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# Microengineered cancer-on-a-chip platforms to study the metastatic microenvironment

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More than 90% of cancer-related deaths can be attributed to the occurrence of metastatic diseases. Recent studies have highlighted the importance of the multicellular, biochemical and biophysical stimuli from the tumor microenvironment during carcinogenesis, treatment failure, and metastasis. Therefore, there is a need for experimental platforms that are able to recapitulate the complex pathophysiological features of the metastatic microenvironment. Recent advancements in biomaterials, microfluidics, and tissue engineering have led to the development of living multicellular microculture systems, which are maintained in controllable microenvironments and function with organ level complexity. The applications of these "on-chip" technologies for detection, separation, characterization and three dimensional (3D) propagation of cancer cells have been extensively reviewed in previous works. In this contribution, we focus on integrative microengineered platforms that allow the study of multiple aspects of the metastatic microenvironment, including the physicochemical cues from the tumor associated stroma, the heterocellular interactions that drive trans-endothelial migration and angiogenesis, the environmental stresses that metastatic cancer cells encounter during migration, and the physicochemical gradients that direct cell motility and invasion. We discuss the application of these systems as *in vitro* assays to elucidate fundamental mechanisms of cancer metastasis, as well as their use as human relevant platforms for drug screening in biomimetic microenvironments. We then conclude with our commentaries on current progress and future perspectives of microengineered systems for fundamental and translational cancer research.

## 1. Introduction

Cancer refers to a group of remarkably heterogeneous diseases that are characterized by the uncontrolled growth and spread of abnormal cells. According to estimates from the International Agency for Research on Cancer, by 2030 both the global incidence and the mortality rates associated with cancer will rise to 22 million new cases, and 13 million deaths per year, respectively.[1] In recent years, the development of novel immunotherapies and molecularly-targeted agents, has improved prognosis in patients diagnosed with cancer.[2,3] As cancer treatments shift from non-specific therapies to more personalized approaches, two aspects that will be crucial for effective therapeutic intervention are: (i) the substantial intra- and inter-tumor heterogeneity that impact all of the clinical aspects of the disease,[4,5] and (ii) the influence of the physical and biochemical features of the tumor microenvironment.[6] Moreover, the interaction between primary tumors with the local microenvironment constitutes just one aspect of this systemic disease. In fact, malignant tumors shed huge numbers of cancer cells into the blood and lymphatic vessels, which can spread to distant sites and develop into clinically-relevant metastases.[4]

Despite great advances in understanding the molecular and cellular biology of cancer, metastases are still responsible for over 90% of the mortality in these patients.[7] Hence, several experimental models have been engineered to reproduce and study the complexity of the metastatic process. However, conventional *in vitro* models are largely unable to accurately mimic the *in vivo* setting.[8,9] In addition, animal models often fail to predict drug efficacy in humans.[10–12] More recently, microengineered systems have emerged as powerful tools to study complex biological phenomena *in vitro*.[13,14] These novel experimental platforms can be engineered to incorporate biomaterials that resemble physiological biochemistries and geometries, as well as microfluidic channel networks that mimic the transport of fluids and soluble factors across the vasculature.[15] Thus, these "on-chip" systems can be used to recreate the critical cell–cell interfaces, spatiotemporal gradients, and dynamics of the tumor microenvironment, with a level of accuracy that cannot be achieved by conventional models.[16]

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In this review, we will discuss the most recent advancements in microengineered models of the metastatic microenvironment, generated through the integration of microfluidics, biomaterials, and tissue engineering approaches. We first give an overview of the events by which invasive cells dissociate from the primary tumor site and migrate towards distant sites in the body. We then discuss the strengths and limitations of conventional experimental approaches to reproduce the complexity of cancer metastasis and the tumor microenvironment. Next, we review state of the art "on-chip" platforms to study the role of the different physicochemical features of the tumor microenvironment, in the various stages of the metastatic process. We conclude by providing future perspectives on the incorporation of advanced bioactive materials, cell reprogramming, and targeted genome editing into microfluidic platforms, and how they can be used to improve on current experimental approaches for the study of cancer metastasis.

