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Review

Evaluating Biomaterial- and Microfluidic-Based 3D Tumor Models

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Cancer is a major cause of morbidity and mortality worldwide, with a disease burden estimated to increase over the coming decades. Disease heterogeneity and limited information on cancer biology and disease mechanisms are aspects that 2D cell cultures fail to address. Here, we review the current 'state-of-the-art' in 3D tissue-engineering (TE) models developed for, and used in, cancer research. We assess the potential for scaffold-based TE models and microfluidics to fill the gap between 2D models and clinical application. We also discuss recent advances in combining the principles of 3D TE models and microfluidics, with a special focus on biomaterials and the most promising chip-based 3D models.

The Importance of 3D *In Vitro* Tissue Models for Advanced Cancer Research

Conventional approaches used in cancer research involve culturing tumor cells on 2D surfaces and the use of animal models, which both poorly correlate with human disease states. 2D cell cultures oversimplify the biological context of a tumor, which is influenced by intrinsic molecular features and external cues from its surrounding microenvironment [1]. Unlike cancer cells grown in 2D, those grown in 3D adopt a rounded shape, forming clusters that are suggestive of tumors *in vivo* [2,3]. Cancer cells grown in 2D versus 3D also exhibit differential gene expression profiles for key genes involved in angiogenesis, cell migration, and invasion [4–8]. *Ex vivo* models or *in vivo* models, such as animal or patient-derived xenograft (PDX) models, are also popular tools for cancer research. Such models have advantages over cell cultures and do not suffer from the lack of 3D context, although they have their own set of limitations (Box 1).

To address the limitations of conventional approaches, the 3D microenvironment of tumors must be taken into account to improve the physiological relevance of *in vitro* models [9,10]. The integration of TE strategies and microfluidic technologies has recently sparked a breakthrough in the design of *in vitro* microfluidic culture models that better adapt to morphological changes in tissue structure and function over time, providing a level of precision control that could not be achieved previously [11]. Here, we review the current 'state-of-the-art' of 3D TE models that have been developed and used in cancer research. We critically assess the relevance of 3D cell models in cancer studies, and discuss the main advantages and limitations, with special emphasis on the biomaterials used. We also highlight new approaches that integrate bioreactors and microfluidic technology, along with the potential impact of 3D TE models on the cancer drug discovery process.

Classical 3D culture systems can be broadly subdivided as scaffold-free or scaffold-based methods [12]. Although scaffold-free 3D cancer models are best exemplified by tumor spheroids

Trends

Conventional 2D approaches used in cancer research poorly correlate with the human condition when compared with the possibilities of 3D models.

We examine how microfluidics can fulfil gaps in 3D models and their other significant advantages, with a special focus on biomaterials.

Using the combination of 3D TE and microfluidics on a multiomics-based approach (i.e., genomics and proteomics) in cancer research can advance our understanding of cancer biology as well as lead to the discovery of novel biomarkers, promising a revolution in the cancer research field. Further development of technologies that are appropriate and sensitive enough to make the most of the new features of microfluidics assays are essential. The development of chip-based 3D cell cultures in cancer research will also be largely dependent on the improvement of biomaterials that emulate the extracellular matrix.

While the physiological architecture of human organs currently exceeds the complexity of all *in vitro* culture systems, microfluidic cell culture devices can be fabricated that capture some of this architectural complexity.

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Box 1. Advantages and Disadvantages of *Ex Vivo* Models, Animal Models, and PDX Models

***Ex Vivo* Models**

Ex vivo tumor culture is performed using a thin slice of tumor tissue collected from human or animal sources and cultured on porous substrates or embedded in ECM-like matrices [55,56]. These models generally preserve the native complex and differentiated 3D cell-matrix architecture, cell phenotype, and complex architecture, logically providing a more accurate mimic of cell behavior.

However, the main drawback of this type of model may be the absence of mechanical forces, such as shear stress and perfusion, as well as surrounding tissue, which may result in changes in the structure and cell behavior compared with the original *in vivo* microenvironment. Another drawback is the need to harvest tissue from human or animal subjects.

Animal Models

Mouse models have proven essential in cancer research. These models yield better prediction of drug behavior and efficacy in humans compared with 2D conventional culture. They are used to understand the genetic basis of tumor development and cancer progression. They can also be used to test the efficacies of different anticancer agents because of their intrinsic microenvironmental complexity. Animal models enable studies of defined mutations, including the analysis of the effects of these mutations on many genetic backgrounds.

