman et al., 2013; Phan et al., 2017; Pradhan et al., 2018; Sobrino et al., 2016; Yang et al., 2018). For instance, Sobrino et al. developed perfused blood vessels on a chip and incorporated various types of cancer cell lines along with stromal cells to generate a realistic tumor model. Higher drug resistance to three drugs, namely, 5-fluorouracil, vincristine and sorafenib, was observed in the tumor-vascular model of colorectal cancer cells as compared to a 2D cancer model (Fig. 5 (ii)) (Sobrino et al., 2016). In the same study, the vascular micromodel without the tumor cells was targeted using anti-angiogenesis drugs. Single treatments with the drugs sorafenib and vincristine, lead to regression of blood vessels and vascular damage, respectively (Sobrino et al., 2016). While in the co-culture vascularized tumor model, a different drug combination worked effectively against both tumor and vasculature (more details in the next section). In another study, endothelial cells derived from human induced pluripotent stem cells (iPSC) were used to create a 3D vascular model and the anti-angiogenic drug sunitinib (100 nM) lead to a significant decrease in the blood vessel area (Kurokawa et al., 2017). Although the use of human iPSCs in that study to generate a micro tumor system is an excellent step towards personalized cancer medicine, the lack of a specific cancer cell type needs to be considered in future studies.

3.1.1.3. Role of intratumoral heterogeneity. Breast cancer reflects intratumoral heterogeneity perfectly with as many as five different subtypes of cells existing at a time, all responding differently to drug treatment (Prat et al., 2013). Even different types of breast cancer cell lines, for example, MCF-7 and MDA-MB-231, show different morphologies in 3D culture and respond to chemotherapy in a way contradictory to each other (Holliday and Speirs, 2011; Kenny et al., 2007). All of these factors need to be considered when designing breast cancer models for drug screening. In this regard, two types of breast cancer cell lines - ER-positive MCF-7 and ER-negative MDA-MB-231 – were homogeneously co-cultured in a 3D microenvironment on a microfluidic chip. The anticancer drug tamoxifen was tested and it significantly reduced the tumor size in monoculture (only MCF-7) but could not do so in the co-culture, where substantial drug resistance was displayed by the 3D co-cultured breast tumor model (Hwang et al., 2013). Even though this device closely mimicked the tumor microenvironment, it did not include endothelial cells or any other component, which ideally would mimic the tumor vasculature. This parameter was added in a first-of-its-kind “Tumor-microenvironment-on-chip” (T-MOC) platform, which more closely mimicked the tumor microenvironment compared to previous studies (Ozcelikkale et al., 2017). Both the ER-positive (MCF-7) and ER-negative (MDA-MB-231) cell lines were cultured in the presence of interstitial flow, plasma clearance, and quick drug transport into the tumor site. Compared to the 2D platform, both cell lines showed higher drug resistance to both doxorubicin as well as doxorubicin-loaded hyaluronic acid nanoparticles on the T-MOC platform. Furthermore, an elevated expression of $CD44^{+}$ and $CD24^{lo/-}$ markers implied the build-up of a drug-resistant phenotype in the MDA-MB-231 cells. This finding was confirmed by similar results obtained from immunocompromised mice experiments (Ozcelikkale et al., 2017).

3.1.1.4. Role of ECM. While the above studies indicate that cancer development is affected by intercellular signals, it is also strongly affected by the cell-matrix interactions and shear stress (Holle et al., 2016). The microdevice developed by Bruce et al. created a tri-culture model to more closely mimic those kinds of cues by controlling the matrix properties and providing shear stress to the cancer cells by use of dynamic fluid flow. This 3D co-culture model consisting of leukemic cells with bone-marrow stromal cells and human osteoblasts was found to be less sensitive to the cytotoxic effects of the anticancer drug cytarabine as compared to the 2D co-culture model (Bruce et al., 2015). This finding was attributed to the changes in the matrix stiffness caused by the remodelling effects of the dynamic flow conditions and the protection provided by the 3D microenvironment.

3.1.1.5. Opinion. The research studies summarized above indicate that *in vitro* on-chip cancer models can potentially help us determine which tumor types are more susceptible to drug-resistance, the extent of resistance build-up to different kinds of drugs, and the use of anti-cancer therapies to exploit new strategies for patient-specific therapies. None of these achievements and insights would have been possible using traditional *in vitro* models. These results are essential, act as successful benchmarks for tumor-on-chip devices, and should pave the way for further technological advances in determining therapies with improved cancer-killing power.

