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Bioengineering for Women's Health

Microengineered 3D Tumor Models for Anti-Cancer Drug Discovery in Female-Related Cancers

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Abstract—The burden of cancer continues to increase in society and negatively impacts the lives of numerous patients. Due to the high cost of current treatment strategies, there is a crucial unmet need to develop inexpensive preclinical platforms to accelerate the process of anti-cancer drug discovery to improve outcomes in cancer patients, most especially in female patients. Many current methods employ expensive animal models which not only present ethical concerns but also do not often accurately predict human physiology and the outcomes of anti-cancer drug responsiveness. Conventional treatment approaches for cancer generally include systemic therapy after a surgical procedure. Although this treatment technique is effective, the outcome is not always positive due to various complex factors such as intratumor heterogeneity and confounding factors within the tumor microenvironment (TME). Patients who develop metastatic disease still have poor prognosis. To that end, recent efforts have attempted to use 3D microengineered platforms to enhance the predictive power and efficacy of anti-cancer drug screening, ultimately to develop personalized therapies. Fascinating features of microengineered assays, such as microfluidics, have led to the advancement in the development of the tumor-on-chip technology platforms, which have shown tremendous potential for meaningful and physiologically relevant anti-cancer drug discovery and screening. Three dimensional microscale models provide unprecedented ability to unveil the biological complexities of cancer and shed light into the mechanism of anti-cancer drug resistance in a timely and resource efficient manner. In this review, we discuss recent advances in the development of microengineered tumor models for anti-cancer drug discovery and screening in female-related cancers. We specifically focus on female-related cancers to draw attention to the various approaches being taken to improve the survival rate of women diagnosed with cancers caused by sex disparities. We also briefly discuss other cancer types like colon adenocarcinomas and glioblastoma due to their high rate of occurrence in females, as well as the high likelihood of sex-biased mutations which complicate current treatment strategies for women. We highlight recent advances in the development of 3D microscale platforms including 3D tumor spheroids, microfluidic platforms as well as bioprinted models, and discuss how they have been utilized to address major challenges in the process of drug discovery, such as chemoresistance, intratumor heterogeneity, drug toxicity, etc. We also present the potential of these platform technologies for use in high-throughput drug screening approaches as a replacements of conventional assays. Within each section, we will provide our perspectives on advantages of the discussed platform technologies.

Keywords—Cancer, Microengineering technologies, Microscale, Microfluidics, Biomaterials, Anti-cancer drugs, Screening.

INTRODUCTION

Cancer is considered a disease with a high mortality rate across the globe. The National Vital Statistics Report found that cancer is the second leading cause of death in the United States.[68] Up till now, approximately equal numbers of cancer cases have been recorded in both men and women, however, recent studies by the American Cancer Society have shown that only 30% females diagnosed with cancer within the last 5 years have survived, while 36% males have survived. Furthermore, for cases diagnosed within the last 10 years, only 21% females have survived, while

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24% males have survived.[138] According to the same study, there will be approximately 0.6 million new cancer cases and 0.3 million deaths in both men and women in the United States in 2020 respectively.[138] The most frequently diagnosed cancer in women is breast cancer with 276,480 new cases, followed by lung/bronchus, colon/rectum, and uterine corpus cancers; whereas, the leading causes of cancer deaths in women (in decreasing order) are expected to be lung/bronchus, breast, colon/rectum, pancreatic and ovarian cancers.[138] Breast cancer is therefore the second leading cause of death in women in the United States, with an average 5-year survival rate of 90%. The risk of breast cancer could be increased due to factors such as family history, gene mutations, endogenous estrogen exposure, obesity, alcohol consumption, and exposure to high dosage of radiation during youth.[21] These figures provide evidence towards a well-established theory which argues that sex may influence chances in responding to treatment and ultimately the surviving outcome.[97,167] To further prove this theory, many studies have been conducted to characterize molecular differences that may be present in cancer between men and women. For example, it has been revealed that specific genes like EGFR may cause better treatment response to non-small-cell lung cancer in women vs. men when there is an elevated rate of mutation.[171] While these sex disparities mostly lead to higher incidence of occurrence of certain cancer types in both sexes, it also contributes significantly to tumor initiation, aggressiveness and prognosis.[171] Other genes that have been identified to be mostly mutated in females are 4q34.3 gene in kidney renal papillary cell carcinoma and 17q11.2 gene in lung squamous cell carcinoma.[171] It is therefore crucial to consider these factors presented by sex disparities when developing drugs for cancer treatment, specifically for women.

Estimated expenditures for cancer treatment in the United States for 2020 were projected to be $173 billion.[87] The National Cancer Institute (NCI) lists a variety of existing approaches for treatment of cancer patients such as surgery, radiation therapy, and chemotherapy, and it is noteworthy to mention that the success of all these treatment approaches heavily rely on drug administration.[140] To that end, developing highly efficient and cost-effective drugs has been at the center of attention in treatment of cancer. Drug discovery is an arduous, meticulous, and expensive process, and it is estimated that the cost of innovating and developing a new drug only in the US is more than $800 million, with an average timeline of 10–15 years.[87] Developing new drugs requires a multiphase process involving drug discovery, preclinical testing, and clinical trials. Preclinical *in vitro* and *in vivo* testings take place to assess the performance of the developed drugs in terms of cytotoxicity, potential side effects, and their tendency to develop resistance mechanisms.[123,152] A study by Malin's group estimated that the success rate for clinical approval of anti-cancer compounds during their full study period is around 13.4%,[26] while another special issue on anti-cancer drugs organized by Meegan *et al.*, stated that the expenditure on drug development was over 1 billion US Dollars, and the success rate was approximately 10%.[91] The low success rate and prolonged testing processes in drug discovery and development could be attributed to multiple factors such as complexities of the disease (i.e. cancer), emergence of drug resistance mechanisms, tumor recurrence and genetic heterogeneity of cells/patient samples. These challenges have therefore inspired researchers to develop reliable platforms that are capable of reducing time and cost of drug screening and validation procedures such as targeted and combinatorial therapies.[173]

To date, significant effort has been devoted to unveiling the cellular and molecular basis of cancer progression and anti-cancer drug resistance utilizing *in vivo* animal models or advanced *in vitro* technology platforms. *In vivo* murine xenografts have been the gold standard models to study the molecular and cellular basis of cancer metastasis and drug resistance.[9,44,88,123] These models have been crucial in highlighting the prominent role of the local tumor microenvironment (TME) in promoting cancer cell behaviors (e.g. invasion).[44,51,53,118] However, *in vivo* models are often based on bolus injection of tumor cells into the subcutaneous tissue, which suffers from lack of precise control over critical aspects of the TME such as specific classes of stromal cells, tumor-stroma architecture or biophysical properties of the stroma. Furthermore, some of the *in vivo* models lack certain key components of the native TME like immune cells, which have been shown to cause drug resistance.[77] In addition, within *in vivo* models, real-time observation of stromal remodeling within the TME, to analyze drug transport, is challenging due to requirement of extensive microscopy, such as multiphoton laser-scanning and second harmonic generation, as opposed to *in vitro* models.[86,136] The limitations of *in vivo* models to accurately recapitulate the unique cellular and molecular profiles of heterogenic tumor-stroma environment may lead to low predictive values of anti-cancer drug discovery during preclinical trials.[41,86] Alternatively, conventional 2D *in vitro* assays or tissue culture plates have a limited capability to recreate the complex physiological environment of the native TME with the necessary cellular and matrix constituents.[5,41,55]

To address limitations of *in vivo* models while complementing their advantages, significant effort has

been devoted to develop sophisticated three dimensional (3D) *in vitro* platforms which comprise of more physiologically relevant components of the TME and could enhance the process of drug screening and discovery. Specifically, in the past few years 3D *in vitro* tissue-engineered[28,130,131,145] microfluidics-based tumor models[17,48,52,96,98,129,143,144,148–150,156,172] or high throughput models and arrays[38,74,109,110] have gained notable attention for mechanistic biological studies on cancer cell behavior and tumor progression.[117,135] These advanced technological platforms have offered better recapitulation with precise control over distinct components of the TME including cells, extracellular matrix (ECM) composition or soluble factors to capture different facets of metastasis,[168] including tumor growth,[80,149] invasion[40,61,149] and intravasation.[30,172] Notably, these sophisticated *in vitro* platforms have gained significant attention for anti-cancer drug discovery due to their inherent time-efficient and cost-efficient characteristics.[129,152]

In this review article, we present state-of-the-art technologies used in the development of 3D microscale tumor models for anti-cancer drug screening in cancers influencing women. We particularly focus on specific types of cancers mainly affecting women to draw attention to the advances in therapeutic regimens and to highlight how research findings have led to increased survival rate of women diagnosed with these types of cancer. While we discuss cancers such as breast and uterine, we also briefly discuss other types of cancers such as colon cancer and glioblastoma due to their high incidence rate in women,[138,171] and proven molecular differences underlying these cancers between men and women which may affect their survival rate. We will first provide an overview of the complexities of the native TME, after which we briefly discuss conventional assays and *in vivo* platforms that have been used for anti-cancer drug screening. We will then expand our discussion on recent advancements in engineering of innovative tumor models based on 3D cell-laden scaffolds, spheroid-based models, and most importantly, microengineered models such as bioprinted platforms and microfluidic assays. We will discuss in detail how these physiologically relevant *in vitro* models have reshaped the field of anti-cancer drug screening and discovery. Within each section, we will provide a brief perspective of significant findings of pertinent studies, highlight advantages and disadvantages of each platform technology and further discuss the challenges and future directions in the use of these advanced assays in cancer research and drug discovery.

TUMOR MICROENVIRONMENT: COMPLEXITIES AND CHALLENGES FOR ANTI-CANCER DRUG DISCOVERY

Despite the inherent characteristics of each cancer type, it is well accepted that there are common ''hallmarks'' which are shared amongst all types of cancer.[43] Regardless of their origin, cancer cells typically develop an uncontrollable replication process, death resistance mechanism, self-sufficient growth signaling, and insensitive response to growth suppressors.[36,43] Cancer cells also present a non-healing wound behavior with persistent inflammation which promote their tumorigenesis and metastatic behavior.[36] Furthermore, cancer cells exhibit the capability to form new vasculature within their proximity tissue, known as tumor angiogenesis.[43]

Tumor progression and formation of lethal entities depend on four major factors, namely, the capability to invade the surrounding tissue, the capacity to degrade the ECM, the ability to intravasate and survive in the bloodstream, and finally, the affinity to establish secondary tumors within distant organs/tissues.[160] Tumor growth is also associated with genetic and epigenetic mutations by regulating factors such as oncogenes[90] as well as biophysical and biochemical signaling cues that the tumors receive from surrounding TME (i.e. resident cells, ECM).[90] Specifically, the role of TME in cancer cell metastasis is a rapidly growing subject of research. The TME is a highly complex and dynamic entity (Fig. 1) consisting of an activated ECM along with numerous cell types, including epithelial cells, immune cells (T-lymphocytes, B-lymphocytes, neutrophils, natural killer cells (NK-cells) and macrophages), cancer-associated fibroblasts (CAFs), mesenchymal stem/stromal cells (MSCs), adipocytes, along with endothelial cells and pericytes.[4,53,82,90] The TME plays a crucial role in the occurrence, progression, and recurrence of cancer.[3] Tumor cells can detect and respond to many signals, involving biophysical and biochemical cues within the TME which all orchestrate to promote the disease.[76,84,131] A large proportion of these signals stem from the stromal cells, ECM architecture, and stiffness within the TME.[57] For instance, ECM remodeling within the TME occurs due to the secretion of chemokines and growth factors, mainly via the crosstalk between CAFs and cancer cells,[131] which ultimately lead to the formation of a fibrotic tissue (i.e. desmoplasia)[130] and cancer cell proliferation and invasion.[45,84] Moreover, protein content of the ECM, such as collagen, could degrade within the TME and lead to increased deposition of proteins.[84]

Due to its biological significance, the TME has been at the center of attention in targeted cancer therapy.[151]

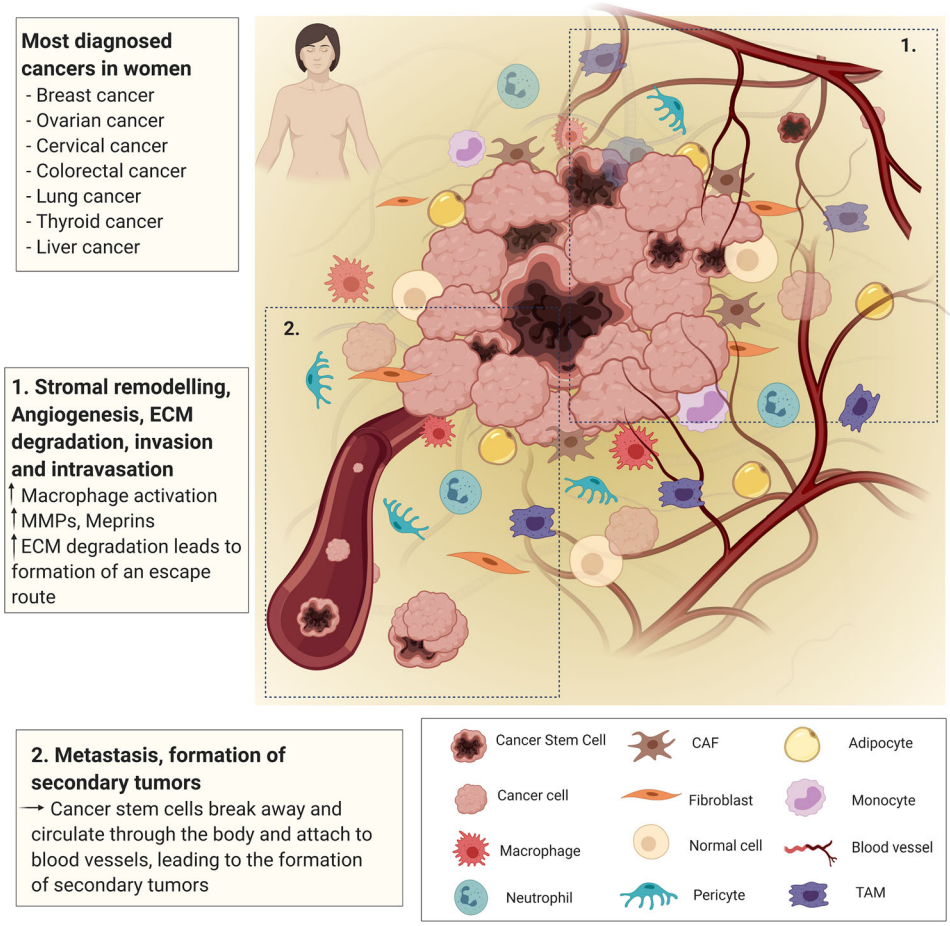

FIGURE 1. A representative schematic of the tumor microenvironment (TME). The TME is a complex biological entity embedded with numerous cells types such as fibroblast cells, vascular cells, immune cell, etc. The biological complexities of the TME has been postulated to be a key factor influencing anti-cancer drug responsiveness in female-related cancers. (Schematic was created using BioRender Software).