However, there is rowing demand from the public to reduce the use of animals as experimental subjects [57–59]. Other limitations involve the inability to mimic human-specific features relating to tumors, autoimmune conditions, stem cell differentiation, and, ultimately, their responses to therapeutic drugs. This is because the physiology, metabolism, tumor cell interactions with the innate immune system, proliferation, metastasis, and the cells themselves are different from those in humans [60,61].

PDX Models

PDX models are models where surgically resected primary tumor samples are engrafted directly from patients onto immunodeficient mice. These enable the molecular, genetic, and histological heterogeneity of their parental tumors to be preserved for longer [62]. PDX models offer a powerful tool for cancer research and a route toward personalized medicine for patients with cancer. They also enable the discovery of biomarkers predicting drug sensitivity and resistance, and possibly the monitoring of the initiation and progression of metastasis as well as the fate of circulating tumor cells using *in vivo* flow cytometry of implanted humor tumors [63].

(Box 2), we focus here on scaffold-based methods because they offer more opportunities for combination with other technologies. Scaffold materials can be synthetic or natural in origin [13]. Synthetic materials typically display better mechanical properties compared with natural ones (Table 1), but we focus our discussion on scaffolds made from naturally derived materials due to their greater physiological relevance. Biomaterials are broadly used for their marked similarities to the extracellular matrix (ECM), and typically have advantageous features, such as biocompatibility, biodegradability, and bioavailability, and also the capability to interact with cells. Additionally, natural polymers can be engineered and their properties tuned to obtain desirable mechanical and physical characteristics [14].

In Vitro 3D Scaffold-Based TE Tumor Models

Scaffold-based models have the advantage of allowing the study of tumor interactions with the microenvironment, in particular, phenomena such as tumor migration and invasion. Another advantage is the possible functionalization of the scaffold materials to obtain desired physicochemical and biological characteristics. For example, it is possible to incorporate bioactive molecules that promote cell adhesion or matrix metalloproteinase (MMP) substrates that render the materials susceptible to degradation by cell-secreted proteases, thus mimicking the naturally occurring interactions of cells with ECM and its consequent remodeling [15]. Great care and attention are required when choosing the biomaterial for culturing cancer cells, to better emulate the physiology of their original ECM, since this facet alone is able to influence tissue organization [11,16].

Models using Matrigel® as reconstituted basement membrane [17,18] can mimic the pathophysiological context of cancer and have enabled advances in 3D tissue engineering. The development of Matrigel grew from pioneering work on the isolation and purification of proteins

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Box 2. *In Vitro* 3D Multicellular Tumor Spheroids

Spheroids form due to the ability of cells in suspension to self-assemble when cell–cell interactions are stronger than those between cells and substrate [64]. Their small size and the absence of vascularization result in limitations to nutrient and oxygen diffusion, requiring the cells of the core of the spheroid to adopt a glycolytic metabolic activity, which results in an increasing pH gradient toward the outside [65]. This mimics what happens in tumors, which have regions of hypoxia and of acidity similarly structured as a function of the distance between the core and the tumor blood vessels [66–68]. Spheroids can be fabricated using several methods: (i) hanging drop method, in which cells can aggregate freely at the bottom of an inverted cell suspension drop; (ii) spinner flask method, which provides constant agitation, allowing spontaneous cell aggregation; (iii) static liquid overlay technique, which enables spheroid formation by preventing cell adhesion to the growth substrate; (iv) centrifugation; and (v) growing cells on nonadherent micropatterned surfaces in microfabricated devices to favor their aggregation [69].

Given their tumor-like features, *in vitro* multicellular spheroids have been particularly useful for studying the efficacy of novel chemotherapeutic agents or drug delivery systems. Significant differences in drug responses have been observed for numerous cancer types in spheroid culture, but increased chemical resistance appears to depend upon the type of cancer cells and the specific treatment under study [70,71].

The emerging resistance to chemotherapies expands the potential application for spheroid cancer models. Breast cancer tumor recurrence, a major cause of death, has recently been attributed to a small population of cells with stem-like characteristics that are able to self-renew and promote tumor progression [72,73]. A 3D spheroid tumor model with stem-like properties was used to discover a new inhibitor of spheroid formation [74]. This stem-like enriched spheroid formation technology could also be applied to drug discovery in other cancer types [75].