3.1.2. Combination drug testing on a chip to tackle drug resistance

The aim of drug therapy (chemotherapy) is essentially to kill the cancer cells that have metastasized to other parts of the body. Empirical clinical data over the last few decades has shown that cytotoxic drugs are most effective when given in combination to achieve additive effects as opposed to single doses (Chabner and Roberts, 2005; Lewis and DeVita, 1979). Each drug has a unique mode of action and the possibility of cancer cells developing resistance to the drug therapy can be decreased by employing a combination of drugs that target different mechanisms (Table 3). The main requirements for successful combination drug testing are: (i) to optimize the effective dosage for each drug, and (ii) to minimize the side-effects (DeVita and Schein, 1973). Combination therapies remain challenging because we must identify new effective combinations of drugs and determine the optimal combination and concentration suitable for each individual patient. Therefore, ideally we need to test a higher number of possible drug combinations on cells *in vitro* before we test them on patients in clinical trials.

Miniaturization of 2D or 3D cell culture systems with microfluidic technologies provides a practical solution to model *in vivo* physiological or pathological conditions for simultaneous testing of a variety of drug combinations at low-cost in a high throughput manner. In this respect, Sun *et al.* designed and developed a microfluidic device for precise combination drug screening of paclitaxel and cisplatin against lung cancer cells. Using the chip together with mathematical modelling, an optimal concentration for both drugs was obtained. The synergetic effect of the two drugs on cell viability reduction was more than each drug alone (Sun et al., 2017). However, this platform is not capable of combining more than two drugs at a time. A microfluidic platform to enable parallel processing of a combination of more types of drugs was demonstrated by Fan et al. by designing a high-throughput drug screening device that included a spheroid-based cell culture system. The device was not made with conventional time consuming silicon

wafer based fabrication methods, but instead used a quicker method based on photolithography of PEGDA (poly (ethylene glycol) diacrylate) to make microchannels (in less than two hours). They cultured brain cancer cells directly in the PEGDA device that were treated with a combination of drugs (pitavasatin and irinotecan) that showed lower cell viability compared to the cells treated with individual treatments of both the drugs (Fan et al., 2016).

Another study used lung cancer cells along with cancer-associated fibroblasts to form gravity-driven multicellular tumor spheroids in microwells surrounded by an endothelial cell monolayer at top with perfused medium flow. (Fig. 5 (iii)) (S.W. Lee et al., 2018). These co-culture spheroids displayed increased drug resistance to individual treatments of paclitaxel and gemcitabine as compared to the mono-culture spheroids. The combination treatment including both the drugs however lead to a significant decrease in the drug resistance in the co-culture fibroblasts (Fig. 5 (iii)), highlighting the synergetic effects of drugs with different modes of action against cancer cells (S.W. Lee et al., 2018).

Cancer drugs cannot distinguish between normal cells and tumor cells in the human body and therefore it is necessary to create co-culture *in vitro* models where the effects of the anticancer drug(s) on healthy non-cancerous cells can be studied (B. Liu et al., 2015). Such models allow optimization of the drug concentrations to achieve better drug efficacy outcomes with high cytotoxicity against cancer cells and lower toxicity against healthy cells. In this regard, Lin *et al.* designed a device to co-culture human breast cancer cells and hepatic cells and screened three anticancer drugs, namely, doxorubicin, cisplatin, and paclitaxel (Lin et al., 2017). The device used was an open-access system containing tissue arrays in the form of spheroids. The drug combination with concentrations - DOX 1 μmol/L, PTX 3 μmol/L and cisplatin 20 μmol/L – was found to be the most effective against the cancer cells (> 90% death rate) and was less toxic to hepatic cells (> 80% survival rate). They also compared the results obtained in the microchip to conventional 3D and 2D platforms and found the drug sensitivity for both the cell types to be lowest on the 3D chip as compared to the 2D or 3D plate culture (Lin et al., 2017).

While it is possible for new drug combinations to be tested, clinically established and successful drug combinations can also be tested and validated on microfluidic devices. One common example is the combination of gemcitabine and oxaliplatin for the treatment of pancreatic cancer. A microfluidic assay containing primary pancreatic cells tested two PKB (Protein Kinase B or Akt) inhibitors, namely, MK-2206 and PHT-427, and it was found they worked in a synergistic manner against the cancer cells (Fig. 6 (iv)) (Eduati et al., 2018). For colorectal cancer, the FOLFOX (5-flurouracil, leucovorin and oxaliplatin) drug combination is the clinical standard, and was tested on a vascularized 3D gel-based tumor model of colorectal cancer cells. FOLFOX proved to be highly toxic to the cells and after 96 h the tumor size was reduced significantly and the cytotoxic effects could be seen even after stopping the drug administration (Sobrino et al., 2016). The same study also targeted the vasculature-on-chip with a wide variety of anti-angiogenic drugs with different mechanisms of action. The drugs with the highest multi-kinase inhibition capacity and most effective in vascular regression and disruption were linifanib and cabozantinib, which inhibited three different types of kinase receptors. The tumor model was also used to determine the most effective drug combination against both cancer cells and the vasculature (Sobrino et al., 2016). In a follow-up study, the same model was modified to test different sets of drug combinations in a high-throughput manner (Phan et al., 2017).