## 2. Overview of the metastatic process

Cancer metastasis is a remarkably complex and multiparametric process, comprised of sequential stages that make up the so-called "metastatic cascade".[17] These stages include: (1) dissociation of cells from the primary tumor (shedding), (2) local invasion through the basement membrane and the extracellular matrix (ECM), (3) entry into the blood or lymphatic vessels (intravasation), (4) circulation through the lymphatic system and the bloodstream, (5) arrest in the capillaries and migration out of the vasculature (extravasation) into the secondary site (seeding), (6) formation of micro and macro-metastasis in the target organs, and (7) induction of new blood vessels (angiogenesis) that will provide oxygen and nutrients to the metastatic tumor[18] (Fig. 1A). This complex succession of events also presents several rate-limiting steps that could be pharmacologically targeted, as potential basis for therapeutic intervention.

For cancer cells to be able to metastasize to distant tissues, they must possess migratory and invasive phenotypic traits.[19] Although these features are acquired early during tumorigenesis, they must remain dynamic and adaptive throughout the journey of migratory cells to the metastatic site. As cancer cells spread, they must be able to selectively overcome different types of environmental stresses, which include cytotoxic immunity, low oxygenation, and the increased acidity that results from enhanced cellular metabolism[20,21] (Fig 1B). In addition, recent evidence suggests that metastatic colonization can only be achieved by rare tumor-initiating cells or cancer stem cells (CSCs).[22] CSCs are able to survive in the circulation, adapt to new microenvironments at metastatic sites and re-initiate tumor growth.[22] Thus, the presence of CSCs within the migratory tumor mass might strongly favor the formation of metastatic tumors, which makes them an attractive target for therapy.

Apoptosis of migrating cells prior to their entry into the metastatic site often prevents the spread of the majority of circulating tumor cells (CTCs)[23] (Fig. 1C). Malignant cells can invade into multiple organs, where they could develop into metastatic tumors or remain dormant for extended periods of time[24,25] (Fig. 1D). Moreover, several studies have demonstrated that cell and microenvironmental signaling can lead to spontaneous regression in several types of primary and metastatic cancers[26–30] (Fig. 1E). This is a remarkably complex process, modulated by several genetic, epigenetic, and cellular factors, through the induction of apoptosis and immune system activation, as well as the inhibition of matrix metalloproteinases (MMPs) and angiogenesis.[18] These observations suggest that microenvironmental signaling at the metastatic site could either lead to the temporal suppression of malignancy, or to the re-activation of dormant metastatic cells.

The successful establishment of metastatic tumors relies heavily on the cumulative ability of malignant cells to adapt to different environments throughout the metastatic