Despite the advantages of using spheroids in cancer research, they are still not routinely incorporated into drug discovery, most likely due to technical hurdles. For example, automated analysis systems are not compatible with loose spheroids, which can clog pipettes and tubing. Tethered spheroids may overcome this hurdle [76].

such laminin and type IV procollagen and the discovery of the biological activity of the reconstituted basement membrane [19,20]. Matrigel has been used to mimic breast cancer progression [5] and help gain understanding of how tissue organization itself influences the development of a malignant phenotype [17]. Despite being the most commonly used biomaterial in cancer research, Matrigel has some disadvantages. Given that it is a biological animal-derived product, it lacks human peptide motifs. There can also be possible growth factor contamination, as well as variation in endotoxin levels and stiffness between batches. These limitations, along with the possible presence of undefined substances, make comparison between studies more challenging [21,22].

Collagen I is a frequently used substrate for cell culture and TE applications because it contains the tripeptide RGD (Arg-Gly-Asp), a short amino acid sequence that preferentially binds to receptors on cell surfaces. Scaffolds made from Collagen I can be synthetically modified to provide a wider range of physicochemical properties. For example, collagen stiffness can be adjusted through covalent crosslinking by nonenzymatic glycation. One of the first organotypic models comprised isolated human fetal colonic epithelial cells seeded on a collagen type I matrix with embedded colonic fibroblasts [23]. Collagen I was also used to produce a biologically relevant 3D tumor model that supported unconfined cellular proliferation and exhibited necrosis beyond a depth of approximately 150 μm and also had angiogenic potential [24]. The biocompatibility and 3D architecture of collagen I hydrogels are suitable for reproducing the microenvironmental conditions of a solid tumor.

Due to the relevance of paracrine function as a major mechanism of cell–cell communication within the tissue microenvironment, both in normal development and cancer, a 3D model to study paracrine function was needed. A recent 3D model, fabricated from alginate, has been used to recapitulate autocrine and paracrine functions in cancer [2]. The model was used to study paracrine interactions between prostate cancer cells and normal prostate stromal cells, in which direct interactions between epithelial and stromal cells are not allowed. Alginate was chosen as the biomaterial because of its hydrophilic nature, allowing a high functional cell loading

Table 1. Naturally Derived and Synthetic Matrices for 3D Tumor Engineering

Material	Characteristics	Cancer Research Applications	Refs
Natural materials			
Matrigel®	3D hydrogel microenvironment for tumor growth and angiogenesis studies; cytocompatibility; cell adhesion sites; tunable physical properties	Human colon adenocarcinoma, colorectal cancer, prostate cancer, breast cancer	[77,78]
Collagen I	Multiple crosslinking methods; biocompatibility; biodegradability; angiogenesis potential	Human breast carcinoma, human hepatocellular liver carcinoma	[24,71,79,80]
Fibrinogen	Blood clotting; cellular and matrix interactions; neoplasia; architecture mimics native ECM; 3D microenvironment for cancer growth	Melanoma, ovarian cancer cells, liver carcinoma	[25,81]
Hyaluronan	Glycosaminoglycan found in extracellular tissue in many parts of body; major component of native brain ECM; used for studying tumor migration processes	Prostate cancer, glioma tumor	[82,83]
Chitosan	Analysis of interaction of prostate cancer tumor cells with immune cells; formation of tumor spheroids	Prostate cancer, glioma tumor	[84–86]
Alginate	Properties for cell transplantation, drug delivery, and TE; suitable for hydrogel microspheres; promotes conversion of cultured cancer cells to a more malignant *in vivo*-like phenotype; nonadhesive to cells	Oral squamous cell carcinoma, human hepatocellular carcinoma	[2,86–88]
Fibroblast-derived matrices	Distinctly different cell morphology, aggregation pattern, proliferation profile and invasive potential; however, these matrices do not fully represent the composition and structure of the tumor microenvironment	Human colorectal carcinoma, human pancreatic carcinoma	[89,90]
Silk fibroin	Unique mechanical properties; good biocompatibility; well-controlled degradability; versatile processability	Human breast adenocarcinoma	[91]
Agarose	Amenable mechanical and biological properties; more stable than traditional natural hydrogels	Osteosarcoma and breast adenocarcinoma	[92]
Synthetic materials			
Polyethylene glycol (PEG)	Biocompatibility; high water content; multitunable properties; specific biological functionalities can be covalently incorporated	Human epithelial ovarian cancer, human pancreatic ductal adenocarcinoma	[15,93,94]
Poly(lactic-co-glycolic) acid (PLGA)	Highly porous scaffolds; convenient to handle; amenable to large-scale use	Oral squamous, cell carcinoma	[95,96]
Poly ε-caprolactone (PCL)	Biologically inert synthetic polymer; high porosity; large surface area:volume ratio for cellular attachment; tunable fiber diameter; low cost	Ewing sarcoma	[97]
Synthetic peptides	Controlled amino acid composition for easy incorporation of specific biological relevant ligands; adequate physiological properties	Human ovarian carcinoma, human breast carcinoma	[98–100]