3.1.2.1. Opinion. For testing of new drugs, microfluidic devices offer a cheaper and easier alternative in order to get more reliable drug screening outcomes on possibly all cancer cell lines, and more importantly with the potential to test patient-specific cells (Table 4). In addition, microfluidic devices offer the possibility to screen thousands of possible combinations of drugs against each type of cancer. This can help clinicians to make an educated and early decision on choosing the right combination and concentration of drugs in order to overcome drug resistance without affecting the healthy cells. Even novel drugs and therapeutics can be screened for their potential and toxicity, which will reduce the high costs associated with new drugs entering clinical trials by significantly improving their safety grades beforehand. However, to achieve this we need to develop large-scale platforms, which can screen drugs not only against single cells or spheroids but also with 3D tumor-on-chip models.

3.2. Cancer Metastasis Assays

The ability of cancer to invade and metastasize distant tissues in the body is often considered as the malignant cancer stage where it is highly unlikely a patient will fully recover without increasing the chances of relapses (Schein et al., 1975). Additionally, recent studies have indicated that metastasis can occur at any stage of the cancer progression and not just towards the end, as previously thought (Friberg and Nyström, 2015; Ji et al., 2003). A wide range of underlying biochemical and genetic factors determine the complex process of cancer invasion and metastasis in a human body (Fidler, 2003; Hanahan and Weinberg, 2000). In this section, we review microfluidic devices designed and developed to observe cancer cell metastasis as well as screen anti-metastatic drugs.

One of the most commonly encountered cancer metastasis leading to a high mortality rate is breast-to-bone (Bussard et al., 2008; Coleman, 2012; Weilbaecher et al., 2011). One of the events in the process of metastasis is extravasation, where cancer cells in the bloodstream transmigrate through the endothelium and invade a new tissue (Strilic and Offermanns, 2017). This process was mimicked on a compartmentalized 3D chip where side channels acted as blood vessels and a central channel held the bone-mimicking tissue (tri-culture of osteoblasts, MSCs, and endothelial cells in fibrin gel). The effect of the biomolecule adenosine was tested on the extravasation capability of breast cancer cells (Jeon et al., 2015). However, further studies are needed that can check the effects of currently available anti-migratory drugs in this microsystem.

The anti-migratory drug artemisinin (a plant-based drug) reduced the migration capability of liver cancer cells (HepG2) cells in a collagen I matrix within a compartmentalized 3D microdevice. This finding was supported by a reduction in the number of cancer cell protrusions as well as increased cadherin expression indicating strong cell-cell attachment. (Kalchman et al., 2013). In another lung cancer study, invadopodia (actin-rich cell membrane protrusions) formation was induced in A549 cancer cells using EGF (epithelial growth factor) within a 3D ECM hydrogel-filled microfluidic environment, and it was found to be significantly inhibited by galardin, a matrix metalloproteinase inhibitor (Wang et al., 2013).

The severity of the cancer also determines the drug response as highlighted in a breast cancer microfluidic study by Mi et al. They co-cultured breast cancer cells with human mammary epithelial cells to create three types of cancer models - mild, moderate and severe – using different ratios of the two cell types (Fig. 6i). Cytotoxicity and anti-metastatic testing with two drugs, namely, paclitaxel and tamoxifen was performed on all three models. The severe cancer model responded least to the drugs and the cells retained the highest migratory capability (Fig. 6 (ii)) (Mi et al., 2016). In a 3D spheroid-based microfluidic device, containing co-culture tumor spheroids of a human pancreatic cancer cell line and pancreatic stellate cells (PSCs), PSCs lead to increased cancer cell migration and EMT (epithelial-to-mesenchymal transition) marker expression. The drug cytotoxicity effect (gemcitabine and paclitaxel) was more on the PSCs and the reduction in PSC cell number lead to reduced migration of the cancer cells (J.-H. Lee et al., 2018). These studies advocate how the heterotypic cell-cell interactions can guide the drug therapy decision-making process.