However, the inherent complexities of TME and the presence of numerous confounding factors have resulted in significant challenges and limitations in efficient treatment of cancer.[3] A key reason has been the lack of mechanistic understanding of the role of the TME components, including resident stromal cells and[3] ECM, on emergence of drug resistance mechanisms. Although the role of certain components in the TME that are responsible for cancer progression and pathogenesis are understood, there still remains confounding factors which need to be thoroughly investigated for certain drug responsiveness.[3]

The functional heterogeneity presented within the TME is among the key major hurdles which have significantly slowed the process of anti-cancer drug discovery.[3,85,133,151] At first glance, the intratumoral heterogeneity itself caused by the presence of a small population of cells known as cancer stem cells (CSCs) hinder the development of specialized treatment.[99,120] For instance, the actual role of stem cells in breast cancer progression is still yet to be fully understood.[119] Recent findings have shown that mesenchymal stromal/stem cells possess a mutual feedback relationship with tumor cells and the ECM in the TME, however, the signaling pathways involved in oncogenesis and tumor progression are not properly understood.[93,124] This presents a significant limitation in drug discovery, specifically in Luminal A and B breast cancer subtypes, since luminal cells are one of the two major cell lineages in the epithelium of the mammary gland.[33,35,119] Apart from functional heterogeneity, the molecular crosstalk between the components of the TME and cancer stem cells also lead to emergence of chemotherapeutic drug resistance.[154] For instance, various studies have shown that CAFs secrete CCL5 and CCL2 which lead to the production of IL6 and consequently cause chemoresistance in certain cancers such as ovarian cancer.[113] Although the full mechanism of action is not fully understood, other studies have shown that CAFs cause chemoresistance to drugs like Paclitaxel and Cisplatin through CAF-endothelial cell crosstalk which involves the regulation of the Li-

poma-Preferred Partner (LPP) gene.[75] A recent study by Liang *et al*. showed that a crosstalk between the p53 tumor suppressor gene and the STAT3 transcription factor was the underlying mechanism of metastasis in ovarian cancer. Phosphorylating STAT3 through gene overexpression and drug treatment assays led to activation of the MAPK and AKT signaling pathways which resulted in increased chemoresistance to Cisplatin drug within an *in vitro* tumor formed with SKOV3-STAT3-DN human ovarian cancer cells.[77]

In addition to chemoresistance emerging from the biological complexities of the disease and specifically the TME, drug pharmacokinetics is another critical factor which may influence the efficacy of therapy.[6,167] When a chemotherapeutic drug is administered to a patient, several factors like liberation, absorption, diffusion and distribution come into play.[6] For a drug to successfully reach the target site, these factors work in a cycle-like manner to break down the drug into a form that can be easily absorbed by the body, ensure that the routes of transport are efficient enough to promote incorporation (e.g. through active transport), and most importantly, ensure that there are no barriers (e.g. blood-brain barrier) to inhibit drug transport.[6] To that end, the TME has also been known to play a crucial role in influencing the drug pharmacokinetics. Specifically, the biophysical alterations within the TME stroma, induced by CAFs due to desmoplasia and ECM remodeling, has been discerned to inhibit drug transport, enabling survival of cancer cells.[83,130] However controlled studies are required to elucidate the mechanism of anti-cancer drug penetration.

Altogether, chemoresistance and drug pharmacokinetics are a major complexity presented by the TME and impeding factors in anti-cancer drug development. Mechanisms like multiple drug resistance (MDR), increased tolerance to drug damage, cellular crosstalk within the TME, and cellular/genetic mutations and heterogeneities are among the causes of chemoresistance in most cancers.[169] To develop dependable and efficient tools for anti-cancer drug discovery and development, it is crucial to ensure that the major characteristics of the native tumor are preserved. Recapitulating the TME is therefore a critical challenge in the development of reliable experimental models for drug discovery which can be overcome by using recent state-of-the-art technologies in development of engineered TME.[117] In the following sections, we will provide a broad overview of the *in vivo* models and conventional *in vitro* assays which have been developed for anti-cancer drug screening in female-related cancers and will highlight the contributions of each to the field.

OVERVIEW OF *IN VIVO* MODELS FOR ANTI-CANCER DRUG SCREENING

For a very long time, cancer was perceived as a death sentence for patients diagnosed due to the very low survival rate which was attributed to the limitation in treatment options (i.e. surgical retraction, radiotherapy).[41] In women specifically, survival rates were recorded to be much lower than men,[138] leading to an argument that sex disparities present a major factor in tumor initiation, progression and treatment.[171] However, recent advances have presented many groundbreaking options for early diagnosis and efficient treatment of cancer in women, specifically. Although relatively new, these advances hold promising results for patients and present an entirely new perspective to cancer therapeutics.[25,28] Various assays have been developed in the last 20 years alone, in an attempt to treat cancer. These new approaches include both *in vivo* tumor models like patient derived xenograft (PDX) models, as well as a plethora of *in vitro* engineered tumor models.[28,70,166] Although each of these models present their own set of advantages and disadvantages, it goes without saying that the field of anti-cancer drug screening has witnessed significant progress which will continue in the coming years.[28,65]

Significant advancements in the use of animal models for anti-cancer drug screening have mainly centered on developing metastatic and orthotopic PDX models as well as transgenic mice models.[66,70,134,166] This focus has been attributed to the need to develop physiologically relevant models to permit efficient drug screening studies. PDX models involve the immediate transfer of tumor samples obtained from human tissues into immunodeficient mice after surgical removal procedures.[166] Although PDX models were initially shunned due to the unavailability of immunodeficient mice which consequently led to immune rejection, they have recently become very useful in mainstream cancer research because of their ability to perpetuate tumor progression along with autocrine and endocrine mechanisms, similar to the native human TME. They therefore have higher predictive values in cancer therapeutics.[166]

Cell-line derived xenograft models include the resection and dissociation of a tumor from a human, and the transfer/injection of the dissociated tumor cell-line into immunodeficient mice.[123] Cell line-derived xenografts have low predictive values in cancer therapy as they do not accurately mimic the complexities of tumors due to the loss of heterogeneity during *in vitro* tumor dissociation.[123] However, using initial tumors to form PDX models remains a huge challenge because tumor formation rates are generally low.[166] Furthermore, the success of forming PDX models primarily

depend on the level of immunodeficiency of the mice, so even though the costs involved in preparing immunodeficient mice samples have reduced over the years, it still takes about three months to develop a successful immunodeficient PDX model for drug screening studies. Hence all in all, PDX models remain technically challenging, time-consuming and often expensive.[166]

Transgenic mice models are also very powerful *in vivo* models that have been utilized for tumor modelling and drug screening because of their highly complex TME.[70,134] Since transgenic mice models are models in which a specific gene is removed or replaced, they have increased the scientific comprehension of certain mechanisms that cause drug induced toxicity or tumor pathogenesis.[70,134] However, the alteration of a specific gene may not necessarily result in the expected phenotypic response. In addition, the phenotypic outcome of gene modification can be influenced by genetic and epigenetic factors, hence, the interpretation of results obtained by using these models are not always reliable.[134] Overall, some of the limitations of *in vivo* models include low tumor formation rates within specimens due to the limited amount of cells obtained from the native tumor masses, as well as low predictive values of drug efficiency due to loss of heterogeneity of the transplanted cells used for experiments.[126] Additionally, *in vivo* models are generally time consuming and expensive and require a high level of technical expertise.

The significant setbacks presented by animal models highlight the possibility and fact that employing *in vitro* models may outweigh the benefits presented by *in vivo* models. Until recently, various experimental assays were mainly centered on utilizing 2D monolayer of cells. Besides being cost efficient, the 2D assays are easy to use and therefore require less expertise. They also permit wide flexibility in designing experiments to investigate a specific hypothesis. Additionally, they require less resources for seeding tumor cells.[28,55,126] However, 2D assays suffer from significant limitations as they do not accurately mimic the complexities of the native TME.[28,111,145]. In addition, they lack a well-defined architecture and therefore inhibit generation of growth factors and drug gradients. Due to these critical limitations of *in vivo* models and 2D conventional assays, the research community has recently witnessed significant emergence of several 3D *in vitro* platforms that have the ability to provide physiological and pathophysiological mimicry of the TME to assess cancer cell behavior and anti-cancer drug responsiveness.[65,126] Amongst these are 3D surface topographies,[11,14,69,103,105–108,122,139,174] 3D cell-laden scaffolds,[13,116,145] spheroid-based models[15,60,92,100] and microengineered or microfluidic models.[1,8,15,22,25,29,52,58,72,78,115,117,137,142,148,161] In the following sections, we present the state-of-the-art engineering of sophisticated 3D *in* vitro tumor models for anti-cancer drug screening in female-related cancers. The uses and advantages of various 3D *in vitro* anti-cancer drug screening models will be thoroughly discussed, and their contributions to cancer research will be highlighted.

3D TUMOR MODELS FOR ANTI-CANCER DRUG SCREENING

Scaffold-Based 3D Cell-Laden Models for Anti-cancer Drug Screening

To properly investigate the efficiency of anti-cancer drugs and ultimately increase the survival rate of women diagnosed with cancer, it is critical to employ translative *in vitro* cell culture models that closely mimic the complexities of the *in vivo* environment to test the efficiency of chemotherapeutic agents. Several factors such as individual cell types, molecular signals, and biomechanical cues like the ECM constituent need to be precisely incorporated within physiologically relevant tumor models. Three dimensional (3D) tumor models permit accurate manipulation of experimental conditions, while allowing precise cell-cell contact and spatial organization of cells and matrix in an organotypic architecture, while they provide the ability for real-time observation of intracellular reactions to drugs.[111,142]

Perhaps the simplest approach to construct a 3D tumor model is mixing cancer cells alone, or with stromal cells with the desired scaffolding biomaterials to mimic a 3D tissue environment and to provide native-like instructional cues to the cells.[96] A commonly used scaffolding biomaterial in this regard, which has been adapted by numerous studies, is collagen, mainly due to its abundance within the TME ECM.[10] In fact, a recent study by Casey *et al.* demonstrated that collagen-based hydrogels are widely accepted as ECM representative matrices for engineering of 3D tumor cell culture models. In this study, an AlamarBlue cytotoxicity assay was used to assess the viability of cervical cancer HeLa (human cervical adenocarcinoma) cells cultured on a 2D standard platform and on a 3D collagen matrix after exposure to Doxorubicin ((DOX), dosage ranging 0.0–100.0 μM). After exposing the 2D standard plates and the 3D collagen coated plates to DOX for 72 hours, it was observed that the 2D platform had a decreased cell viability of about 10% as compared to the 3D platform which had an average viability of about 20%. The higher viability observed in the 3D hydrogel model was proven

through spectroscopy to be as a result of matrix alteration rather that cellular metabolic changes. Furthermore, the effects of the cell culture environment on drug resistance was investigated using spectroscopy by comparing the absorbance spectrum of the obtained culture media from the 2D and 3D platforms in both control (no drug exposure) and standard conditions (exposure to 100 μM DOX). It was confirmed that the collagen matrix in the 3D platform indeed influenced toxicity of the DOX drug which consequently resulted in higher cell viability as against the 2D platform.[13] These findings therefore confirmed that the response of tumor cells to anti-cancer drugs is highly dependent on the dimensionality (2D vs. 3D) of the culture model.