in culture. Alginate hydrogels have the appropriate characteristics for cell transplantation, drug delivery, and TE because their production and cell encapsulation can be achieved under mild conditions.

Fibrin (purified from fibrinogen) hydrogels are also used as a biomaterial in cancer research, supporting the growth of cancer cells into colonies that resemble embryonic stem cell colonies [25]. Fibrin is present in connective tissue stroma in human malignant tumors, and fibrin and fibrinogen can increase the survival and metastatic potential of circulating tumor cells [25]. *In vitro* scaffold-based cancer models have been used in drug discovery to understand mechanisms of action, find novel targets, or address drug efficacy, toxicity, and resistance events. For instance, the efficacy of three anticancer drugs (i.e., camptothecin, docetaxel, and rapamycin) in the treatment of bone metastatic prostate cancer was assessed using hyaluronic acid (HA)-derived hydrogels to grow the lymph node carcinoma of the prostate (LNCaP) [3]. The difference in efficacy between the three drugs may reflect their different mechanisms of action and chemical properties, emphasizing the importance of a proper microenvironment in anticancer drug efficacy assessments. HA allowed cells residing in the hydrogel matrix to form distinct clustered structures that grew and merged, reminiscent of real tumors [3].

Other biocompatible hydrogels, such as those made of gellan gum or silk [26,27], can be used in the development of complex microtissues for cancer research. These two naturally derived hydrogels attract special interest since they may provide important chemical cues to the cells due to their resemblance to the natural ECM and their ability to easily achieve a 3D model architecture. Their biodegradability and biocompatibility have been extensively validated *in vitro* and *in vivo* [28–30]. Moreover, they have tunable mechanical properties, which are ideal for cell encapsulation. Therefore, cancer models can be created by including cancer cells, stromal fibroblasts, macrophages, or growth factors.

Novel Applications for Scaffolds

Scaffold-based TE strategies were also applied to cancer immunotherapy to develop an *in vitro* 3D scaffold model for examining the interaction of tumor-associated fibroblasts (TAF) with breast tumor cells and breast-specific, neu antigen (p98)-reactive T cells [31]. Breast cancer cells seeded on 3D chitosan-alginate (CA) scaffolds exhibited productive growth and formed distinct tumor spheroids. Antigen-specific p98 T cells, but not naïve T cells, bound better to tumor cells on scaffolds. The p98 T cells induced potent tumor cell killing, but T helper cell cytokine function was impaired in the presence of TAF co-seeding on scaffolds. From a biomaterials perspective, CA scaffolds are important because they are biocompatible and nonimmunogenic, they have the proxy structure of glycosaminoglycans (GAGs) (a major component of native ECM), and are approved by the US Food and Drug Administration (FDA) for numerous biomedical applications. These types of strategy can bridge the gap between *in vitro* and preclinical testing of novel immunotherapies by enabling researchers to probe individual cell types and factors in a more physiologically relevant tumor-like microenvironment.

Chip-Based 3D TE Models in Cancer Research

TE-based models still face difficult challenges, such as the requirement of functional vasculature networks to regulate the transport of nutrients and oxygen and the need to control metabolic or mechanical functions of the encapsulated cells within biocompatible scaffolds [32]. Combining TE principles with microfluidic technologies has the potential to fill such gaps, since microfluidics allows the fabrication of 3D architectures with controlled spatial relations between cells, the presence of flow-induced signaling and transduction, and the capacity to introduce the chemical gradients necessary to reproduce the architecture of the *in vivo* microenvironment [33–35].