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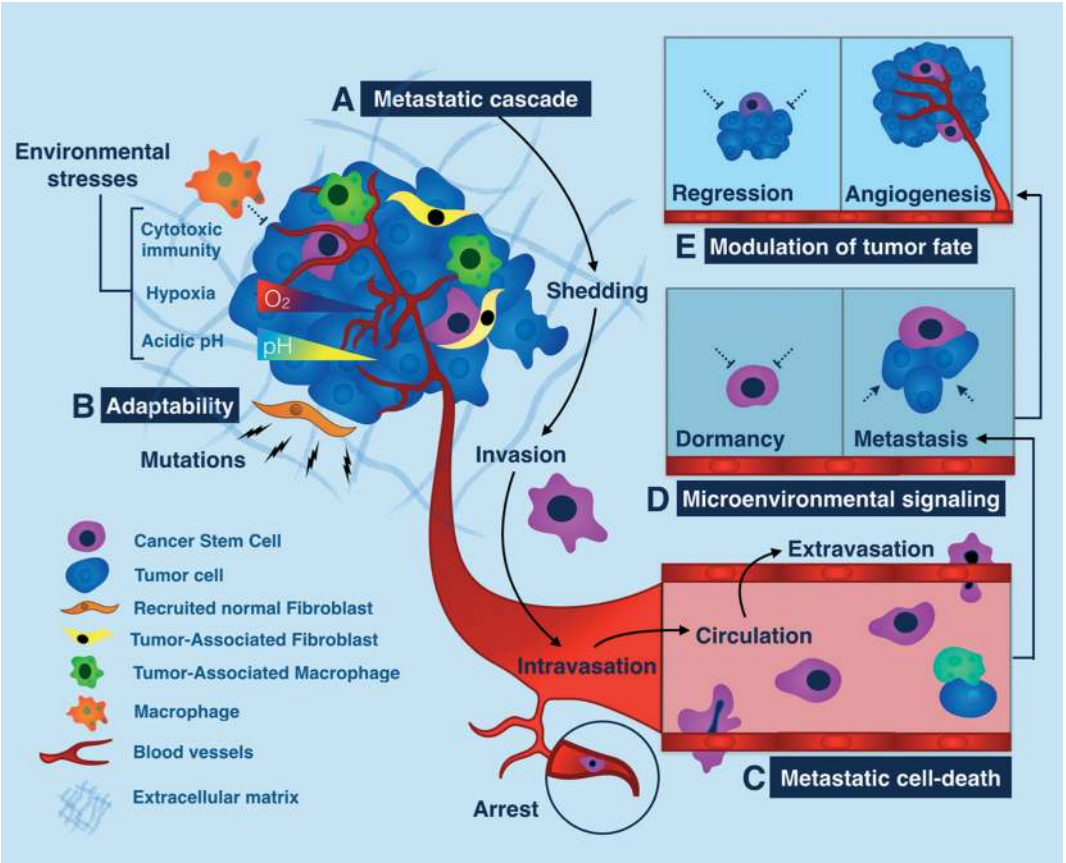


**Fig. 1** Overview of cancer metastasis. A. The metastatic cascade. Cancer metastasis involves the sequential progression of different stages, which include: (1) dissociation of cells from the primary tumor (shedding), (2) local invasion through the basement membrane and the ECM (invasion), (3) entry into blood or lymphatic vessels (intravasation), (4) circulation through the lymphatic system or the bloodstream (circulation), (5) arrest in capillaries and migration out of the vasculature (extravasation) into the surrounding distant tissue (seeding), (6) formation of micro and macro-metastasis in the target organs, and (7) induction of new blood vessels (angiogenesis). B. Cancer cell adaptability. Tumor-initiating mutations promote cancer cell survival by increasing their ability to overcome different environmental stresses (*i.e.*, cytotoxic immunity, and hypoxic and acidic environments). C. Death of metastatic cells. Apoptosis of migratory cells, immune surveillance in the circulation, and microenvironmental signaling prevent the spread of the majority of circulating tumor cells. However, cancer stem cells are able to survive in the circulation and adapt to new microenvironments at metastatic sites. D. Microenvironmental signaling modulates malignancy. The interplay between cancer cells and the new metastatic microenvironment could promote the development of clinically relevant metastasis, or prevent further tumor growth by inducing dormancy in invading cells. E. Modulation of tumor fate. Established metastatic tumors can induce the formation of new blood vessels that provide them with oxygen and nutrients. However, genetic, epigenetic and microenvironmental factors can also lead to the spontaneous regression.

cascade.[31] The molecular pathways and cellular interactions that modulate the regression and dormancy of invading cells, as well as those involved in their eventual re-activation, represent attractive targets for anti-metastatic drug development. Therefore, the study of these and other aspects of cancer metastasis will help identify relevant targets, which could potentially be used to improve prognosis and treatment outcome.