In a pioneer study by Mina Bissell's team, it was revealed that special genes relating to apoptosis were regulated differently when the tumor cells were cultured in a 3D Basement Membrane (BM) platform, relative to a 2D platform.[159] For example, in one of the initial studies, two different cell types (non-malignant S-1 and malignant T4-2 microvascular endothelial cells (MECs)) were cultured in 2D and 3D fashions, and their responsiveness to apoptotic stimuli was observed after they were exposed to chemotherapeutic agents such as Paclitaxel. It was shown that, a 3D polarized architecture conferred protection against apoptosis to the cells regardless of their growth status, while non-polarized cells which mostly grew as a monolayer were more susceptible to cell death after exposure to certain chemotherapeutic agents. Additionally, the gene expression profiles for breast cancer cells grown in 2D and 3D platforms were significantly different in terms of their ability to resist apoptosis.[158] In a later study, Bissell's team further established evidence that cells grown in different architecture reacted differently in terms of certain genes and proteins expression.[56] Specifically, distinct morphological differences were revealed when different breast epithelial cell lines were grown in a 3D collagen based model as against a monolayer fashion. It was observed through phase contrast microscopy and F-actin fluorescence imaging that, cell lines grown in a monolayer largely expressed the same morphological patterns, whereas cell lines grown in a 3D substrate adopted four major morphologies namely; round, stellate, mass and grape-like colonies. Furthermore, a western blot assay revealed significant differences in protein expression across the four different morphologies. For instance, it was observed that the stellate cell lines mainly lacked E-cadherin and ErbB2 genes, while the other morphologies expressed it in different quantities. Furthermore, gene expression analysis revealed that a significant number of genes, like the CDH1 and EGFR genes, were differentially expressed in the 2D culture condition as against the 3D collagen-based condition. Interestingly, some of the genes like IL16, IL21, and AGTR2, that were expressed differently were identified to be responsible for signal transduction, confirming results obtained from the previously discussed study where different signal transduction activities were observed in 3D conditions relative to 2D conditions.[56] These results indicate that the 3D vs. 2D microenvironment significantly influence the behavior of cancer cells such as their morphology and also lead to a differential response of the cells to chemotherapeutic agents, highlighting the importance of employing physiologically relevant platforms in anti-cancer drug screening.[56,71,89,159] In the following sections, we will outline recent advancements in engineering of 3D spheroid based as well as microengineered (i.e. micropatterned, microfluidic) tumor models for anti-cancer drug screening.

Spheroid Based 3D Tumor Models

Certain types of cancer cells are capable of aggregating and forming tumor spheroids ranging from a few hundred to a few thousand microns (e.g. MCF7 and T4-2 breast cancer cells, TOV-21G malignant ovarian cancer cells).[153] Spheroid-based models have been widely used for drug screening studies due to their ease of formation, multi-cellular components and their resemblance to certain characteristics of the native TME such as three dimensionality and the formation of a hypoxic core. Numerous techniques such as hanging drops and low adhesion well plates have been commonly used for formation of tumor spheroids for different types of cancer.[100,153]

To validate the relevance of spheroid-based tumor models for drug screening, Kim *et al.*, developed a microfluidic device which consisted of four sets of 80-microwell pits connected via a microchannel network. MCF7 breast cancer cells were injected into the devices and allowed to form spheroids over a course of 3 days. The efficiency of the proposed device was confirmed by simultaneously forming tumor spheroids side-by-side via the conventional hanging drop (HD) method. Spheroids formed from the two methods (Microfluidic device vs. HD) were compared, and it was observed that while the spheroids formed from the two methods had similar dimensions, the spheroids formed with the microfluidic device had an almost perfect spherical shape from day 1 of culture. This observation further proved the efficiency of microfluidic devices, as physiologically platforms, for anti-cancer drug development. After establishing a suitable platform for spheroid formation, the cytotoxicity of MCF7 spheroids was investigated and compared with a 2D monolayer cell culture platform to determine the efficacy of specific anti-cancer drugs, as well as the

response of the cells under the two conditions (2D vs 3D) to drug exposure. Results indicated that the formed MCF7 spheroids were more resistant to anti-cancer drugs such as Mitomycin C, 5-FU (5- Fluorouracil) and DOX in comparison to the 2D culture assays due to the high cell-cell interactions within the 3D spheroids.[60] Similar findings were reported in another study by Imamura *et al.*, for invasive ductal carcinoma cells such as T-47D, BT-549, and BT-474, in which dense multicellular spheroids were formed in low adhesion 3D-culture plates over a 3 day time period. Their findings indicated an increase in resistance to chemotherapeutic agents such as DOX and Paclitaxel[46] within the 3D spheroid model as compared to the 2D culture models. In a similar study by Liu's *et al.*, a microfluidic device consisting of 4 layers and 8 chambers was used to form tumor spheroids as shown in Fig. 2a. Briefly, cell suspensions at 5 million cell densities were introduced into the inlets of the devices using a syringe pump, and a cell trapping mechanism was employed to trap the cells in specific Pluronic F127 treated chambers which allowed them to form spheroids. Monitoring the apoptotic activity of the glioblastoma cells (U-251) treated with Vincristine and Bleomycin in the novel pneumatic microstructure revealed an increased caspase-3 activity and mitochondrial depolymerization in the 3D spheroids which confirmed the hypothesis that 3D spheroid models possessed higher resistivity due to the establishment of a hypoxic core.[81]

In a different study by Chen *et al.*, a PDMS based microwell array was designed to screen the dose-dependent response of uniformly formed T-47D breast cancer cell line spheroids to DOX and Paclitaxel (Fig. 2b). DOX treatment showed higher cell viability; however, with the same dosage treatment of Paclitaxel, the spheroid completely degenerated. To further validate the capability of this multicellular tumor spheroid (MTS) platform for physiological drug screening, the mechanism of action of Paclitaxel and DOX were examined by monitoring the expression of proteins related to apoptosis. After 24hrs of drug exposure, the expression levels of cleaved PARP and cleaved caspase-8 antibodies were elevated, with a simultaneous increase in both DOX and Paclitaxel concentrations. The authors concluded that the proposed MTS platform could imitate an *in vivo* microenvironment and could therefore be used as a reliable model for drug cytotoxicity analysis.[15]

More recently, a novel approach to quantify and predict treatment outcomes of drug tests on HT-29 colorectal cancer spheroids in an ultra-low adhesive aqueous two-phase system (ATPS) plate platform, was developed by Thakuri *et al.* Although colorectal cancer leads to high mortality rates in both men and women, recent studies have shown that women have a lower 5-year survival rate as well as a higher mortality rate compared to men.[63,162] One of the reasons for this is the lack of efficient screening models that can distinguish the more aggressive types of colon cancer which often occur in women.[63] In this study, a PrestoBlue biochemical assay was used to establish a linear correlation between spheroid size and cellular metabolic activity. This assay allowed the authors to accurately predict drug resistance of the formed HT-29 spheroids after kinase inhibition. Specifically, acquired 2D images of HT-29 spheroids showed that the formed spheroids maintained their well-defined boundaries and densities during the culture period, and that the volume of the spheroids correlated with the fluorescence intensity obtained from the PrestoBlue assay. Drug testing on the formed spheroids revealed that Trametinib was the most effective kinase molecular inhibitor at the nanomolar concentration range, followed by Sorafenib, Dactolisib, and Ponatinib in micromolar concentrations.[146] This model was therefore useful in quantifying and predicting treatment outcomes in colorectal cancer samples, and can be even more useful as a model system to quantify and distinguish proximal colon cancers from distal colon cancers to improve the survival rate of women diagnosed with colorectal cancer.

To further increase the relevance and sophistication of spheroid based models, various scientists have considered incorporating other cell types (e.g. stromal) which have been shown to influence tumor progression in the native TME.[49] For example, CAFs have been found to modify the stromal ECM matrix and consequently promote tumor invasion and metastasis in many types of cancer.[130] In a recent study, a 3D co-culture system for short term screening of anti-cancer drugs was developed by Zoetemelk *et al.* A variety of colorectal adenocarcinoma cell lines such as HCT116, DLD-1, and SW620 were cultured in 2D and 3D fashions. Normal human fibroblasts (CCD-18Co) were co-cultured with the cancer cells to assess the influence of multicellular interactions on drug performance. The 3D co-culture condition indicated greater physiological relevance by providing more translational information and enabling more relevant patient-specific treatment plans as compared to the 3D unicellular condition.[175] In another study, the influence of multicellular reactions and their response to drugs were investigated by establishing a 3D co-culture platform comprising CCD-18Co human normal fibroblast cells and human colorectal spheroids (HT-29 cells) (Fig. 2c). The HT-29 spheroids were formed in a collagen-based scaffold, and co-cultured with CCD-18Co cells which resulted in significant morphological and phenotypic changes in the spheroids. A higher expression of α-SMA was

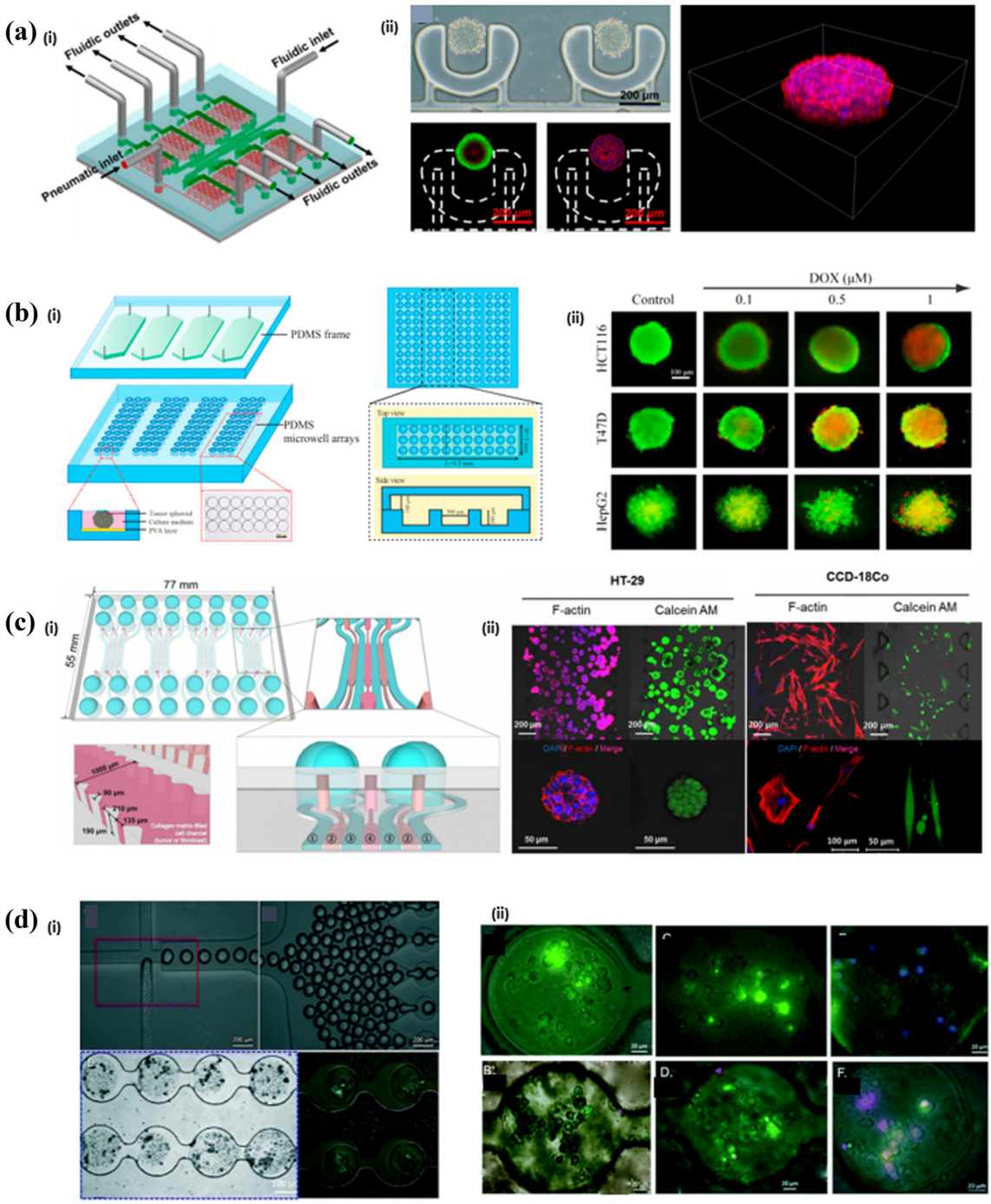

FIGURE 2. Representative figures of spheroid-based models used for anti-cancer drug screening. (a) (i) Schematic of recyclable microfluidic device used for drug screening. (ii) Investigation of microfluidic device efficiency in culturing varied sizes of spheroids. Adapted with permission from American Chemical Society: [Analytical Chemistry], copyright (2015).[81] (b) (i) Schematic showing microfluidic device used to form multicellular tumor spheroids (MTS). (ii) Fluorescence images showing viability of spheroids after exposure to Doxorubicin. Adapted with permission from Elsevier: [Analytica Chimica Acta], copyright (2015)[15] (c) (i) Schematic of microfluidic platform used for 3D co-culture of human colorectal cancer cells and fibroblasts. (ii) Representative fluorescent images of spheroids expressing F-actin and high viability. Adapted with permission from PLoS One: [PLoS One], copyright (2016).[49] (d) (i) Schematic showing microfluidic device with docking array for spheroid formation of MCF7 breast cancer cells. The device consisted of a T-junction where cell droplets were allowed to form before gelation. (ii) Fluorescent images of viability assay of formed spheroids after treatment with Doxorubicin. Adapted with permission from Royal Society of Chemistry: [Lab on a Chip], copyright (2016).[127]

observed in the co-culture condition as against the mono-culture conditions, indicating that the fibroblasts had been activated into CAFs. Furthermore, the presence of CAFs in the co-culture condition appeared to increase growth levels of the HT-29 spheroids as compared to the mono-culture spheroids. Exposing the spheroids to Paclitaxel revealed lower survival rates in the co-culture condition. Additionally, profiling the apoptosis-related proteins revealed that five factors were significantly downregulated in the co-culture condition, indicating that the CAFs indeed played a critical role in tumor survival and progression.[49] The aforementioned cancer spheroid models developed to quantify and test anticancer drugs can be further improved to investigate the underlying mechanisms of sex disparities in colorectal cancer, and ultimately improve the survival rate of women diagnosed with this cancer.[63]

Overall, 3D spheroid models are very attractive platforms for studying the biology of the TME due to their three dimensionality, the ability to incorporate heterotypic and or homotypic cellular components as well as the formation of nutrient, oxygen, and signaling gradients. These model systems are also relatively inexpensive and have served as appropriate models/assays for anti-cancer drug responsiveness studies.