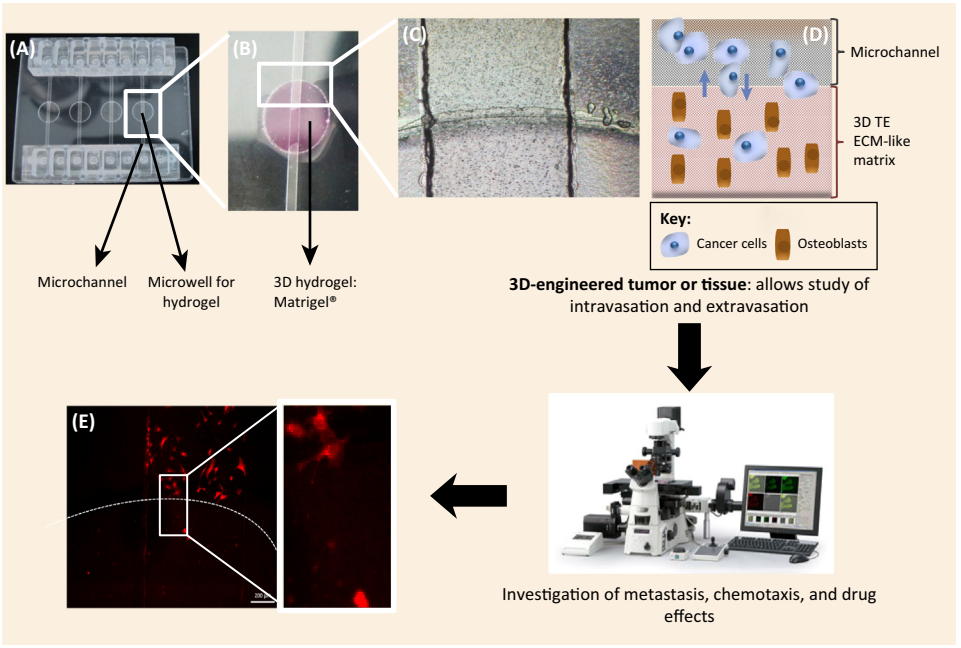


Figure 1. Chip-Based 3D Models in Cancer Research. Scheme of an example of a chip-based 3D model mimicking the native extracellular matrix (ECM) tumor microenvironment. The entry of tumor cells into the blood stream and then the micrometastasis process in a variety of tissues, or in the presence of other relevant cell types, can be studied. The chip microwells could contain a 3D engineered hydrogel representing an ECM-like matrix, such as Matrigel®, in direct contact with microchannels mimicking blood vessels for intravasation and/or extravasation studies. The small blue arrows represent the migration of (cancer) cells from the microchannel and into the 3D tissue-engineered ECM-like matrix and vice versa. (A) Cellix VenaT4 chip; (B) The microwell filled with Matrigel® (3D); (C) Microscopy image of the channel–microwell interface; (D) Representation of cancer cells migrating from the microchannel to the 3D TE ECM-like matrix in the presence of osteoblasts; (E) fluorescence microscopy image of HeLA cells adhered to the channel migrating into the ECM-like matrix (Matrigel) (M.R. Carvalho, unpublished data, 2015).

The combination of TE principles and microfluidic technologies can take the form of so-called 'biochips' for 3D cell culture that better mimic the physiological environment and interactions observed *in vivo* [36,37]. *In vitro* multitissue 3D tumor models on a chip can make it possible to obtain quantitative measurements on circulating tumor cells, extravasation and micrometastasis (Figure 1). For example, a microfluidic platform was built to emulate the dynamic physiology of the bone marrow microenvironment, allowing realistic interactions of bone marrow cells and osteoblasts, so as to investigate multiple myeloma [38].

In combination with biomaterial-based approaches, several chip-based models (Figure 2) have provided invaluable knowledge and previously unmeasurable or unobservable data (Figure 3) about cancer-related processes, such as angiogenesis and metastasis, and have proved instrumental in drug discovery.

Angiogenesis

Angiogenesis is a prerequisite for tumor growth, invasion, progression, and metastasis, and, thus, is crucial to include in cancer models. Organ-on chip models that integrate vasculature have the potential to transform *in vitro* approaches for the study of cancer [39,40], offering the possibilities of spatially resolved delivery and extraction of solvents and solutes to control the biochemistry of the microenvironment of the tumor, growth of appropriate endothelium, and delivery of circulating cells, as well as the possibility to control tension and shear stress during angiogenesis, tumor growth, and drug delivery [41].