## 3. Influence of the tumor microenvironment on cancer metastasis

The bi-directional communication between cancer cells and the surrounding tumor microenvironment endows primary tumor cells with the ability to proliferate, migrate, invade and colonize distant tissues to establish metastatic tumors.[32] The tumor microenvironment refers to the actual physiological setting that supports and modulates the proliferation, function and fate of cancer cells. This complex ecosystem comprises various soluble factors, nutrients, metabolic components, ECM proteins, and several non-malignant cells that are supported by vascular networks (Fig. 2). The cellular component of the microenvironment consists primarily of stromal cells that are able to recruit endothelial cells and fibroblasts, as well as leukocytes, as well as leukocytes and other immune cells that constitute the inflammatory component of the microenvironment (Fig. 2A).[33] Fibroblasts in particular are in close association with cancer cells throughout all stages of tumorigenesis, where they exert functional and structural roles including the production of growth factors, chemokines, ECM proteins, and remodeling enzymes that promote angiogenic recruitment.[34,35] The interaction between tumor cells and

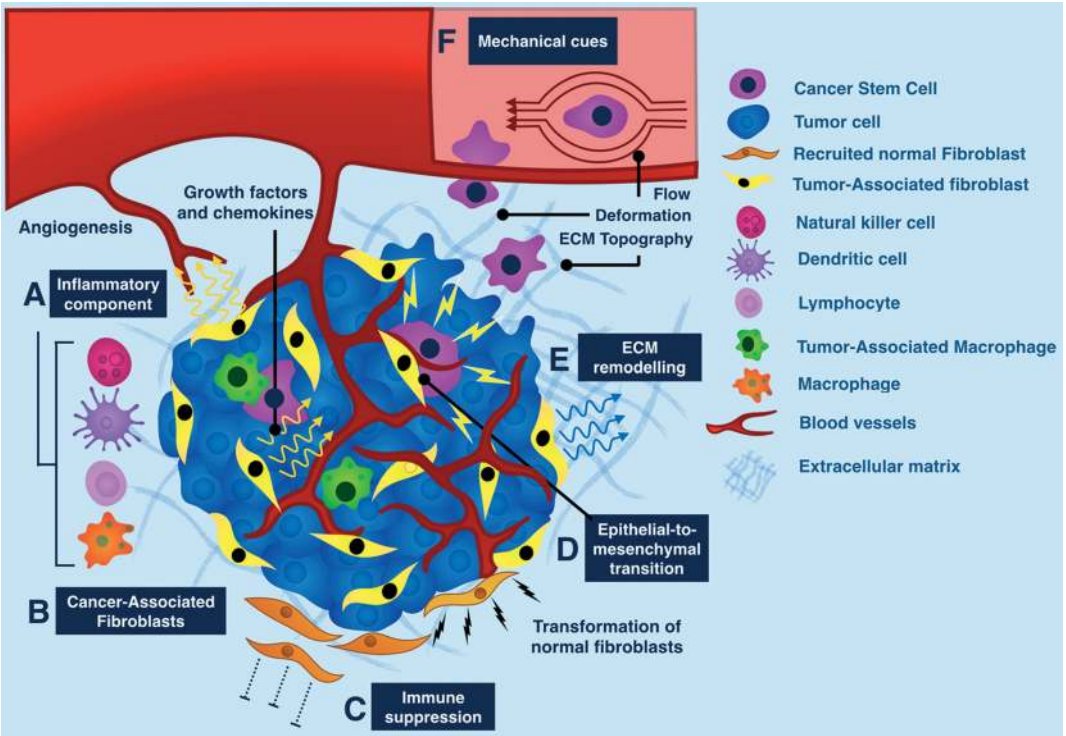


**Fig. 2** The influence of the tumor microenvironment in cancer metastasis. A. Inflammatory component. Cells from the immune system infiltrate into the tumor site in response to chemotactic signals secreted by the microenvironment, where they not only fail to exercise anti-tumor effector functions, but they are co-opted to promote tumor growth. B. Cancer-associated fibroblasts (CAFs). CAFs can originate from multiple resident precursor cell types, including endothelial, smooth muscle, myoepithelial, and mesenchymal stem cells. They exert structural and functional roles, which include the production of growth factors, ECM proteins, and remodeling enzymes that promote angiogenic recruitment. C. Immune suppression. CAFs not only secrete pro-tumorigenic growth factors, but they also express pro-inflammatory gene signatures, which induces neo-vascularization and recruitment of immune cells. D. Epithelial-to-mesenchymal transition (EMT). The tumor-associated stroma and the hypoxic environment promote EMT at the primary site, which allows cancer cells to break free from epithelial cell–cell junctions and acquire an invasive phenotype. E. ECM remodeling. The synergistic action of stromal and tumor cells leads to the active remodeling of the physical properties of the ECM, such as matrix stiffness, pore size, and viscoelasticity. In turn, the topology of the ECM modulates cell motility, tumor progression and metastasis. F. Mechanical cues. In order to migrate across a variety of environments, metastatic cells must be able to alter their cell shape and squeeze through small gaps in the ECM, or extravasate into the blood or lymphatic vessels. Similarly, circulating tumor cells that enter into the bloodstream must tolerate several hemodynamic stresses, before they can extravasate to establish new metastatic tumors.