3D Spheroid-Based Models for High-Throughput Drug Screening

The emergence of microdroplet generators has attracted significant attention from the research community due to their potential for manipulating miniaturized volumes and translation to a variety of biological and drug discovery applications. Recently, microfluidic droplet generators have also been utilized for single-cell analysis, miniaturized cell culture/suspension as well as spheroid generation. The microdroplet technology could aid in the formation of high-throughput platforms for drug screening and discovery applications in female-related cancers.[16] Specifically, cancer spheroid-based cultures could be combined with a droplet generator to analyze anti-cancer drug cytotoxicity in a high-throughput fashion.[54,110] One of the early studies in this regard was conducted by, Yu *et al.*, where a microfluidic setup was used to generate tumor spheroids of MCF7 breast cancer cells embedded in an alginate hydrogel shell. Results demonstrated that the viability of the cells encapsulated within the hydrogel matrix could be maintained by adding an alginate shell structure. Moreover, the generated spheroids were exposed to Tamoxifen and Docetaxel, and the results showed reduced toxicity for the cellular aggregates in the 3D culture condition compared to the monolayer culture condition.[170]

Since the dynamics of drug uptake at a single-cell level could provide information about drug resistance mechanisms, another study conducted by Konry's group utilized a microdroplet generator to assess DOX uptake in drug-resistive and drug-sensitive breast cancer cells, MCF7-R and MCF7-S respectively. Their study demonstrated that MCF7-R cells exhibited a low level of DOX accumulation, while in contrast, MCF7-S cells showed a higher level of drug uptake and retention.[132] Although this study shed some light on the drug uptake capabilities of MCF7 breast cancer cells with different drug-responsiveness background, further studies are required to understand the underlying mechanisms linking drug resistance to uptake for improved therapeutic outcomes. In a follow up study, the same group demonstrated the advantages of high-throughput spheroid-based models for anti-cancer drug screening applications. In this study, a microfluidic droplet generator platform was developed to enable parallel tracing of each assembled spheroid through spatiotemporal monitoring (Fig. 2d). Two types of MCF7 cells, drug-sensitive and drug-resistive (MCF7-R and MCF7-S), were co-cultured with HS-5 bone marrow fibroblasts and the formed spheroids were treated with DOX and Paclitaxel chemotherapeutic drugs. Co-cultured spheroids (MCF7-S cells + HS-5 cells) illustrated a higher overall cell viability as compared to MCF7-S drug-sensitive mono-culture spheroids. Moreover, combining Paclitaxel and DOX reduced the viability of the tumor spheroids (MCF7-S). Therefore, the cytotoxicity of the combinatorial drug scheme was higher than the single drug treatment when MCF7 and HS-5 cells were co-cultured.[127]

Popova *et al.* recently reported development of another high-throughput drug screening platform by engineering a droplet microarray (DMA) using superhydrophobic surfaces to generate cancer cell spheroids (Fig. 3A). By using minute amounts of cells (150 cells in 100 nL droplets) they were able to establish a platform for precision medicine, and this platform permitted the study of the microtumors response to chemotherapeutic drugs. Specifically, HeLa cell spheroids were formed within the proposed platform and were further exposed to different chemotherapeutic drugs such as DOX, Oxaliplatin, and 5-Fluorouracil. The observed cell viability percentage for all of the administered drugs confirmed the fact that the 3D spheroid culture was more drug resistive compared to the conventional 2D culture platform. The proposed DMA platform was an important contribution to anti-cancer drug studies as the authors were able to address one of the major limitations associated with culturing patient derived cells *in vitro* by using their platform to generate tumor spheroids with a limited number of cells.[121]

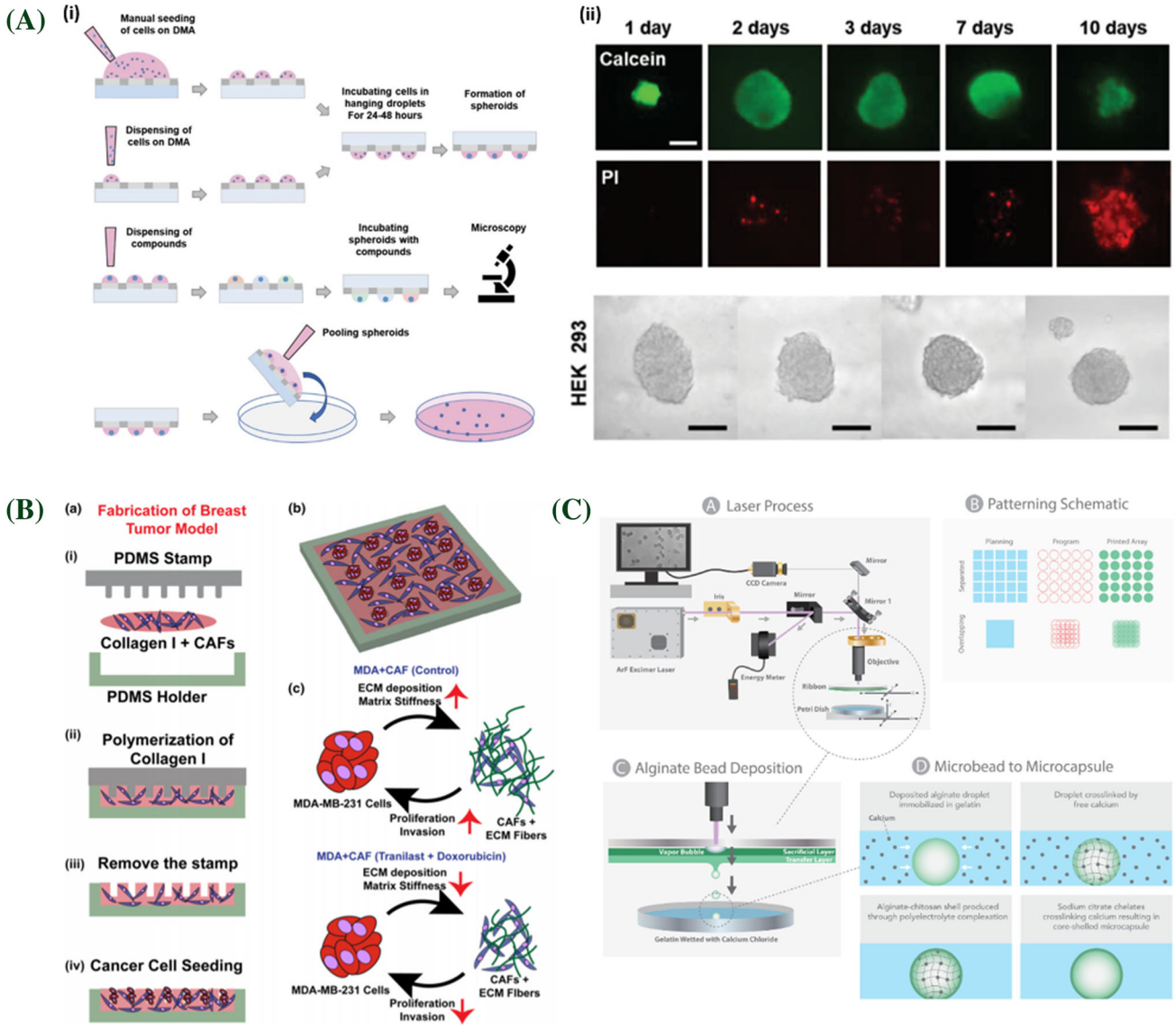

FIGURE 3. Representative figures of micropatterned tumor models. (A) (i) Fabrication schematic of Droplet Microarray (DMA) platform showing cell seeding process, compound dispensing and screening process, and spheroid extraction process. (ii) Fluorescent and phase contrast images showing viability and sizes of formed spheroids. Adapted with permission from Wiley: [Small], copyright (2019)[121] (B) (a) Fabrication process of the 3D micro-engineered breast tumor model. (b) Isometric view of the micropatterned breast tumor model. (c) Representative illustration of the role of desmoplasia on tumor progression and drug responsiveness. Adapted with permission from Springer: [Cellular and Molecular Bioengineering], copyright (2018)[131] (C) Representative diagram of laser-direct write (LDW) platform for the growth of well-defined multicellular tumor spheroids (a, b) Workflow of laser process for patterning and fabrication of microbeads on a petri dish or glass dish. Green array representative of final device design (c.) Schematic showing how alginate beads were deposited in the LDW technique and processed into core-shelled constructs (d.) Schematic showing how microbeads are crosslinked by calcium and eventually formed into core-shelled microcapsules. Adapted with permission from Elsevier: [Acta Biomaterialia], copyright (2019).[64]

Perspective: Advantages and Limitations of Spheroid Models

Spheroid-based 3D tumor models could be engineered with precise geometry and cellular composition and provide biological information about cell-cell and cell-ECM interactions and respective drug responsiveness. Moreover, 3D spheroids could establish a necrotic core that is physiologically relevant to the TME, although the development of necrotic core is highly dependent on the size of the spheroids. To that end, spheroid models provide straightforward, efficient, and relatively inexpensive assays for high throughput anti-cancer drug screening. Furthermore, their straightforward design permits side-by-side investigation of tissue samples obtained from different sources for the study of gender-based disparities in

cancers like colorectal cancer and their response to anti-cancer drugs. Despite their advantages, it is often challenging to use spheroid models to recapitulate complexities of native TME such as tissue architecture, spatial cellular organization and ECM composition. In addition, the reconstruction of an internal vasculature, an essential source of nutrition to the engineered tissue, might be a challenging task due to the size limitation of the spheroids. Incorporating 3D tumor spheroids in microfluidic platforms might enhance the physiological relevance of 3D spheroid-based models for anti-cancer drug screening by applying dynamic culture media perfusion to control shear stress. In addition, the functionality of spheroid models could be improved by encapsulating the engineered spheroids in an ECM mimicking environment matrix (e.g. hydrogels), which could enable the co-culture of multiple cells types within the same assay for a more predictable and accurate anti-cancer drug testing. Table 1 provides a summary of representative studies using 3D spheroid based model systems for anti-cancer drug screening applications.

MICROENGINEERED TUMOR MODELS FOR ANTI-CANCER DRUG SCREENING

Developing anti-cancer drugs with high efficacy is by no means a simple feat in cancer therapy. Understanding metastatic behavior of tumor cells and their mechanism of drug resistance, as well as optimizing combinatorial treatment and chemotherapeutic drug efficacy are just a few significant challenges that need to be addressed to conquer cancer. Since sex disparities are also a major cause of concern in cancer treatment,[97] development of relevant models which enable the manipulation of specific sex-biased genes, to investigate the underlying mechanisms of specific mutations, could be beneficial in improving the survival rate of female-related cancers. To that end, the development of physiologically relevant *in vitro* models of the TME could be a reliable approach to address currently existing challenges in the field of anti-cancer drug development and therapy. In recent years, integrating micro- and nanoscale technologies along with advanced biomaterials have offered enormous opportunities to develop tissue models for disease modeling and regenerative medicine applications.[5,117,176] Specif-

TABLE 1. Summary of representative 3D spheroid-based tumor models for anti-cancer drug screening.

Cancer type	Cell line	Drug	Key point	References
Breast	T-47D, BT-474, BT-549	Paclitaxel DOX	Increase of drug resistance in 3D compared to 2D culture	46
Breast	MCF7	Tamoxifen Docetaxel	Reduced toxicity in the 3D culture compared to the 2D culture	170
Breast	MCF7	DOX	Phenotypic drug profiling	132
Breast	MCF7	DOX DOX+ Paclitaxel	Decrease of CD137 and CD40 during combinatorial drug scheme	128
Colon	LS174T, HT-29/SW620, HCT116, Caco-2, DLD-1	Rapamycin Torin1 PF4706867 MK2206 AZD6244	Drug response to AKT signaling pathway	125
Colon	PANC-1	Gemcitabine Oxaliplatin Paclitaxel	Exposure of mono- and co-cultured tumor spheroids to Gemcitabine did not significantly influence their viability across both conditions	72
Colon	HTC116, DLD-1, SW620	Erlotinib HCL 5-Fluorouracil Regorafenib	Antagonistic drug response observed for drugs at higher concentrations, that otherwise express synergistic responses at low concentrations	175
Colon	HT-29	Trametinib Dactolisib Ponatinib Sorafenib	Correlation between spheroid size and drug response; combinatorial treatment of Trametinib and Dactolisib resulted in increased resistance of spheroids to drugs	146
Human cervical adenocarcinoma	HeLa	DOX Oxaliplatin 5-Fluorouracil	Higher IC50 values for the 3D culture compared to the 2D culture	121

ically, *in vitro* 3D tissue-engineered, micro-patterned or microfluidics-based tumor models have gained significant attention to address limitations of 3D conventional or spheroid-based models, for mechanistic studies on cancer progression and anti-cancer drug responsiveness.[117] These technological platforms have provided unique flexibility in designing precise experiments to test the efficacy of multiple compounds in a timely and resource-efficient manner. In the following sections, we will provide a detailed overview on recent advancements in engineering of 3D micro-patterned tumor models, bioprinted models as well as microfluidic platforms for anti-cancer drug screening and discovery.