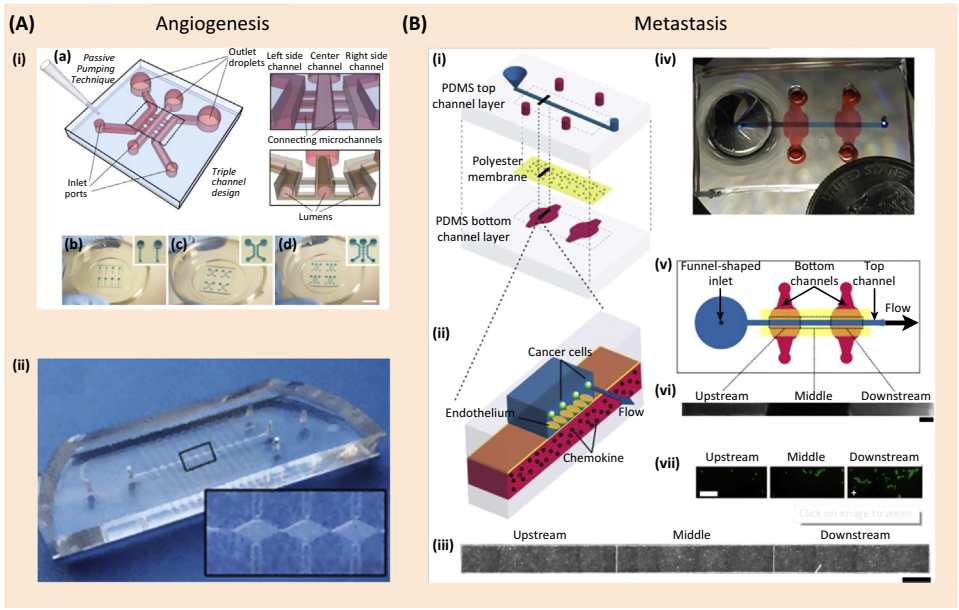


Figure 2. Microfluidic Devices Used in the Study of Angiogenesis (A) and Metastasis (B). (Ai) Passive pumping-based microfluidic angiogenesis assay with 3D cylindrical lumens. (a) Illustration of a triple channel design with connecting microchannels. (b–d) Microchannel systems can be (b) single, (c) double, or (d) triple channel designs, and are arrayable. (Aii) Perfusable 3D microvessels are generated using an optically clear polydimethsiloxane microfluidic-based platform. (B) Microfluidic vasculature enables the region-specific activation of endothelium under physiological flow conditions [101]. Reprinted with permission from [102] (Ai) and [47] (Aii). Abbreviation: PDMS, polydimethylsiloxane.

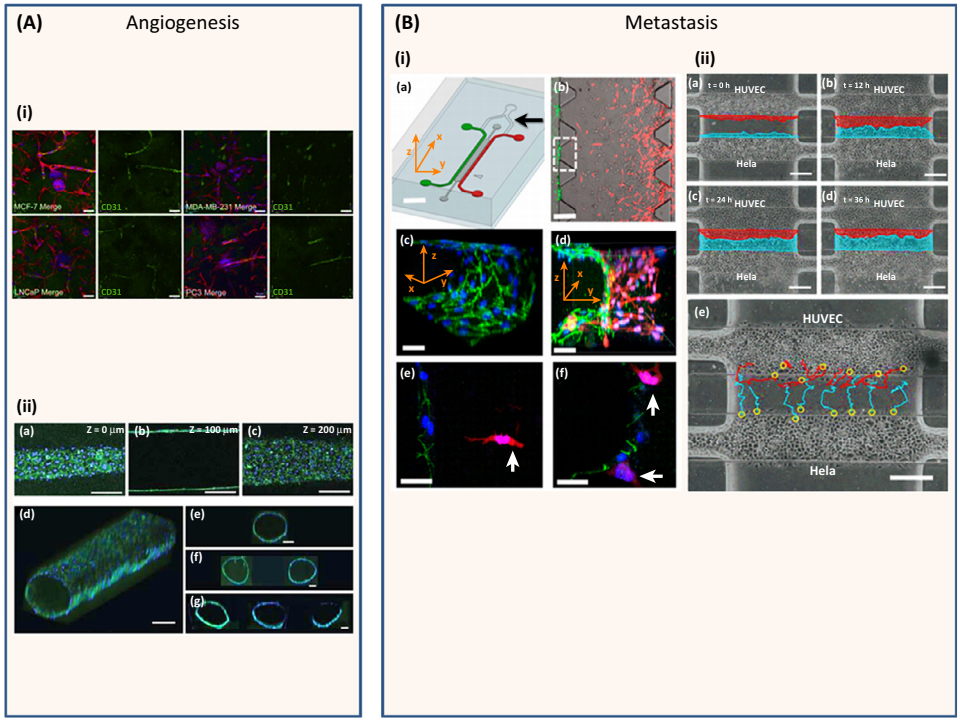
Physiological vascularized tumor conditions can be achieved by including mixed cell populations in the device: tumor cells, stroma cells, endothelial cells that line the vessels, and also immune cells. Such an approach would enable the analysis of any circulating molecular and cellular components that may promote tumor angiogenesis [42]. The biomaterials that form the microfluidic scaffold would have to recapitulate matrix stiffness and withstand interstitial pressure, as well as convey mechanical cues that modulate cell signaling via mechanoreceptor signal transduction.