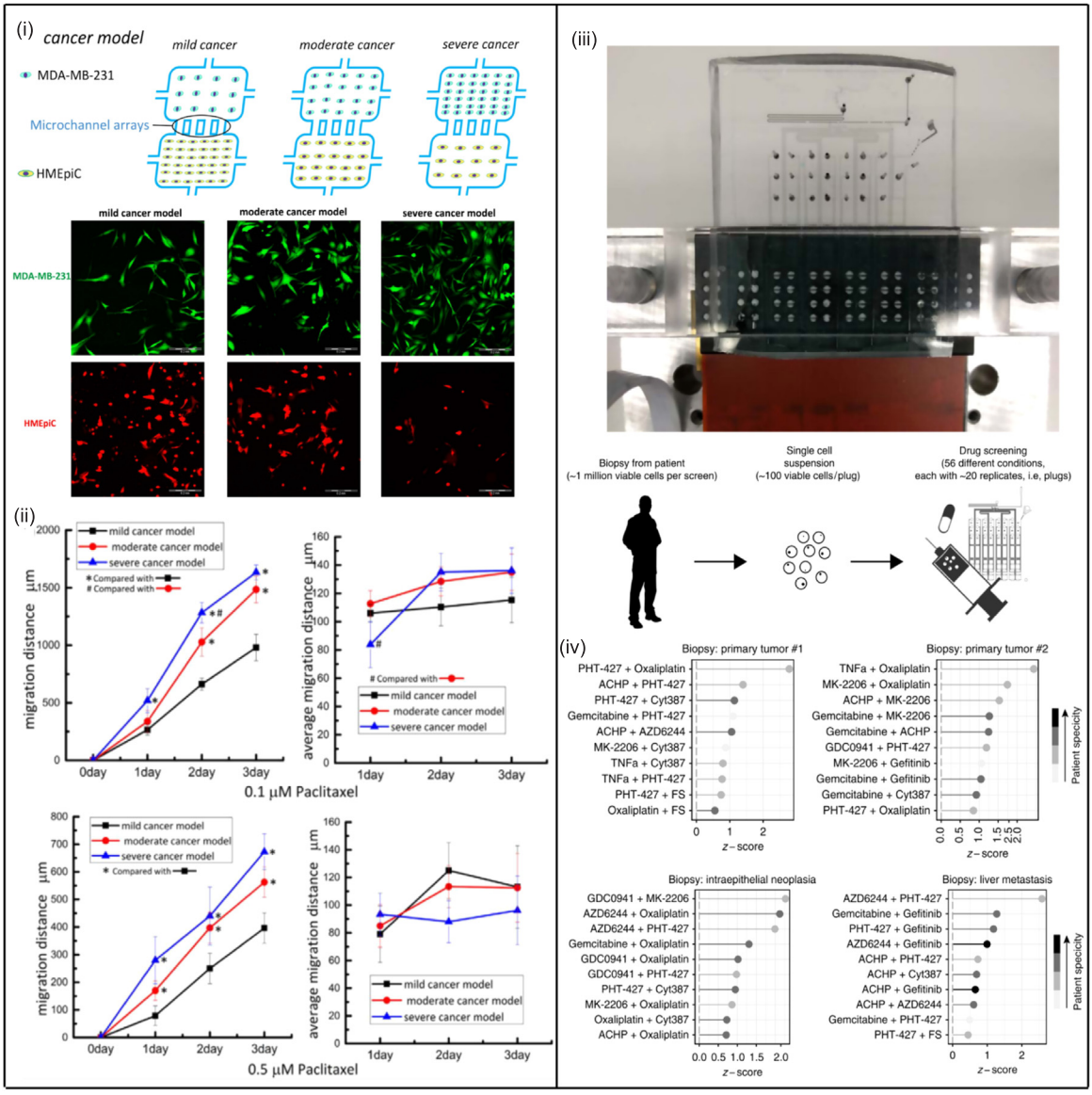

Fig. 6. Anti-metastatic drug screening: **(i)** Top: Schematic diagram showing three types of cancer models containing metastatic breast cancer cells (MDA-MB-231) in co-culture with human mammary epithelial cells (HMEpiC). Bottom: Both the cell types fluorescently labelled as seen in all the three different cancer models (Scale bar of all images: 200 μm). **(ii)** Migration distance analysis of all three cancer models when treated with the drug paclitaxel at two different concentrations (n = 3; *p < 0.05, #p < 0.05.). *Reproduced from* Mi et al. (2016) *under the Creative Commons Attribution 4.0 International Public License* (https://creativecommons.org/licenses/by/4.0/). Personalized medicine: **(iii)** Microfluidic chip connected to a Braille display so that the channels are aligned with the braille valves. This chip was treated the primary cells obtained from patient biopsies with ten different drugs and biomolecular agents. **(iv)** For each biopsy sample screened for anticancer drugs and biomolecules, the graph shows the most effective drugs or combination of drugs. Light grey color indicates drug combinations which were effective in all the samples and black color represents drug combinations which were highly specific, i.e., effective in only one patient sample. *Reproduced from* Eduati et al. (2018) *under the Creative Commons Attribution 4.0 International Public License* (https://creativecommons.org/licenses/by/4.0/).