these cancer-associated fibroblasts (CAFs) leads to epigenetic alterations in the latter that disrupt the modulation of cell division[36,37] (Fig. 2B). In turn, CAFs are able to suppress the normal function of immune T cells in the microenvironment, through the release of pro-inflammatory cytokines such as IL-6[38,39] (Fig. 2C). Moreover, they are also able to promote the upregulation of transcription factors that are involved in epithelial-to-mesenchymal transition (EMT)[40,41] (Fig. 2D). During EMT, tumor cells undergo a rearrangement of cytoskeletal organization and cell–cell junctions, which results in the acquisition of migratory abilities and the invasion of local and distant tissues.[42,43] Recent evidence also suggests that the direct association with the stroma, renders some populations of cancer cells resistant to chemotherapeutic drugs.[44] Hence, the study of the interactions between tumor and stromal cells is crucial to design more efficacious therapies, and to improve the effectiveness of current approaches.

The synergistic action of stromal and tumor cells also leads to the active remodeling of the ECM (Fig. 2E). Recent studies have demonstrated the role of the physical properties of the ECM in the modulation of cancer metastasis.[45,46] Differences in ECM topology modulate metastatic cell motility through physical cues that guide the directionality of cell migration.[47,48] Increased ECM stiffness has been associated with the induction of stem cell differentiation, modulation of cell adhesion, ECM deposition, and upregulation of genes that promote invasion and metastasis.[49,50] In addition, the higher mechanical resistance associated with increased ECM stiffness could determine the specific mechanisms used by metastatic cells to invade the surrounding tissues.[51] Similarly, ECM density has also been shown to influence the cooperativity between invasive tumor cells with mesenchymal phenotypes.[52] Therefore, the physical interactions of tumor cells and their modulation by mechanical cues from the

 

microenvironment are determinant to the progression of the metastatic process (Fig. 2F).

The heterotypic interactions between cancer cells with the tumor microenvironment leads to the recruitment of different cell types, the modulation of cell fate, and the dynamic modification of the physical features of the ECM. In turn, these modifications influence cancer cell behavior, and trigger several mechanisms associated with tumor invasiveness. Elucidating the different types of microenvironmental cross-talk will provide a better understanding of the mechanisms that modulate cancer cell metastasis, as well as their implications in patient outcome and treatment. In addition, these advancements in tumor and environmental mechanobiology also highlight the importance of representing these interactions accurately, with the use of adequate experimental models.

# 4. Strengths and limitations of conventional models of cancer

Experimental models of cancer allow the dynamic visualization of the evolution of the disease, as well as high-throughput and reproducibility for hypothesis testing, drug screening, and biomarker discovery. To this date, the use of different *in vitro* and *in vivo* tumor models has provided invaluable mechanistic insight into different phenomena associated with cancer cell proliferation and metastasis, ECM remodeling, tumor dormancy and angiogenesis, as well as drug resistance. However, conventional models of cancer exhibit several intrinsic shortcomings, which limit their potential to study more complex phenomena associated with human diseases.