Microfluidic vascular models can be divided in two categories [43]: microfabricated molds that confine biological hydrogels between parallel microfluidic channels [44], or *bona fide* vascular structures fully embedded within 3D ECM [45]. In an example of the microfabricated mold approach, a device was developed with a central microchannel embedded within a collagen hydrogel, which allowed tumor-relevant hydrodynamic stresses to be introduced and quantified using microparticle image velocimetry (μ-PIV) [46].

As an example of the second approach, a 3D microphysiological system is being developed. The model uses iPSC technology (i.e., the development of vessel networks derived from human iPSC-derived endothelial cells in a cardiac-derived ECM to simulate the microcirculation), the cardiac muscle, and the solid tumor into a single integrated microphysiological system [47]. As a 3D matrix, a co-culture of endothelial colony-forming-derived endothelial cells and normal human lung fibroblasts are mixed with fibrin matrix.

Metastasis

Metastasis is one of the most complex processes in cancer and likely one of the most difficult to study and mimic using *in vitro* models [48,49]. Therefore, it is crucial to understand the molecular



Trends in Biotechnology

Figure 3. Outputs of Angiogenesis (A) and Metastasis (B) Studies in Tissue-Engineered (TE) Designs. (Ai) Cultured breast or prostate epithelial carcinoma cells (MCF-7 and LNCaP) with human umbilical vein endothelial cells (HUVECs) and mesenchymal stromal cells (MSCs) within matrices fabricated from synthetic star-polyethylene glycol (PEG) and maleimide-functionalized heparin to study 3D tumor angiogenesis microenvironments after 14 days: extended focus confocal images displaying phalloidin (red), Hoechst (blue), and CD31 (green) showing HUVEC and MSC to cancer cell interactions for each tumour cell type. (Aii) 3D endothelial-lined lumens (ELL) obtained by seeding HUVECs in a microfluidic chip. (Bi) A microfluidic tumor–vascular interface model: endothelial channel (green), tumor channel (green), and 3D extracellular matrix (ECM; gray) between the two channels. White arrow shows fibrosarcoma cells (HT1080) invading in 3D toward the endothelium [52]. (Bii) Migration of HUVEC and Hela cells during co-culture on-chip. Reprinted with permission from [103] (Ai), [102] (Aii), and [104] (Bii).

and cellular phenomena involved in the metastatic cascade [50,51]. Invasion of cancer cells through the basal membrane into a blood or lymphatic vessel (intravasion) followed by entrance in other tissue and/or organs (extravasion) are critical steps [52,53]. Although significant progress has been made in visualizing tumor cell motility *in vivo*, the underlying mechanism of cancer cell intravasation is largely unknown. Few studies have addressed the development of 3D models for metastasis studies. However, an *in vitro* 3D microfluidic model of the tumor–vascular interface was designed to integrate live imaging, precise control of microenvironmental factors, and endothelial barrier measurement [52]. The chip comprised two independent channels in which tumor and endothelial cells are seeded, interconnected via a 3D ECM hydrogel made of collagen type I. This work sheds light on the influence of macrophage-secreted factors in the intravasion processes. Thus, the formation of an endothelial monolayer on a 3D collagen type I hydrogel mimicking ECM enabled the precise quantification and control of critical microenvironmental factors.