3.2.1. Opinion

Cancer metastases is a very complicated and multi-step process and studying the effects of drugs on this process definitely requires better *in vitro* models and more research. We have summarized above only those studies that have screened drugs using cancer models designed to study metastasis. 3D organ-on-chip consisting of different organ types and functionalities would be required to investigate and understand the metastasis of cancer cells from one site to the other. “Metastasis-on-chip” platforms with cancer organ tissue and tubes of endothelium-lined vasculature connecting the cancer organ to other organs cultured on the same chip or on different chips should be the target. This would help identify drug targets and develop personalized therapies.

3.3. Cancer heterogeneity: personalized medicine testing

All human beings are genetically different and so are the tumors they develop if they get cancer (Kelloff and Sigman, 2012). Cancer biology research has led us to understand that cancer is a heterogeneous disease (Hanahan and Weinberg, 2011; Polyak et al., 2009; Polyak and Kalluri, 2010) and there is a growing need to address drug development in a tumor-specific and patient-specific manner (Fujii et al., 2016; Hutchinson, 2014). Most of the anticancer drugs that reach the stage of clinical development do not get approved due to failures in later stages of clinical trials (esp., phase-III) (Paul et al., 2010), which leads to slow drug development and overall higher costs. One of the major reasons for drug failure is the unexpected and varied response of the drug between tumors of different organs as well as between different patients

Table 4
Anticancer drug combination screening and drug comparison studies performed on microfluidic devices highlight their relevance as effective *in vitro* drug screening models for chemotherapy.

Cancer Cell Types	Anti-Cancer Drug(s) Tested	Highlights of Drug Screening	Reference
Type of Drug Screening: Drug Combination			
Human glioblastoma cells (brain)	Pitavastatin and irinotecan	Combination treatment more cytotoxic than single drug treatment	(Fan et al., 2016)
Human primary multiple myeloma cells and human myeloma cell lines	Bortezomib and melphalan	Bortezomib helped increase melphalan sensitivity in co-culture	(Khin et al., 2014)
Human breast adenocarcinoma cells	Mitomycin C, 5-fluorouracil and doxorubicin	Mitomycin C more cytotoxic followed by fluorouracil and doxorubicin	(Kim et al., 2012)
	Doxorubicin, cisplatin, paclitaxel	Drug combination of all three drugs more cytotoxic than single drug treatments	(Lin et al., 2017)
	Doxorubicin and paclitaxel	Combination treatment increased cell mortality by 12% as compared to single treatment	(Sabhachandani et al., 2016)
Human hepatocellular carcinoma cells	Doxorubicin and paclitaxel	Both drugs effective at high dosage leading to complete disintegration of spheroids	(Chen et al., 2015)
	Cisplatin, resveratrol, tirapazamine	No conclusive results on the best drug combination, but all three possible drug combinations lead to low cell viability as compared to single drug treatment	(Patra et al., 2016)
	Genistein and dacarbazine	Combination drug treatment inhibited cell metabolism and enhanced apoptosis as compared to single drug treatment	(Jie et al., 2018)
Primary lung cancer cells; Non-small cell lung cancer cell line	Gefitinib, paclitaxel, cisplatin, and gemcitabine	For cell lines, paclitaxel and cisplatin combination worked effectively. For primary cell co-culture, combination of cisplatin and gefinitib was most effective	(Xu et al., 2013)
Human non-small cell lung cancer cell line	Flavopiridol, paclitaxel and 5-fluorouracil	Most effective combination was observed to be 200 nM flavopiridol followed by 100 μM 5-fluorouracil	(Du et al., 2013)
	Gemcitabine and paclitaxel	Lower cell viability in the co-culture spheroids due to combination drug treatment than single drug	(S.W. Lee et al., 2018)
Human pancreatic cancer cell lines; human colorectal cancer cell line	Gemcitabine, paclitaxel and oxaliplatin	Combination of gemcitabine and paclitaxel lead to 50% cell mortality in spheroids	(J.-H. Lee et al., 2018)
Human colorectal carcinoma cells	5-Fluorouracel, oxaliplatin, vincristine, sorafenib, papozanib	5-Fluorouracil and papozanib combination most effective against vascularized tumor model	(Sobrino et al., 2016)
	Linifanib, tamoxifen, bortezomib, vincristine, CP − 673451, axitinib, sorafenib, mitomycin C, vorinostat, and gemcitabine	Combination of tamoxifen, mitomycin C, gemcitabine, and vorinostat, vincristine and axitinib most effective against vascularized tumor model	(Phan et al., 2017)
Patient pancreatic tumor biopsies	Gemcitabine, oxaliplatin, gefitinib	Varied drug response in each patient sample. Gemcitabine and oxaliplatin most effective in combination.	(Eduati et al., 2018)
Human Prostate Cancer Cells	Curcumin and TRAIL	Curcumin led to the enhancement of TRAIL cytotoxicity (> 90% cell death)	(An et al., 2014)
Type of Drug Screening: Drug Comparison			
Human metastatic breast adenocarcinoma cells	Cisplatin, epirubicin, bortezomib, vorinostat	Cisplatin more effective than the other three drugs	(Wong et al., 2017)
Human breast cancer cells (T47D)	Doxorubicin and paclitaxel	Paclitaxel found to be more cytotoxic than doxorubicin leading to complete disintegration of spheroids	(Chen et al., 2015)
Human colorectal carcinoma cells	Doxorubicin and paclitaxel	Large spheroids less sensitive to both drugs but least to paclitaxel than doxorubicin	(Chen et al., 2015)
	Doxorubicin and paclitaxel	Presence of fibroblasts reduced sensitivity of both the drugs; Paclitaxel highly cytotoxic to the fibroblasts	(Jeong et al., 2016)
Human hepatoma cell lines	Doxorubicin and etoposide	Doxorubicin more cytotoxic as compared to etoposide	(Lei et al., 2018)
Human non-small cell lung cancer cells	Erlotinib and rociletinib	Rociletinib more cytotoxic than erlonitib on the breathing lung-on-a-chip	(Hassell et al., 2017)
Human endothelial cells	Carbozanitib, Axinitib, apatnib, linifanib, sorafenib, papozanib, vandetanib, CP − 673451, nilitinib, vemurafinib	Linifanib and cabozantinib most effective in restricting 3D vasculature	(Sobrino et al., 2016)