3D Micro-Patterned Tumor Models

Micro-patterning is an efficient way to mimic the highly structured microenvironment of native tissues within which cells reside.[23,101,104,147,176] Micro-patterning strategies have been also shown to enable the study of cellular morphogenesis and test the efficacy of anti-cancer drugs in a well-structured and spatially organized tissue at the microscale level.[135] The pioneering study by Bhatia *et al*. in 1993 demonstrated that selective micropatterning of hepatocytes, surrounded by co-culture of fibroblast cells, promotes physiologically relevant cellular response in the co-cultured hepatocytes, relative to hepatocytes in the mono-culture condition. These responses were detected by significantly promoted secretion of protein synthesis markers like albumin and urea, as well as nitrogen metabolism in the hepatocytes.[7] The proposed platform was compatible with conventional plate readers and was amenable for assessment of hepatotoxicity and combinatorial drug interactions.[7,59] Since then, several micro-patterned platforms have been developed for drug screening purposes. For instance, Shen *et al*. developed one of the early micro-patterned tumor models by applying collagen coated round glass coverslips onto laser engraved stencils. The stencils consisted of a repellent silicone mask engraved with circular apertures for the culture of MDA-MB-231 breast cancer cells. After stencil removal, normal human dermal fibroblasts (NHDF) were seeded around the micropatterned tumor cells on the glass substrate. This design was created to mimic the *in vivo* spatial organization of tumor and stromal cells. Two tumor inducing factors were upregulated with higher tumor-stromal interactions, indicating that stromal cells were activated by direct contact of tumor cells. This model was further used to investigate the efficiency of the chemotherapeutic drug, Reversine, in reducing bone metastasis by targeting tumor-stromal activation pathways in MCF7 cells.[135]

The Nikkhah lab recently developed a 3D micro-patterned tumor-stroma model for 3D cancer cell migration studies.[116] Specifically, a novel two-step photolithography fabrication technique involving crosslinking and micro-patterning of gelatin methacrylate (GelMA) was used to fabricate the tumor models. MDA-MB-231 and MCF7 cells were cultured and the migration of the cells monitored over a five-day period. Results indicated that cancer cells cultured within the tumor model maintained high cellular viability, and that the novel fabrication technique was instrumental in recapitulating the initial architecture of tumor tissue. MDA-MB-231 breast cancer cells exhibited an invasive pattern toward the stroma, while MCF7 cells demonstrated clustering tendencies. F-actin immunofluorescent images revealed that each of the cell lines (MDA-MB-231, MCF7) exhibited different morphologies within the microfluidic device, and that the MDA-MB-231 cells had the highest proliferation as compared to the other cell lines.[116] In a follow up study, the same group utilized micro-molding technique to generate a TME model in collagen and to investigate the role of stromal fibroblast cells on breast cancer invasion and drug responsiveness (Fig. 3B). This study primarily hypothesized that the presence of stromal cells, specifically CAFs, within the breast TME would lead to ECM remodeling and stiffening (i.e. desmoplasia) and consequently enhance the growth and invasion of the highly metastatic and tumorigenic breast cancer cells (MDA-MB-231, MCF7).[130,131] The proposed model was further utilized to assess the role of anti-cancer combinatorial drug treatment on cancer cell behavior and stromal matrix desmoplasia. Specifically, the platform consisted of a PDMS holder which was filled with CAFs-encapsulated collagen hydrogel. A PDMS stamp was further utilized to create high density microwells within the CAFs-encapsulated collagen. Upon formation, MDA-MB-231 cancer cells were seeded immediately within the microwells. This strategy led to the formation of an organotypic tumor-stroma model with highly organized tumor entities surrounded by CAFs. Upon formation of the model, the effects of anti-fibrotic and chemotherapeutic drugs, Tranilast and DOX, were investigated on cancer cell invasion and growth as well as regulating desmoplasia and ECM remodeling. The result of this study demonstrated a significant reduction in invasive behavior of cancer cells under combinatorial treatment. Notably, there was also a significant decrease in stromal ECM stiffness upon combinatorial administration of Tranilast and DOX. Further molecular analyses on Matrix Metalloproteinase (MMPs) and TIMPs indicated a significant reduction in MMP1 expression upon addition of Tranilast to the drug treatment regime.[130]

3D Printed Tumor Models for Drug Screening

Three dimensional (3D) printing provides another powerful strategy to develop complex microengineered platforms for disease modeling and multi-drug screening.[37,94] Creating a standardized test platform for anti-cancer drug screening could be achieved by utilizing 3D bioprinting technologies such as laser-based bioprinting, which is a non-contact method and allows spatial patterning of different cell-embedded biomaterials to test the toxicity of different anti-cancer drugs.[38] For instance, Kingsley *et al.* utilized a laser-direct write (LDW) bioprinting technique to pattern microbeads and produced size-controlled core-shelled structures in alginate hydrogel to improve the size controllability and targeted placement of microfabricated beads which could consequently contribute to the study of localized cell signaling and drug discovery (Fig. 3C).[64] They utilized this strategy to generate microbeads for the formation of self-aggregates of cells to produce multicellular tumor spheroids (MCTSs) to investigate the influence of spheroid size on drug delivery. The viability assessment of the generated MDA-MB-231 breast cancer spheroids revealed a significant decrease in viability for the microcapsule culture condition (core-shelled spheroids) in comparison to the printed ribbon and microbead culture conditions. However, formed microcapsules maintained viability during the experiments. Furthermore, due to the role of ligands in receptor-mediated drug delivery, the influence of the size of formed microcapsules (MDA-MB-231 spheroids) on ligand uptake was investigated by selecting Transferrin, (the ligand used to deliver anti-cancer drugs) where a larger spatial heterogeneity in ligand uptake was observed by increasing the size of formed tumor microcapsules.[64]

In another study, Xie *et al.* developed a novel 3D tumor array chip (3D-TAC) to screen anti-cancer drugs, namely, Epirubicin and Paclitaxel. The setup comprised of a printing unit which was used to generate GelMA based microdroplets as well as a culturing chamber. MDA-MB-231 breast cancer cells were encapsulated in the GelMA based microdroplets and seeded into the culturing chamber for drug screening tests. Laser scanning confocal microscopy indicated that Epirubicin exhibited a higher anti-tumoral effect in comparison to equal concentrations of Paclitaxel. Furthermore, flow cytometric analysis was used to test the apoptosis of the MDA-MB-231 cells. It was revealed that Epirubicin could cause apoptosis at the core of the cell clusters at lower concentrations as compared to Paclitaxel. Based on this study, it was concluded that Epirubicin was a more effective anti-cancer drug as compared to Paclitaxel.[165]

Microfluidic Platforms for Anti-cancer Drug Screening

Microfluidic platforms have provided powerful platform technologies for numerous applications in biomedicine from fundamental biological studies to disease modeling, regenerative medicine, organ-on-chip, and drug discovery.[5,29,58,141,155] Due to their attractive advantages such as inexpensive cost of production, creation of a physiologically relevant environment, and its transparent nature which permits real-time imaging of intercellular reactions, microfluidic platforms have been widely utilized to screen and determine the effects and cytotoxicity of certain anti-cancer drugs with high fidelity and precision.[1,15,22,25,29,39,52,58,72,78,115,117,137,142,148,161] These microfluidic platforms have shown the ability to recapitulate the complexities of the TME and investigate the role of its different components on cancer progression.[117] For instance, analyzing critical factors that cause cancer cells within the TME to build immunity and develop resistance to certain drugs during preclinical testing is essential. Therefore, customizable platforms such as tumor-on-a-chip (TMOC) models could optimize the preclinical processes and efficiently identify underlying causes of chemoresistance and ultimately lead to the development of better drugs for chemotherapy.[2,126] The TMOC models are particularly suitable for drug screening due to their capabilities in mimicking the influence of interstitial flow, plasma clearance, and drug diffusivity on the tumor *in vitro*.[126]

Recent studies have shown that tumor cells in ductal carcinoma *in situ* (DCIS) can obtain aggressive phenotypes and invade the basement membrane of the TME due to factors such as ECM remodeling, paracrine signaling, and immune response.[3,19,36,73] To further validate this finding and investigate possible treatment options for DCIS, a 3D microsystem that enabled the replication of the DCIS TME by co-culturing mammary fibroblasts with breast cancer cells, as well as mammary ductal epithelial cells with breast cancer cells was developed. For this purpose, a two-layered microchannel patterned PDMS device was fabricated (Fig. 4a). The two layers within the microfluidic device were separated by an ECM-derived membrane to form an upper channel to mimic the ductal lumen and a lower channel to mimic the vascular region of the *in vivo* mammary stroma respectively. The recreated ductal lumen was formed within the upper channel of the device by attaching human mammary epithelial cells to the upper side of the ECM membrane, while the stromal tissue was formed in the lower channel of the device by embedding human mammary fibroblasts in a collagen solution. DCIS spheroids (150 μm diameter) were then injected into the upper channel and allowed to incorporate into the

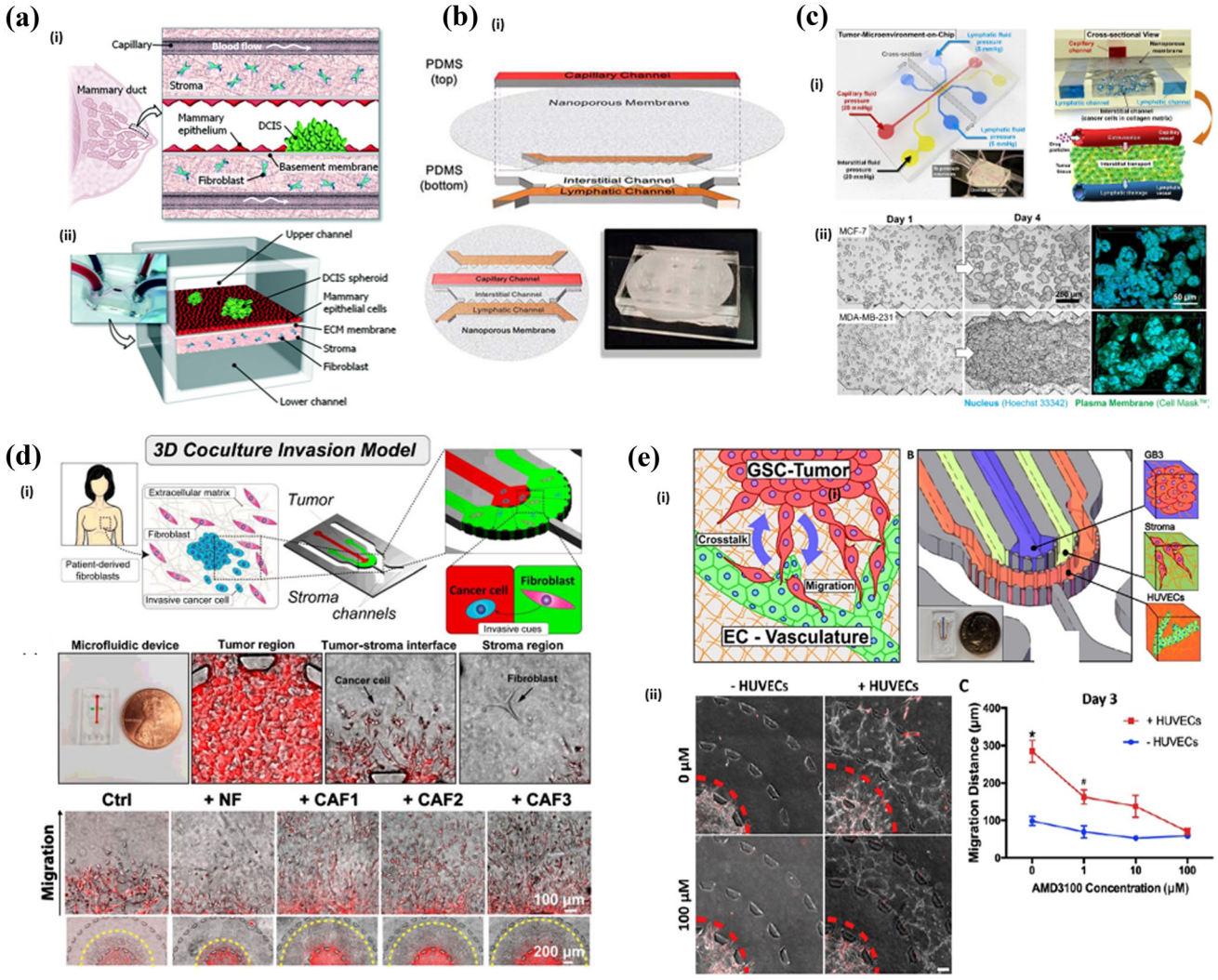

FIGURE 4. Representative figures of some microfluidic devices developed for drug screening studies. (a) Development of a human breast-on-a-chip model using microfluidic technology. (i) Representation of ductal carcinoma *in situ* (DCIS) within the mammary duct. (ii) Schematic of proposed microarchitecture for the breast-on-chip model. Adapted with permission from Royal Society of Chemistry: [Lab On a Chip], copyright (2015).[19] (b) (i) Schematic showing TME-on-a-chip model to assess drug transport and resistance in breast cancer cells. The device consisted of a capillary channel, interstitial, and a lymphatic channel. Adapted with permission from American Chemical Society: [Molecular Pharmaceutics], copyright (2016)[136] (c) Compartmentalized microfluidic invasion platform. (i) Capillary culture region (red), the lymphatic region (blue), the interstitial region (yellow). Scale bar is 125 μm. (ii) Phase contrast and fluorescent images showing morphology of breast cancer cells within the platform. Adapted with permission from Elsevier: [Journal of Controlled Release], copyright (2017).[112] (d) (i) Representation of microfluidic breast tumor-on-a-chip platform for cancer cell invasion studies. Second and third rows show cancer cells (red) invading adjacent stromal region, embedded with CAFs. The presence of CAFs significantly promoted cancer cell invasion. Adapted with permission from American Association for Cancer Research (AACR): [Cancer Research], copyright (2019).[149] (e) (i) Illustration of glioblastoma tumor microenvironment (TME) and schematic of 3D microfluidic device for engineering of the glioblastoma perivascular niche on-chip for invasion and drug response studies. (ii) Phase contrast images and graphs showing attenuation of migration distance in glioma stem cells exposed to AMD-3100 drug. Adapted with permission from Elsevier: [Biomaterials], copyright (2019).[148]

epithelial layer. Generating a continuous flow of Paclitaxel for treatment revealed increased cytotoxicity percentage in the group consisting of DCIS spheroids as compared to the control condition where there were no DCIS spheroids, indicating that Paclitaxel had no cytotoxic influence on normal epithelial cells. Furthermore, proliferation studies also confirmed that exposure of DCIS to Paclitaxel reduced tumor growth and thereby limited tumor progression within the device.[19]