Drug Discovery

Developing *in vitro* 3D chip-based tumor models will not only aid in investigating angiogenesis and metastasis, but can also help provide a realistic preclinical assessment of anticancer drug efficacy and resistance.

For example, a microfluidic chip-based, 3D co-culture drug-sensitivity test platform was developed in which a mono-lung cancer cell line, a mixture of lung cancer and stromal cell lines, and cells from fresh lung cancer tissues were treated with anticancer drugs [54]. A gradient concentration generator inside the chips allowed the reconstruction of tumor microenvironments *in vitro* with continuous nutrient and oxygen supplementation. Cell culture medium was introduced into the microchannels to generate a set of gradient concentrations for each drug or combination of drugs. Moreover, these drug-sensitivity tests were carried out on the fresh cancer tissues. which enabled the screening of single or combination anticancer drug schemes efficiently and accurately for patients.

In addition to the chips independently developed by researchers in academic laboratories, companies are emerging that manufacture microfluidic-based 3D cell culture devices that are approved for commercialization. For example, MIMETAS produces OrganoPlates™, which are microfluidic-based culture plates that enable culturing and screening of a range of organ and tissue models. These platforms allow the precise deposition of cells in a 3D culture matrix, and their patented liquid-handling technology results in better readout and quantification compared with conventional culture systems. The cells are contained in a gel that allows 3D tissue configurations and cell–cell interactions. A continuous perfusion of media through the plate mimics blood flow and the exchange of nutrients, oxygen, and metabolites.

Other companies in the market are also following this new trend. Cellix's VenaT4 chip is suitable for ECM, collagen gels, hydrogels, Matrigel®, or similar aqueous biomaterials, and ideal for invasion assays in an *in vivo*-like settings. Such companies will revolutionize the field by working closely with researchers and clinicians to develop customized *in vivo*-based accurate biomimicry.

Concluding Remarks and Future Perspectives

Bioengineered 3D microsystem technologies are relatively new and still require great effort to validate and characterize their properties and suitability for practical biomedical applications. Despite the promise of microfluidic chip-based 3D cell culture systems for cancer research, there are some disadvantages compared with conventional techniques and challenges that need to be addressed (see Outstanding Questions). Moving *in vitro* culture from macroscopic culture to Polydimethylsiloxane (PDMS)-based devices can come with unforeseen challenges. Changes in device material, surface coating, cell number per unit surface area, or per unit media volume may all affect the outcome of otherwise standard protocols and, in this sense, surface materials and treatments deserve special attention. Although these systems present numerous advantages in terms of their ability to mimic what happens *in vivo* and to visualize cell growth, cell migration, and cell–cell interactions, the small volumes used as well as the low numbers of cells within the microfluidic devices make conventional biochemical assays more challenging due to detection limits, making it difficult to generate statistically significant amounts of data. Advancements in technologies that are sensitive enough to detect all-important genetic and transcriptomic changes are required for the discovery of novel biomarkers and critical events in cancer development. Regardless, their potential in predicting clinical responses could have great effects on the way in which drug discovery and bioequivalence studies, and the pathogenesis of relevant diseases, such as cancer, can be investigated.

Further development of chip-based 3D cell cultures in cancer research will largely depend on the improvement of biomaterials that emulate the ECM, and the capacity to scale up these complex technologies. Integrating TE approaches and microfluidics into easy-to-use, scalable, reproducible, and cost-effective systems will be the key to their success and future translation to the market. A 3D multitissue *in vitro* tumor model on a chip could contribute to accelerating the time-to-market for anticancer drugs, along with a well-defined regulatory and development strategy.

Outstanding Questions

There is a need to develop microfluidic devices to achieve high detection efficiency that could make use of the multiple biochemical and biophysical cues that are distinctive in cancer.

Can we apply the already developed microfluidics to patient's samples to bridge the gap to the clinics?

Are we taking advantage of the right biomaterials? How can we improve ECM-like features and avoid batch-to-batch variations and compare and correlate work from different groups?

3D *in vitro* models could demonstrate whether different formulations of the same drug are bioequivalent. A straightforward bioequivalence trial comparing relevant pharmacokinetic parameters of both formulations would be instrumental in gaining FDA approval.

There is now a huge demand for new strategies and more suitable biomaterials to interface with microfluidic chips for cell biological studies. Clearly, there is still a long road ahead for this to become reality, but we believe that this is the right direction to pursue in the search for novel and more efficacious treatments for cancer.

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