or patient subgroups (Hutchinson, 2014; Wang et al., 2011). The underlying cause is the variability of genotypic and phenotypic characteristics among different human sub-populations, which the *in vitro* models based on easily available human cancer cell lines cannot address (McLean et al., 2011; Niu and Wang, 2015). Thus, the best and potentially most practical way to address this is to use pre-clinical cancer models based on individual patient-specific cancer cells that can screen all potential drugs at low cost and in less time before they reach clinical trials (Ronaldson-Bouchard and Vunjak-Novakovic, 2018).

An ideal pre-clinical model for such purposes would require screening of a specific tumor's response to a particular drug, testing cytotoxicity of the drug and the time dependent drug resistance development. Microfluidic models provide all these possibilities enabling construction of human populations-on-chip in a more practicable way to predict a patient's clinical drug responses (Mertz et al., 2018). Even though manufacturing such a huge number of chips encompassing all the relevant subpopulations (sex, age and ethnicity) seems to be a highly difficult and time-consuming challenge, initial steps to design and fabricate chips to test anticancer drugs using patient derived primary cells are already underway, as summarized below.

Chang et al. demonstrated high-throughput chemosensitivity data generation, where they attached an "organotypic slice culture" of mouse brain via a PTFE membrane to a microfluidic chip, containing a PDMS microchannel network with a bottom-less 96-well plate for cancer drug screening and therapy. The working device can generate quick drug screening data and provide real-time patient-specific therapy using tumor slices (Chang et al., 2014). However, the study did not use human samples or include any anticancer drug assays.

In a lung cancer study, homogenous 3D spheroids consisting of primary lung cancer epithelial cells and primary pericytes (sorted using

FACS) were screened using the drug cisplatin on a perfused microwell-based PDMS microfluidic chip. The homogeneity of the spheroids was achieved using a tree-like design (inspired from the *in vivo* microvasculature) to distribute and trap cells evenly in eight microwells. Pericytes are one of the elements of the tumor microenvironment that can act as a barrier for the drug to reach the tumor cells. A similar observation was made in this study, where a lower chemo-sensitivity to cisplatin was observed in co-cultured spheroids compared to the mono-cultured spheroids (Ruppen et al., 2015). In another study by Xu *et al.*, primary lung cancer cells were found to be less responsive (lower apoptotic rate) to a combination of drugs as compared to the lung cancer cell line at the same dose (Xu et al., 2013). Both the studies indicate the variable drug responses of primary cancer cells.

Wong *et al.* developed a centimeter-sized PDMS-based droplet microfluidic chip for rapid single cell analysis using cells dissociated from primary nasopharyngeal tumors of human patients. Drug screening was performed on 16,000 cells, one cell at a time, and two anti-cancer drugs (bortezomib and cisplatin) were compared for efficacy and toxicity on the chip platform. While bortezomib was more effective and cytotoxic than cisplatin, varied dose-dependent drug responses in all seven primary tumors were observed (Wong et al., 2017). Despite the fact that the exact biological mechanism behind varied drug responses between the primary tumor samples could not be explained using this device, it clearly underlines the tumor heterogeneity. In a very recent study, pancreatic cancer biopsies from five patients were subjected to three anticancer drug treatments, gemcitabine, oxaliplatin and gefitinib, and seven other biomolecules to test several drug combinations. The microfluidics platform was based on plug technology and included braille valves which provided plugs to enable collection of 1200 data points (62 different drug combinations with n = 20 each) (Fig. 6 (iii)). The cytotoxicity exerted by different drug combinations was found to be non-generalized but was specific to each patient sample. (Fig. 6 (iv)) (Eduati et al., 2018). All of the studies above show that the dose-dependent drug response varies significantly among different patient samples validating the significance of individualized therapy for cancer patients.