In another study, Shin *et al.* engineered a breast cancer TMOC platform to investigate the response of specific breast cancer subtypes to certain anti-cancer drugs (Fig. 4b). Specifically, they developed a TMOC

platform consisting of a double-layered microfluidic device to mimic blood vessels, tumor interstitium and lymphatic vessels. A nano porous membrane was used to separate the first channel (capillary channel) from the second channel, designated as interstitial and lymphatic channel. Three different breast cancer cell lines (SUM-159PT, MDA-MB-231, MCF7) were then embedded in a collagen type I matrix and embedded into the devices, after which they were either exposed to the free form of DOX or DOX encapsulated in hyaluronic acid in nanoparticle form (DOX-HANP). Results from a viability assay indicated that SUM-159PT and MDA-MB-231 cells had a higher survival rate in comparison to the MCF7 cells treated with the same DOX dosage. It was concluded that DOX impeded cell growth of triple-negative breast cancer cell lines (MDA-MB-231 and SUM-159PT), and was effective in causing ultimate cell death in the MCF7 group.[136]

A similar study was conducted by Ozcelikkale *et al.* where a TMOC platform was fabricated to study the efficacy of free form of DOX and DOX encapsulated in hyaluronic acid (DOX-HANP) as a potential treatment for two different breast cancer types, namely MDA-MB-231 and MCF7 cell lines representing triple-negative and luminal A subtypes respectively. The fabricated TMOC sandwiched a porous membrane between two PDMS layers (Fig. 4c). The first PDMS layer represented the capillary vasculature around the tumor, while the second channel represented a lymphatic drainage system. A polycarbonate porous membrane with a pore size of 400 nm was used to mimic a leaky vasculature. The phenotypic changes of the cells cultured in the TMOC model was compared to an *in vivo* xenograft model as well as a 2D culture model to prove that the TMOC model was a physiologically relevant platform for drug transport screening. Employing flow-cytometry revealed that MDA-MB-231 had higher $CD44^{+}CD24^{lo/-}$ expression (associated phenotype with drug-resistant cancer cells) in TMOC rather than the 2D culture. However, MCF7 had a low expression of $CD44^{+}CD24^{lo/-}$ in both culture models, which validated the reliability of the TMOC for drug screening. Additionally, the efficacy of the DOX and DOX-HANP, which perfused for 1 h vs. 24 h, illustrated that free DOX accumulated relatively faster in the interstitial channel and disappeared gradually over the following hour. In the slow clearance scenario, the DOX-HANP accumulated faster than free DOX. However, DOX-HANP had more limited penetration, due to a larger molecular size of the drug.[112]

In a more recent study, Nikkhah's group developed a novel compartmentalized microfluidic tumor model, with well-defined architecture, consisting of adjacent tumor and stroma regions.[149] Specifically, the device consisted of two adjacent layers (i.e. tumor and stroma) separated by trapezoidal microposts which permitted the tumor cells to interact with stromal cells (Fig. 4d). Breast cancer cells (SUM-159, MDA-MB-231, MCF7) were co-cultured with either patient-derived CAFs or normal fibroblasts (NFs). This study unveiled the role of a gene of interest namely glycoprotein non-metastatic B (GPNMB), which emerged due to the presence of CAFs from triple-negative breast cancer patients in the stroma, in significantly promoting the invasion of tumor cells. Notably, knocking down of GPNMB led to significantly reduced tumor cell invasion in both mono-culture and co-culture conditions.[149] In another study, the same group utilized this microengineered TME platform to investigate the role of SAHA, an HDAC inhibitor on cancer cell progression. The influence of SAHA treatment on the SUM-159 cells, as well as the morphological and phenotypic changes caused by co-culturing of breast cancer cells with CAFs, were further investigated. A viability assay illustrated a decrease in survival of the peripheral tumor cells (near the media channels) at 10 μM concentration. Interestingly, a wound assay analysis showed an increase in migration of SUM-159 cells due to an increase in SAHA concentration, which contradicted 3D migration assay results that showed a decrease in migration under high SAHA concentrations. F-actin immunofluorescence assay was further used to observe the morphological patterns of the treated cells, where results illustrated that SAHA treated cells showed a more elongated structure, in comparison to a more rounded structure of untreated ones, which confirmed the role of SAHA in promoting the mesenchymal morphology of the cells.[115] Addition of CAFs to the tumor model enhanced tumor cell invasion and influenced the morphology of the cancer cells by increasing their protrusiveness in the untreated condition. These findings were consistent with other literature suggesting the role of HDAC inhibition on the upregulation of the mesenchymal markers [32,164] and morphological changes of the cells.[139] In another study by Konstantopoulos' team, a microfluidic assay (MAqCI) was developed to quantify breast cancer cell invasion in the presence of select therapeutic agents undergoing clinical trials.[168] The device consisted of two parallel channels which were connected by a Y-shaped microchannel. Metastatic MDA-MB-231 breast cancer cells were co-cultured with non-invasive MCF7 breast cancer cells. It was observed that, even when MDA-MB-231 cells were significantly diluted, about 20% of them remained migratory. Following establishment of the microfluidic device, SUM159, BT-549 and MDA-MB-231 cells were treated with BKM120 and Trame-

tinib drugs or a combination of the two. It was observed that, Trametinib, an MEK1/2 inhibitor, was successful in reducing the migratory potential of SUM159, BT-549 and MDA-MB-231 cells, while BKM120, a PI3K inhibitor, was mostly effective in reducing the migratory potential of MDA-MB-231 cells. These observations elucidated the potential of the engineered microfluidic model for screening therapeutics without the need to conduct further genetic testing.[168] In a more recent study by Nikkhah group, the previously developed two-layer tumor-stromal model[115,149,150] was further advanced into a three-layer microfluidic platform to investigate the influence of the perivascular niche on glioma stem cell stemness and the role of AMD-3100 drug on their invasion. Briefly, the microfluidic device consisted of three cell culture regions, namely tumor, stroma and vascular regions, each separated by trapezoidal microposts fabricated using soft lithography technique (Fig. 4e(i)). A vascular network was formed by embedding HUVECs in a fibrin gel and injecting the gel solution into the third layer (i.e. vascular) of the platform, after which patient-derived glioma stem cells (GB3 cells) were injected into the tumor region of the device to form the co-culture condition. It was primarily observed that GB3 cells migrated significantly in the co-culture condition as compared to the mono-culture condition. Moreover, the cells were exposed to AMD-3100 (Fig. 4e(ii)), a drug which targets the C2CX12 signaling pathway. Migration distance in the co-culture condition was significantly reduced after administration of this drug, and hence it was confirmed that cellular crosstalk within the perivascular niche is a crucial contributing factor in promoting glioblastoma tumor progression.[148]

In another study, Wang *et al*. developed a slightly different bilayer TMOC platform to recreate the vascular environment in a breast TME. This TMOC platform consisted of a bottom chamber for anti-cancer drug perfusion such as DOX and a polycarbonate membrane where NIH-3T3 fibroblasts were seeded on. Human umbilical vein cells (HUVECs) were also seeded beneath the PC membrane (Fig. 5a). The PC membrane had a pore size of 5 μm and was glued to a surface channel containing two distinctive chambers where drug-sensitive MCF7 and drug-resistive MCF7/ADR cell lines were cultured simultaneously. In this platform, the poor organization of vasculature beneath the PC membrane limited the access of nutrients and oxygen to the tumor, thereby causing hypoxia in the tumor. The presence of fibroblasts above the PC membrane also created a barrier that reduced the DOX uptake of the tumor, resulting in drug resistance. Moreover, exosome secretion by fibroblasts contributed to drug resistance development, which was observed by TEM imaging. It was concluded that the existence of the TME improved the viability rate of the treated cells with the same DOX concentration.[157]

The TMOC platform could also be used to address complexities within the TME, such as host immunological occurrences. To that end, Nguyen *et al*. proposed a 3D multi-culture system integrating cancer cells (BT474 cell line as a $HER2^+$ representative), immune cells (PBMCs, peripheral blood mononuclear cells), CAFs (Hs578T), and endothelial cells (HUVECs) to reconstruct an *in vitro* breast tumor ecosystem. The response of this ecosystem to Trastuzumab, as a clinically administrated drug for treating $HER2^+$ cancer, was further investigated. Observations revealed that interactions between BT474 and CAFs were transitory which indicated a long-distance communication mechanism. Furthermore, Trastuzumab exposure hindered the tumor growth within the platform. However, the presence or absence of PBMCs and CAFs did not significantly affect the tumor growth nor apoptosis mechanism. Interestingly, CAFs antagonized the effects of introduced Trastuzumab, thereby confirming the role of stromal cells in tumor progression.[102]

In another recent work, Haase *et al*., examined drug delivery in a 3D vascularized TMOC model (Fig. 5b). In the proposed system, two types of tumor spheroids were cultured within a perfusable microvascular network to investigate drug delivery mechanisms in a physiologically relevant TME by understanding the role of the endothelium. Ovarian (Skov3 cell line) and Lung (A549 cell line) tumor cells were selected to examine morphological changes and permeability differences related to the microvasculature network (MVNs). To find differences in drug uptake, P-glycoprotein (P-gp), an efflux pump linked to the multidrug resistance mechanism, was combined with Paclitaxel to enhance cellular drug uptake. Measuring fluorescent intensity distribution suggested that transfer of both Paclitaxel and possibly P-gp inhibitors, were impeded by the presence of the MVNs, as opposed to the control condition (2D assays or tumor spheroids alone). Therefore, P-gp-inhibitors had minimal effect on the chemotherapeutic agent accumulation in the cancerous tumor. In the 2D condition, after Paclitaxel treatment, CD44 expression was increased in both ovarian and lung cell types. However, flow cytometry measurements for tumor spheroids within MVNs illustrated similar CD44 expression for both treated and non-treated conditions, which further validated the necessity of a physiologically relevant platform for drug screening.[39] Although combinatorial anti-cancer drug schemes has become popular due to their ability to generate synergistic anti-cancer responses along with reducing single drug toxicity and suppressing the