## 4.1. Two-dimensional (2D) and three-dimensional (3D) *in vitro* models

*In vitro* models are remarkably practical and can be designed to incorporate various cell sources (*e.g.* patient cells, commercially available cell lines, stem cells, stromal cells, immune cells, *etc.*), biophysical properties (*e.g.* oxygen partial pressure, pH, interstitial flow, *etc.*), ECM features (*e.g.* fiber density, stiffness, surface patterns, *etc.*), and biochemical stimuli (*e.g.* chemoattractants, chemokines, angiogenic and growth factors, *etc.*).[8] In particular, 2D cultures have been widely implemented in several different areas of fundamental and applied cancer research, as well as in drug discovery and efficacy testing, and translational studies. This large spectrum of applications is strongly related to the low cost, reproducibility, accessibility, and ease of handling associated with 2D cultures. However, the shape and fate of cells propagated under 2D conditions differ radically from those growing in 3D native tissues.[53] This is particularly critical for the study of cancer biology, since cell morphology has been shown to determine cell behavior, signal transduction, responsiveness to external stimuli, and resistance to radio- and chemotherapy agents.[54]

Pre-clinical cancer research is usually carried out using 2D cancer models. However, this simplified geometry translates to variations in cell–cell and cell–ECM interactions that lead to discrepancies in drug responses *in vitro*.[55,56] Because of this, 3D *in vitro* models have been increasingly used as an alternative approach between 2D cultures of isolated cancer cells, and *in vivo* models with higher complexities. Similar to 2D models, 3D culture systems can also be engineered to be biomimetic, and to incorporate cancer cells alone or in heterogeneous co-cultures. In particular, the spatially relevant arrangements of 3D cultures help mimic native cellular interactions, which promotes the acquisition of phenotypic features associated with tumors *in vivo*. Comparative studies have demonstrated that proteins associated with metabolism, cell stress response, signal transduction, protein synthesis, and cellular transport are over expressed in 3D tumor spheroids (tumorspheres), when compared to 2D cultures.[57] Other groups have also demonstrated that tumorspheres are comparatively more resistant to chemotherapeutic drugs, and possess an increased number of CSC-like populations.[58] Due to its relevance to basic and clinical cancer research, several methods have been developed to generate tumorspheres, including spontaneous aggregation, rotary cell culture systems, hanging drops, matrix encapsulation, magnetic levitation, 3D bioprinting, as well as low binding and micro-patterned plates.[59] However, 3D spheroid models still present several technical challenges, as well as intrinsic limitations to mimic the more elaborate features of complex tissues.

## 4.2. *In vivo* animal models

Although organotypic 3D models are increasingly being used in translational cancer research, the lack of more sophisticated whole-organ culture systems still makes animal models necessary for drug toxicity and efficacy validation. *In vivo* animal models have been used extensively to recapitulate the natural history of human cancers, as well as their clinical responses to investigational anti-cancer therapies that have shown promise *in vitro*.[60–62] However, the use of animals for experimentation is often restricted by the access to test subjects and the feasibility of probing methodologies, as well as the ethical concerns related to the discomfort and pain inflicted on live subjects. In addition, *in vivo* models might not be accurate enough to predict the efficacy of therapies directed against certain types of human tissues.

Traditionally, rodents have been the most frequently used animal models in cancer research, and have enabled the study of human disease function *in vivo*. Among these, the most common rodent cancer models are chemically or genetically induced cancer models, and xenograft models.[63] In particular, xenograft cancer models are generated when human tumor cells are transplanted either under the skin (ectopic) or into the organ of origin (orthotopic) in immunocompromised animals.[60] These models constitute a relatively inexpensive approach for generating *in vivo* tumors, which can be formed from human primary cells or established cancer cell lines. Because of this, xenografts are frequently used for several purposes in drug discovery and development, which include: characterization of disease pathophysiology, evaluation of the mechanisms of action of existing drugs,

discovery of new drug targets and biomarkers, establishment of pharmacodynamic/pharmacokinetic relationships, estimation of clinical dosing regimens, and determination of safety margins and toxicity.[53] However, they still exhibit disadvantages that often prevent the efficient translation of novel research to the clinical setting. These are mainly associated with discrepancies in the effectiveness of anti-cancer drugs and the lack of human stroma–tumor interactions, as well as the absence of an immune component due to the compromised immune systems of experimental animals.[60] Despite their important contributions to cancer research, animal models still constitute imperfect representations of human disease, which often leads to errors in the selection and approval of drugs in clinical trials. Furthermore, the highest probability of attrition for all drugs used in oncology occurs during the more costly phases II and III of clinical development, which is a reflection of the limited clinical relevance and predictive power of current pre-clinical models.[61,63]