3.3.1. Opinion

Individualized or personalized drug therapy for patients is the ultimate goal of tumor-on-chip models. As the number of cells acquired from primary tumor tissues is quite low, microfluidic devices provide a suitable platform for analyzing several permutations and combinations of drugs at the preclinical stage. The main goal of microfluidic technology would be to incorporate primary cancer cells in 3D environments along with other cell types (or induced pluripotent stem cells) from the same patient in order to obtain a true biomimicking cancer model. The drug or therapy responses obtained from this model would significantly enhance the reliability of drug response predictions made for human clinical drug trials. There are however, other important factors to consider such as addition of more organs and functionality (such as the immune system) on the chip to form a complete human-on-chip since cancers are affected by systemic signals inside the human body. Integrating cancer biopsy tissues directly into microfluidic devices need to be studied in more detail before they can be tested in preclinical settings.

4. Summary and conclusion

Over the past 4–5 years, a major driving force for the evolution of microfluidic device technology has been in the area of anticancer drug screening and discovery. We already have the right types of technologies on hand, with complex organ-on-chip designs (2D and 3D) being available that just require some modifications based on the type of tumor type that needs to be modelled. Based on the speed and number of drugs tested, drug screening systems are roughly classified into two types: low-throughput and high-throughput. Low-throughout systems can test only a few drugs at a time, and have designs similar to the compartmentalized organ-on-chip models where the engineered tumor tissue faithfully mimics the complexity of the native tumor. The drugs take time to interact with the cancer cells present inside the 3D multiculture tumor-on-chip. Such systems can help us study overtime resistance development towards cancer drugs, testing of drug combinations that are effective against cancer cells and not too toxic to healthy cells, and investigating all the hallmarks of cancer. High-throughput systems, as reviewed, are the microwell-design chips, which can host hundreds of 3D tumor spheroids at a time (Mosaad et al., 2018; Mulholland et al., 2018), or the droplet microfluidic devices that can quickly generate single cell droplets or spheroid-droplets. Such systems enable faster drug screening and can help determine intratumoral heterogeneity and help design better drug combinations to use. So far, the literature indicates that these two systems are separate paradigms and the gap needs to be bridged in order to speed up the drug discovery process.

Almost all types of cancer have been studied using microfluidic chip technologies in the past five years. Hence, it is remarkable that no matter which cancer cell type and anticancer drug(s) are used, the most important parameter that affects the drug dosage and its response is the tumor or patient drug specificity. That is, the most critical goal of this technology should be to incorporate and study the patient's own tumor cells in a microfluidic device. It is possible to even use a patient's own iPSCs to engineer other cell types (stromal cells, endothelial cells) necessary to create organ models along with the cancer model to fabricate a completely humanized microfluidic chip. This way of studying cancer tumor-on-chip models has provided unparalleled prospects to identify key factors contributing to cancer progression and gain new insights on how to control or target those factors, which would not have been possible with conventional *in vitro* models (Sontheimer-Phelps et al., 2019). Even though one chip/device alone cannot have all the relevant *in vivo* parameters, nevertheless, a minimal required tumor functionality per device to ascertain a reliable drug response should be the goal when designing future cancer models. The demand to screen anticancer drugs is certainly going to increase in the future, which puts immense pressure on the research community to advance this highly challenging and interdisciplinary field. The pharmaceutical corporations need to come forward on a larger scale now and work along with the scientists and engineers to develop high-throughput systems. These should integrate many aspects including, robotic systems, patient genomes, patient-specific microfluidic chips, biosensors to detect cancer cell metabolism and drug responses, feedback control systems, the ability to screen thousands of drug combinations as well as provision of cyclic or sequential combinatorial chemotherapy, and communication of the results generated to the patient's physician(s) for smarter clinical management.

5. Future directions

Microfluidic technology is a versatile platform for intensive anticancer drug screening with the capability to make better predictions of clinical trial outcomes compared to animal models. Validation of microfluidic tumor models would need continuous demonstration that they can indeed mimic the pathophysiological aspects of native tumors and help predict drug responses as observed *in vivo*. This implies that research involving the applications of the tumor-on-chips needs to be carried out as much as possible in present and in the future (Rothbauer et al., 2019). However, there are still several challenges that need to be overcome before such devices can find widespread use. Those limitations are both technical and specific to drug screening applications.