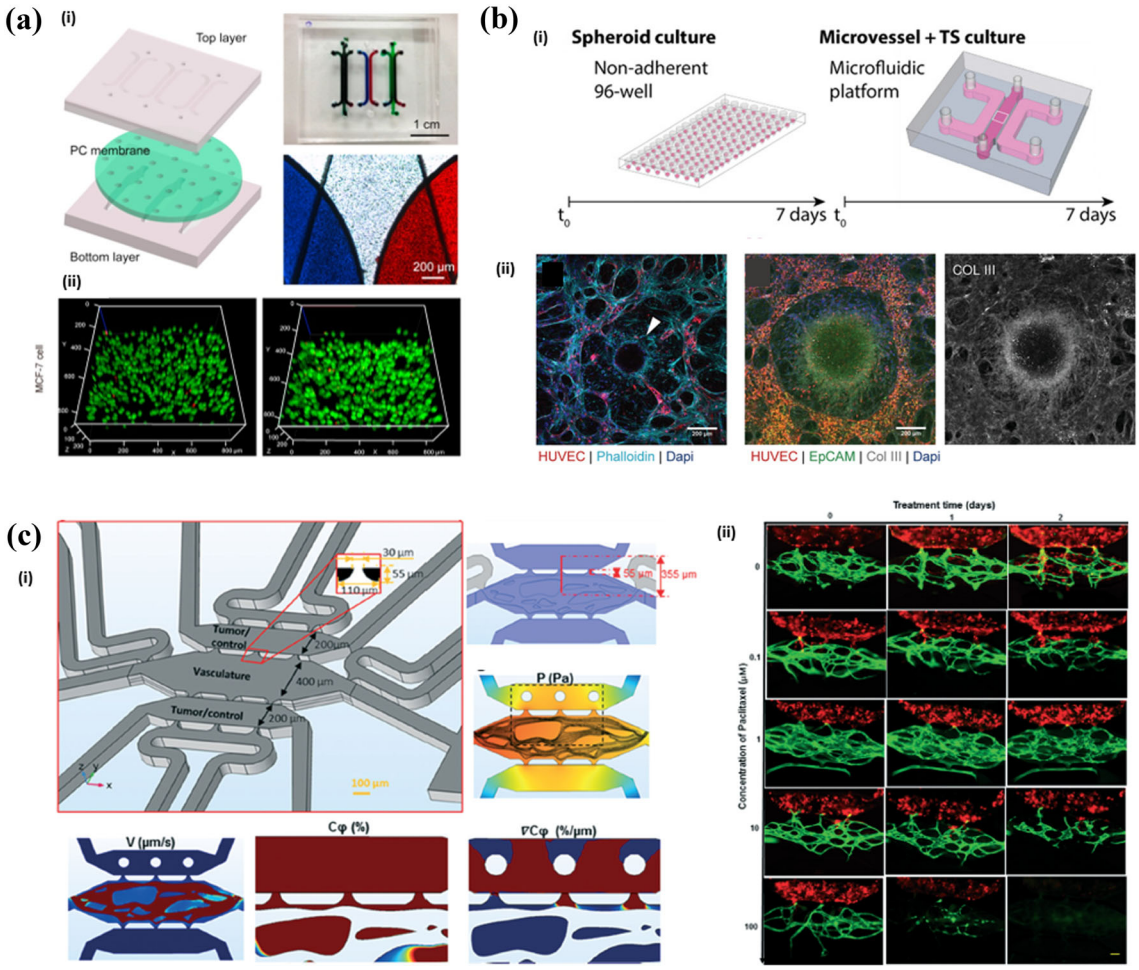

FIGURE 5. Representative images of engineered microfluidic device models for the recapitulation of native TME for drug screening (a) (i) Exploded view of microfluidic device showing bilayer design separated by polycarbonate membrane. (ii) Fluorescent images showing viability of breast cancer cells embedded in difunctional polyethylene glycol—glycol chitosan (DF-PEG-GCS) hydrogel Adapted with permission from Elsevier: [Talanta], copyright (2019).[157] (b) (i) Schematic of 96-well non-adherent assay used to generate spheroids for culture in engineered microfluidic platform. (ii) Fluorescent images showing vessels (blue-phalloidin, green-EpCAM, gray-Collagen 3) surrounding the formed spheroid within microfluidic device. Adapted with permission from Wiley: [Advanced Functional Materials], copyright (2020).[39] (c) (i) Schematic showing microfluidic platform with vasculature and tumor regions (ii) Fluorescent images showing MDA-MB-231 cells (red) and formed vasculature (green) being treated with Paclitaxel under different drug concentrations. Adapted with permission from American Chemical Society: [Lab on a Chip], copyright (2018).[137]

development of different drug resistance mechanisms, it is still challenging to conduct in-depth drug screening *in vitro* due to the lack of adequate 3D tumor models.[29,141] To investigate the underlying reasons and address some of these challenges, like the lack of relevant tumor models, Lin *et al.* developed a microfluidic chip containing a porous PC membrane, which was sandwiched between two PDMS layers (forming a 10 × 10 array). To increase the relevance of this platform, an endothelial barrier was recreated by forming a nutrient diffusion gradient on one side of the membrane and creating a flow-stop valve on the other side of the membrane by adjusting the air–liquid flow pattern. Breast cancer cells (MCF7 cell line) and hepatic cells (LO_2 cell line) were exposed to six chemotherapeutic drugs containing different concentrations of DOX, Paclitaxel, and Cisplatin as possible treatment scenarios. A dose-dependent response was observed for both MCF7 and LO_2 cell lines. The novel feature of this platform was the ability for orthogonal drug screening with a reduced number of required experiments.[78]

George's group recently reported the development of an on-chip microfluidic vascularized TME model to assess the role of physiological delivery of nutrients as well as anti-cancer drugs through the vascularized regions to various types of tumor organoids including colon and breast. The presence of the tumor organoids triggered angiogenesis within the proposed model. Notably, they also demonstrated the utility of the

proposed physiological tumor on-chip model for anti-cancer drug response studies, where there was a significant reduction in MDA-MB-231 cell growth in dose response studies using paclitaxel (Fig. 5c).[137]

Recently, a high-throughput two-layer microfluidic platform, consisting of a fluidic layer and an array of microwells, that could be utilized for simultaneous drug screening and assessing the influence of tumor size on drug resistivity, was developed by Wang's team (Fig. 6a(i)). The microfluidic model contained eight parallel chambers, a microchannel layer with 60 μm height and forty-nine pillars to prevent the channel from collapsing. The influence of two anti-cancer drugs, Bleomycin (BLM, targeting normoxic tumors) and Triapazamine (TPZ, targeting hypoxic tumors) and their influence on U251(human glioma) cells were investigated. Cell death was maximized at the peripheral region of the 3D tumor, which showed higher molecular diffusion at the curved region of formed tumors. Moreover, three days of drug treatment illustrated that drug efficacy was dose-dependent, similar to previous studies. Small sized tumors (100–300 μm) treated with BLM and TPZ depicted a similar cell death pattern. However, TPZ was more effective for larger tumors (500–600 μm) (Fig. 6a(ii)), and this was attributed to the formation of a hypoxic core in large 3D tumors.[79] In another study, a microfluidic flow cell array (MFCA) consisting of 48, 830 μm $\times$ 500 μm wells was designed to simultaneously test 5 different concentrations of the Cisplatin drug on human ovarian cancer cells. MTT and viability assays were initially performed, and cell survival and dose response curves were obtained. Results revealed that the dose response curve from the MFCA assay was similar to that of the MTT assay, indicating that the engineered MFCA assay was a useful screening tool for anti-cancer drug screening.[1] In a similar study by Ding *et al.*, a print-to-screen (P2S) microfluidic platform was designed to conduct high throughput drug screening on SKOV-3 ovarian cancer cells. Ten drugs, namely Actinomycin D, Adriamycin Hydrochloride, BCNU, Celecoxib, Ellipticine, Methotrexate, Mitomycin, Streptonigrin, Tamoxifen and Thalidomide were screened in a combinatory manner using the P2S device, and it was observed that some drugs had an antagonistic effect, while others exhibited a synergistic cytotoxic effect on the SKOV-3 cells. This high throughput model therefore served as a high efficiency, low cost platform for screening of various anti-cancer drugs in a timely manner.[27] Further multiplexed devices have also been designed to screen anti-cancer drugs on ovarian cancer cells with potential for personalized therapy.[22,24] Recently, Wang's team developed a three-layer high throughput microfluidic device to investigate the influence of CAFs on drug pharmacokinetics in a triple negative breast cancer model. MDA-MB-231 tumor cells and human microvascular endothelial cells (HMVECs) were embedded into the device, along with either normal fibroblasts, CAFs, or mesenchymal stem cells (MSCs) and then treated with DOX. It was observed that, the drug induced apoptosis while delaying pharmacokinetics due to the presence of the HMVEC monolayer, indicating that this platform could be as a screening tool for multiple anti-cancer drugs.[18]

The malignancy of cancer directly correlates with the ability of the tumor to metastasize and invade distant tissues which sometimes makes it improbable for the patients to recover fully.[3] However, recent studies have shown that metastasis could happen at any stage of cancer progression, and not necessarily at the final stages only.[34] Microfluidic technology could provide valuable information about cancer metastasis, drug response, and resistance mechanism in metastatic sites by serving as a physiologically relevant platform for in-depth studies. For instance, Mi *et al.* presented a microfluidic platform for the *in vitro* co-culture of MDA-MB-231 breast cancer cells with human mammary epithelial cells (HMEpiC) to study cell migration in different metastatic models by changing the densities of MDA-MB-231 and HMEpiCs and assess the response of these models to the chemotherapeutic drugs such as Paclitaxel and Tamoxifen (Fig. 6b(i)). Utilizing this platform revealed that the densities of the cancer cells determined the probability of metastasis occurrence. It was observed that increased secretion of IL-6 in the co-culture condition promoted the migratory behavior of the MDA-MB-231 cells (Fig. 6b(ii)). Moreover, higher concentrations of both drugs led to shorter migration distance.[95] However, comparing migration distance for the same concentration amongst different metastatic models illustrated a longer migration distance for higher MDA-MB-231 densities.[95]

Extravasation is another phenomenon that takes part in the metastatic cascade, where cancer cells invade a new tissue by transmigrating in the bloodstream.[117] Besides serving as a medium of transportation for extravasating cells, the vessel networks within TME are a major source of nutrients for most cancer types. Therefore, researchers have conducted in-depth studies to understand their influence on tumor invasion, and assess how certain drugs can be used to target these underlying mechanisms to reduce tumor persistence and recurrence. For instance, in a recent study by Ko *et al.*, a PDMS-based microfluidic model consisting of a standardized, ready-to-use 96 well plate was developed (Fig. 6c(i)).[67] Due to the standardized nature of the platform, the model was utilized in a high throughput fashion to establish a vascularized tumor model for drug screening. Specifi-

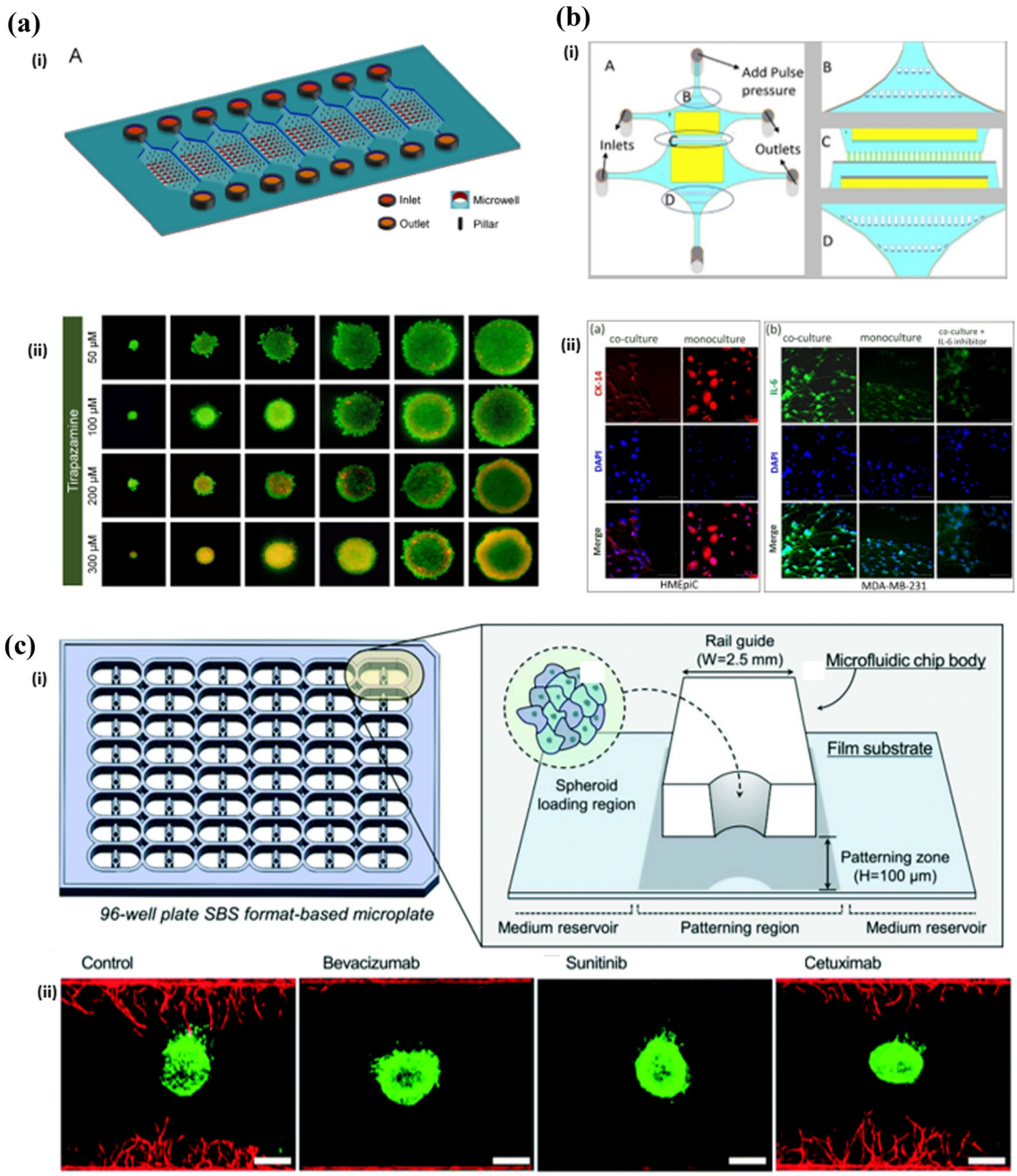

FIGURE 6. Representative high-throughput platform utilized for anti-cancer drug screening studies. (a) (i) Illustration of high-throughput microfluidic platform utilized for drug screening of DOX and Etoposide after exposure to human hepatoma and glioma cells (ii) Fluorescent images showing chemosensitivity of different spheroid sizes to Tirapazamine (TPZ). Adapted with permission from Elsevier: [Sensors and Actuators B: Chemical], copyright (2019)[79]. (b) Representation of a PDMS-based microfluidic platform showing co-culture chambers separated by microchannel arrays through surface tension. Devices were used to culture uniformly dense MDA-MB-231 breast cancer cells for screening of Paclitaxel and Tamoxifen (ii) Immunofluorescent images showing expression of CK-14 and IL-6 in HMEpiC and MDA-MB-231 breast cancer cells. Adapted with permission from Nature Publishing Group: [Scientific Reports], copyright (2016)[95] (C) (i) Schematic showing semi high-throughput microfluidic platform utilized for the investigation of the influence of anti-angiogenic drugs on formed vessels. (ii) Fluorescent images showing exposure of co-culture platform to anti-angiogenic drugs and inhibitors. Administration of drugs prevented HUVECs (red) from undergoing angiogenesis. Adapted with permission from Royal Society of Chemistry: [Lab on a Chip], copyright (2019).[67]

cally, culturing glioblastoma U87MG cells with HUVECs in a fibrin gel, to form a 3D vascularized-tumor model, and further treating them with anti-angiogenic drugs/inhibitors (i.e. Cetuximab, Bevacizumab, Sunitinib) significantly reduced the vascular network area within the devices (Fig. 6c(ii)). This model can be further utilized to investigate the influence of blood vessels within the TME on tumor formation, progression and recurrence.[67]