More recently, microfluidic systems have been used in conjunction with tissue engineering approaches and advanced biomaterials, in order to develop more sophisticated platforms to successfully tackle the complexity of human cancers. These novel experimental platforms exhibit several technical and scientific advantages over conventional models of cancer[64] (Fig. 3). Furthermore, these "on-chip" systems can be used to study treatment-induced responses at the tissue and organ level for drug screening, while also allowing the dissection of physiological complexities into more discrete units to ease their analysis.

## 5. Microengineered models of the metastatic microenvironment

All of the major events that are relevant to proliferation, survival, and metastatic spread of malignant cells, are dependent on the interactions between the cellular and physicochemical components of the tumor microenvironment. Due to their complexity, the study of these processes has been majorly conducted using animal models. Recently, novel "on-chip" cancer models have emerged as an efficient alternative to conventional *in vitro* and *in vivo* approaches, since they can recapitulate the native microenvironment of human tumors in a comparatively simpler, more accurate, and human relevant fashion. With the advent of microengineered systems, several studies have reported the application of these novel platforms in different areas related to cancer cell biology, including tumor cell detection, isolation and characterization,[65–68] modeling of cancer–immune interactions,[69] mechanics,[70] drug screening and development,[71,72] as well as the study of the tumor microenvironment.[53,73–75] Similarly, several microfluidic systems have been developed to study the dynamic events that constitute the metastatic