Technical limitations include, firstly, the techniques for fabricating the microfluidic chips in a high-throughput manner. Currently, photolithography is widely used but it is time-consuming, low-throughput, expensive (strictly requires a clean-room environment) (Huh et al., 2013). The process related limitations include expensive silicon wafers,

photoresists (which usually have a limited shelf life), high-end equipment, and extensive training to handle that equipment and to fabricate the high-resolution silicon master to be used to generate PDMS microfluidic chips. In addition, microfluidic devices can be a single-layer or multi-layered depending on the design and application. While the single layer devices require just one silicon master to replicate a PDMS device, multi-layer devices require a silicon master for each layer. Even though the soft lithography process of replica molding using PDMS is simple and quite economical, the production capacity is quite low. For instance, it requires manual labour and can take several days on average to fabricate sufficient numbers of devices for a single cell-culture experiment. Moreover, if design changes are required at a later stages of the experiment, the entire process needs to be repeated, starting with the first step of designing the CAD mask followed by photolithography, all of which lead to more expenses (Jafek et al., 2018). In addition, the silicon masters are fragile and have a tendency to fracture over multiple rounds of replica molding. To address this, plastic masters can be produced using the PDMS replica of the microfluidic device (Desai et al., 2009). These plastic masters are highly durable, long lasting and easy to fabricate, but nonetheless require photolithography to generate the first silicon master. A potential solution to circumvent this issue is to use three-dimensional printing (3D printing). 3D printing can provide a high-throughput way to fabricate microfluidic devices once the challenges associated with the technology are addressed (for example, material selection, printing resolution, biocompatibility with the cells, etc.) (Au et al., 2016). 3D printing can also help in generating non-fragile durable masters for PDMS molding and reduce the costs associated with producing prototypes for initial experimentation (Kamei et al., 2015; Villegas et al., 2018). Nevertheless, the challenge of automating the whole process remains.

Secondly, the difficulty to collect enough cells out of the 3D tumor-on-chip for quantitative analysis, such as, qPCR, and immunohistochemistry (Bhatia and Ingber, 2014) poses a challenge as the engineered 3D tumor is housed inside the enclosed channels. A lot of the sample is lost and potentially contaminated in the process of acquiring it by cutting open the device. Currently, the most successful and widely used way to analyse the cell responses in a tumor-on-chip device is imaging (e.g., using immunofluorescence), but getting quantitative data out of these images requires extensive post imaging software analysis. Potentially that can be solved by integrating the analytical capabilities on the chip itself (such as PCR-on-chip, ELISA or other lab-on-chip parameters for long-term observations) but for cells embedded in a hydrogel, this can be technically tough and needs further research. However, for tumor spheroids or single cell analysis, droplet microfluidic systems offer a great potential to integrate the abovementioned analytical capabilities on the same device in a high-throughput manner (Refer Section 2.3 for details).

Thirdly, microfluidic tumor models must be technically robust. That is, for successful and long-term use as drug screening models, they should not face issues that can halt the whole experiment such as bubble formation in the microchannels or microbial contamination over the long-term culture of cells. The cancer cells along with the other cell types cultures inside the chip should not lose their functional and structural characteristics overtime. A lot of time-consuming experimentation and optimization to formulate a media composition that is optimal for all the types of cells inside the chip is required but so far remains a challenge. Further research on the development of multi-organ-on-chip systems is a potential solution to the above problems (Edington et al., 2018; Satoh et al., 2018; Xu et al., 2016).

Fourthly, the preferred choice of material for fabricating microfluidic devices is currently PDMS. That remains a big limitation in drug screening models since PDMS is known to non-specifically absorb certain types of drugs (Halldorsson et al., 2014). Development of new materials that have all the good properties of PDMS such as optical transparency, flexibility, biocompatibility and low cost, but do not absorb drugs and other biomolecules is a future requirement. However, as reviewed in this paper, other materials/polymers have been explored such as PEGDA (Fan et al., 2016), borosilicate glass (Weltin et al., 2014), and cyclic olefin polymer (Beer et al., 2017). A lot more research is required to find the suitable material that has none of the above limitations.

All the challenges discussed open up vast opportunities for future research aimed at developing low-throughput cancer-on-chip models or high-throughput devices based on microwells or droplet technology. The vision of this technology is to develop human-on-chip or patient-on-chip models based on the cells obtained from the patient. Here, the challenges are to isolate all different types of cells, culture them in separate organ-specific compartments, link the compartments with fluid channels, and ensure the successful long-term culture of cells from different organs/cancers. Future microfluidic technology for developing anticancer drug screening models needs to consider all these scientific and engineering issues to become an effective contributor to healthcare.

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Declaration of interests

None.

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