Breast to bone metastasis is a common occurrence which happens in later stages of breast cancer, and therefore has a considerably higher mortality rate.[12] Jeon *et al*. engineered a compartmentalized microfluidic chip capable of modeling a human quad-culture platform where breast cancer cells (MDA-MB-231) could flow, adhere to, and metastasize in the formed microvascular network. The presented platform consisted of side channels acting as blood vessels and a central channel as the bone-mimicking site where HUVECs, primary human bone marrow-derived mesenchymal stem cells (hBM-MSCs), and osteoblast-differentiated cells (OBs) were tri-cultured in a fibrin gel. Results indicated that the permeability and extravasation rates were significantly different in the control condition (hBM-MSCs) compared with conditions that had the bone-mimicking sites. Moreover, the influence of adenosine on extravasation was investigated by using a blocking agent (A_3 adenosine receptors), where the outcome demonstrated that adenosine had a potential to diminish extravasation.[47] In another study conducted by Clark *et al*., breast cancer metastasis to liver was mimicked in a microfluidic chip by utilizing a modified polyethyleneglycol-based hydrogel, SynKRGD. The platform incorporated a tri-culture region containing donor isolated primary human hepatocytes, donor isolated nonparenchymal cells (NPC), and MDA-MB-231 breast cancer cells to examine the inflammatory phenotype of the cancer cells, as well as their response to DOX and Cisplatin. Their results indicated lower levels of pro-inflammatory analytes secreted in the hydrogel-supported scaffold (SynKRGD) compared to the control condition (polystyrene scaffold). The proportion of MDA-MB-231 cells entering dormancy was decreased in the polystyrene scaffold compared to the SynKRGD scaffold. Interestingly, a dose-dependent response against MDA-MB-231 cells was not observed in the SynKRGD scaffold which might be due to patient pathophysiology.[20]

Perspective: Advantages of Microfluidic Models

Advances in sequencing technologies have significantly benefited personalized medicine for the development of tailored treatment plans for individual patients based on their genomic data.[33,62,114] Cancer genomics is complicated, and so even though there has been increased knowledge regarding mutation occurrence, the underlying mechanisms of drug response are still not fully unveiled. The genomic data need to be potentially merged with other technologies, such as advanced 3D *in vitro* tumor models, to enhance the efficacy of personalized therapy in cancer.[117,163] To that end, in recent years, there has been tremendous effort in development of high-throughput microfluidic platforms to screen various drug combinations on tumor biopsies from patients *in vitro*, integrated with gene expression analyses.

The reviewed studies illustrates that microfluidic platforms provide efficient and competent assays for drug screening applications. Microfluidic platforms are capable of eliminating some of the current drug screening challenges by utilizing low working volumes, low cell numbers, high-throughput experimentation, and reliable data generation. Several studies have shown the aptitude of cancer-on-a-chip models in determining the susceptibility of different cancer cell lines to chemotherapeutic agents and combinatorial drug schemes. For new drug development, microfluidic platforms provide an inexpensive and more reliable anti-cancer drug screening studies due to its ability to investigate the emergence of drug-resistive mechanisms within different cancer types.

Acquiring cancer cells from a primary tumor biopsy is a laborious task and the number of cells obtained is quite low, which makes the microfluidic platform an exceptional candidate for individualized treatments. Microfluidic platforms have the capability to offer tools for analyzing several combinatorial drug scenarios at the preclinical stage on patient samples. Patient-derived cancer cells could be incorporated with induced pluripotent stem cells and other cell types derived from the same patient to recreate a patient specific cancer on-a-chip.[88] The patient specific biomimicking cancer models could be utilized to test the response of specific chemotherapeutic agents before starting the actual clinical treatment plan. Furthermore, these microfluidic models can be used to predict the response of patients in the clinical drug trials for FDA approvals. Table 2 summarizes the

TABLE 2. Summary of representative microfluidic tumor models for anti-cancer drug screening.

Cancer type	Cell line	Drug	Key point	References
Breast	MCF10 DCIS	Paclitaxel	Paclitaxel has negligible cytotoxic effects on normal epithelial cells. However, the presence of DCIS increases the cytotoxicity.	19
Breast	SUM-159PT, MDA-MB-231, MCF7	DOX DOX-HANP	DOX impedes cell growth of triple-negative breast cancer cells. However, it causes ultimate death for $HER2^-$ subtype. Cellular drug transport could be quantified by rate constants representing the uptake and efflux processes across the cellular membrane.	136
Breast	MDA-MB-231	Paclitaxel Tamoxifen	A synergetic effect between Paclitaxel and cisplatin	141
Breast	MDA-MB-231, MCF7, SUM-159, BT-549	BKM120, Trametinib	Trametinib was effective in reducing migration of invasive cells	168
Breast	MCF7, MDA-MB-231	DOX DOX-HANP	Higher CD44 expression in TMOC platform compared to 2D	112
Breast	SUM-159	SAHA	Compartmentalized microfluidic platform to visualize the invasion profile and promoting role of CAFs	115
Breast	MCF7, BT474	Trastuzumab	CAFs antagonized the effects of trastuzumab	102
Breast	MCF7, MCF7/ADR	DOX	Exosome secretion by fibroblasts contributed to drug resistance	157
Breast	MCF7	DOX	Fabrication of orthogonal drug screening platform	78
Breast/colon	MDA-MB-231, MCF7, Caco-2, patient--derived tumor organoids	Paclitaxel	Physiological delivery of Paclitaxel through vascular network significantly reduced tumor growth.	137
Ovarian	SKOV-3	Paclitaxel	Presence of vasculature network alters drug efficacy	39
Ovarian	SKOV-3	Cisplatin	Fabrication of MFCA assay for parallel drug screening	1
Ovarian	SKOV-3	Actinomycin D, Adriamycin Hydrochloride, BCNU, Celecoxib, Ellipticine, Mitomycin, Methotrexate, Streptonigrin, Tamoxifen, Thalidomide	High throughput print-to-screen microfluidic device for combinatorial drug testing	27
Ovarian	TOV112D	Carboplatin Paclitaxel	Microfluidic multiplex model to screen chemoresistance of cancer cells	24
Ovarian	PH039, PH592, PH704, PH757, PH398	DOX Gemcitabine	Response of ovarian cells to select drugs in microfluidic multiplex platform	22
Glioblastoma	U87	DOX	Genomic aberrations responsible for the development of the DOX resistance could be identified by performing exome sequencing	42
Glioblastoma	U87	Pitavastatin Irinotecan	Combinatorial drug scheme decreases cell viability	31
Glioblastoma	U251	BLM (Normoxic targeting) TPZ (Hypoxic targeting)	TPZ was more effective for larger tumors due to targeting hypoxic tumors	79

outcomes of major drug testing experiments performed in recent years, while Table 3 summarizes the advantages and disadvantages of the aforementioned 3D models for anti-cancer drug discovery.

CONCLUSION AND FUTURE PROSPECTS

The development of drugs with enhanced efficacy and outcome has been a key factor in treatment of cancer and increased survival rate of patients. In the past decade, men diagnosed with cancer have experienced a higher survival rate than women.[138] This observance has been attributed to several factors, including sex-biased genes that are mutated in certain types of cancer, leading to a better response in men than women.[171] By reviewing therapeutic applications of 3D models in drug development in female-specific cancers, we can appreciate how far the research field has come in increasing the life expectancy of females diagnosed with cancers. Although there has been significant increase in survival of women with cancer

TABLE 3. An overview of the advantages and disadvantages of different types of 3D models for anti-cancer drug development.

Model type	Advantage	Disadvantages	Cost
Scaffold based 3D cell-laden models	Could be incorporated with key ECM components of the native tumor microenvironment, Straightforward and easy to construct	Low degree of complexity, Limited capability for real time observation of intercellular processes due to relatively large size, Random co-culture and distribution of cells, Limited throughput	Low cost
Spheroid-based 3D models	Could establish a hypoxic core within the tumor Can be used for high throughput screening of various therapeutic drugs due to ease of construction and time efficiency	Low degree of complexity, Lacks physiological relevance due to absence of ECM and organotypic tissue architecture, Random co-culture and distribution of cells	Low cost
Micropatterned tumor models	High degree of complexity and control in spatial organization of cells and ECM at microscale level, Enables real-time observation of intercellular processes due to small (i.e., microscale) size of the model, Requires low working volumes and cell numbers	May require higher degree of expertise for fabrication compared to scaffold-based and spheroid-based models	Relatively low cost
3D bioprinted models	High degree of complexity and control in spatial patterning and organization of cells/ECM with organotypic architecture, Permits real-time observation of intercellular reactions due to small size of model, Utilizes low working volumes and cell numbers	Limited degree of manipulation after model is printed, i.e., establishment of gradient of biochemical factors or drugs may be challenging, cells may not be efficiently extracted for further molecular-level analysis, May require an expensive setup	Requires relatively expensive bioprinting setup
Microfluidic models	Very high degree of complexity and control in spatial organization of cells and ECM at microscale level, The ability to construct organotypic architecture of the TME, The ability to generate gradient of biochemical factors or drugs, Enables real-time observation of intercellular processes and high-resolution imaging due to small (i.e., microscale) sample size, Utilizes low working volumes and low cell numbers	May require high degree of expertise and training to fabricate model	Relatively low cost

within the last few years, various challenges such as tumor heterogeneity, chemoresistance stemming from either tumoral heterogeneity and/or drug induced heterogeneity, as well as the lack of adequate platforms to properly mimic the complexities of the TME have been major barriers in successful drug development processes.[3] Traditional 2D cell culture assays do not accurately present the spatiotemporal complexity of the native TME since the cells are grown on flat surfaces and are therefore not exposed to a 3D physiologic environment, to reliably advise on the effects of anti-cancer drugs during pre-clinical studies.[55] *In vivo* animal models have long provided gold standards for drug discovery screening, however, these models exhibit certain disadvantages a such as high cost and being non-translative due to their dissimilarity to the human physiology.[126] Most *in vivo* models like transgenic models also lack the immunity presented by the native TME due to alterations made to certain genes during experimental procedures, and therefore have low predictive values when used for anti-cancer drug screening.[126]

Recently, three dimensional (3D) spheroid-based models and scaffold-based models have been introduced to circumvent the disadvantages of *in vivo* models, while simultaneously being cost-effective and relatively easy to use.[15,60,92,100,111] Although these platforms have been useful in improving our understanding of various molecular mechanisms underlying cancer progression, they still lack certain key advantages like permitting real-time visualization of cell-cell interactions, random co-culture with lack of tissue architecture, amongst others.[55,126,142,153] Recent technological advances such as state-of-the-art microengineered platforms (e.g. microfluidics) and high throughput microscale platforms have improved the efficiency and workflow of drug testing in cancer research due to their sophisticated characteristics and the unique ability to adequately recapitulate the complexities of the native TME.[28,29,126,153] These micro-

engineered technologies are easy to use, cost-effective, and allow scientists to investigate the role of specific components of the TME on tumor progression. For instance, tumor-on-a-chip (TMOC) models have been used widely to model several cancer types in a time and cost efficient manner. By using these models, it has been possible to improve understanding on the biological complexities of the native TME and how these complexities influence tumor progression, drug responsiveness as well as the mechanism of anti-cancer drug resistance.[50,112,113] More importantly, these state-of-the-art technologies and high throughput platforms have enabled simultaneous testing of multiple drug compounds to investigate how they synergistically or antagonistically influence tumor progression before proceeding to clinical trials.

These developments have enormous potentials to advance preclinical combinatorial therapies and personalized medicine.[3,54,119] For instance, these models could be further employed to investigate molecular mechanisms induced by sex disparities which lead to a lower survival rate of cancer in women. However, further improvements in the current technologies are still required to ensure more mechanistic understanding of underlying mechanisms surrounding tumor biology, progression and chemoresistance. Some of these advances could for instance be incorporation of multi-culture and patient-derived cells, long term culture, the ability to incorporate hypoxia and gradient of drugs as well as addition of more biomimetic ECM within the engineered tumor models to precisely assess drug distribution, penetration and pharmacokinetics.

In summary, in this review article, we discussed the use of various *in vitro* tumor models for drug discovery in female-related cancers such as breast, cervical and ovarian which are collectively the second leading cause of death in women. We also briefly discussed other types of cancer such as colon adenocarcinomas and glioblastoma due to the high rate of occurrence in women as well as the high rate of sex-biased mutations which occur in these types of cancers and potentially influence the survival rate in women. By focusing specifically on female-related cancers, we have demonstrated how research advancements have led to the development and discovery of new drug compounds for treatment of cancer in women.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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