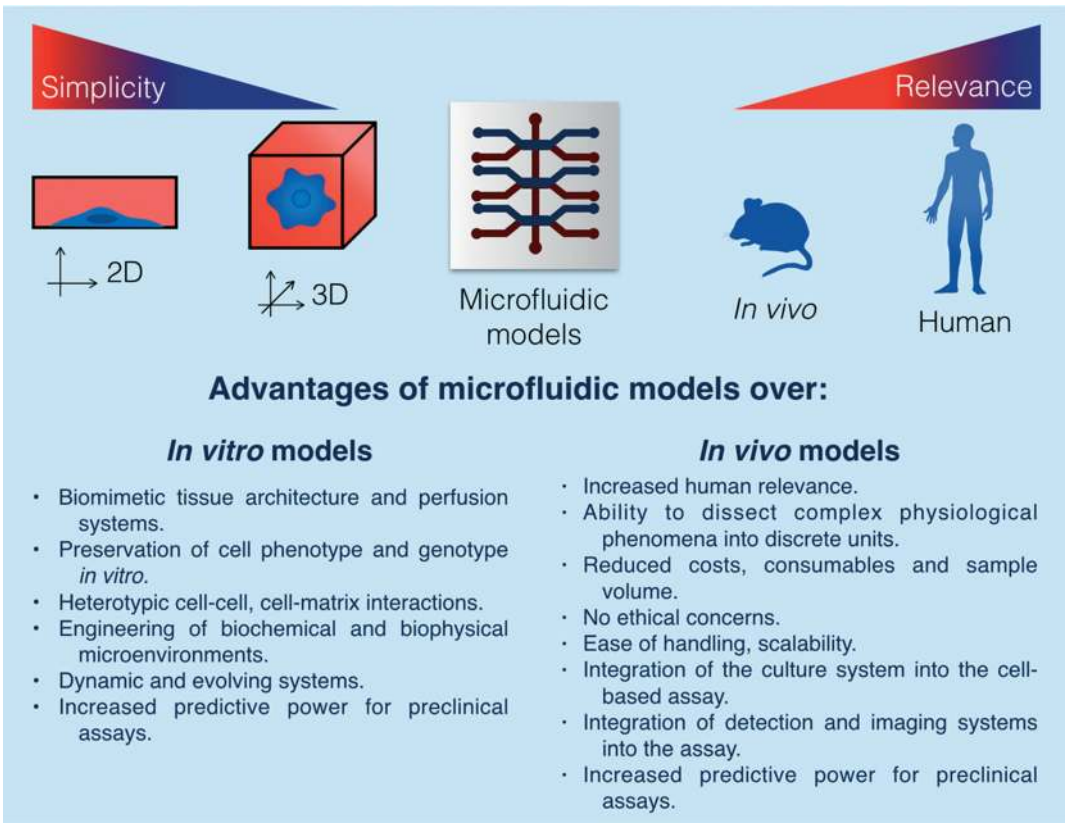


**Fig. 3** Advantages of microfluidic models over conventional *in vitro* and *in vivo* models. Comparative schematic of the different types of models that are available for fundamental cancer research and preclinical drug screening.

 

process, including 3D invasion,[76,77] intra- or extravasation,[78,79] and angiogenesis.[80,81]

Two of the most remarkable aspects of microengineered systems are: (i) the ability to integrate complex cell-based assays with real-time and non-invasive monitoring systems for quantitative measurements of relevant cellular parameters, and (ii) the ability to reproduce the complexity of the biophysical, biochemical and cellular components of the microenvironment in human tumors. Moreover, the integration of multiple microengineered components provides the unique opportunity to monitor highly complex phenomena in real-time, using non-invasive probing techniques. Thus, in the next section we will review the most recent developments in integrative microengineered platforms to study the roles of various microenvironmental features, throughout the different stages of cancer metastasis.

### 5.1. Models of cancer cell invasion

Cell invasion, the first step of cancer metastasis, involves the adhesion, proteolysis, and migration of metastatic cancer cells through the ECM. Throughout this process, cancer cells engage in a bidirectional communication with their surrounding microenvironment through both physical and biochemical cues. Pro-inflammatory cytokines secreted by locally activated stromal cells from the microenvironment, such as TGF-β, TNF-α, and IL-6, are capable of inducing EMT in carcinoma cells and facilitate local invasion and metastatic dissemination.[82] Although blockade of IL-6 signaling in preclinical models has been proven to prevent tumor growth and metastasis, its success in clinical trials has been limited.[83] Therefore, identifying features that predict response to anti-IL-6 therapy are needed. Recently, Lei *et al.* described the development of a microfluidic device that incorporates impedance measurement for the quantitative determination of cell invasion, under biomimetic cytokine stimulation[84] (Fig. 4Ai). This device consisted of two reservoirs connected through a microchannel that mimicked the basement membrane *in vivo*. Malignant NPC-BM1 cells were seeded in one reservoir, and allowed to invade through a microchannel filled with a methylcellulose hydrogel to another reservoir (Fig. 4Aii). Electrodes embedded on the bottom of the microchannel allowed for impedance measurement of migrating cells. Using this device, they investigated the influence of IL-6 cytokine signaling in cell invasion, which demonstrated that invasion rates were directly proportional to the IL-6 concentration[82] (Fig. 4Aiii). The approach presented by Lei *et al.* enables the quantitative study of cell invasion under extracellular biochemical stimulation, which might facilitate the quantitative assessment of relevant clinical parameters in translational cancer studies. More importantly, the design of the device eliminates the influences of membrane pore size and gravity, which are the major technical concerns associated with conventional Transwell invasion assays.

Biochemical signals from the tumor microenvironment are mainly secreted by CAFs. However, the molecular mechanisms by which CAFs influence tumor behavior are still not fully understood. Recently, Yu *et al.* reported the engineering of a microfluidic device for the 3D co-culture of non-small cell lung carcinoma (NSCLC) cells and CAFs, to study the interactions between tumor and stromal cells in the microenvironment[85] (Fig. 4Bi). Normal human fibroblasts were first activated to CAFs through their co-culture with A549 and SPCA-1 lung cancer cells, and then CAFs-conditioned growth medium was assessed for the promotion of NSCLC cell invasion (Fig. 4Bii). Their results demonstrated that NSCLC cell migration in response to stromal signaling from CAFs was promoted in part through the up-regulation of the stress-induced chaperone protein GRP78. Interestingly, GRP78 is significantly upregulated in various human cancers, where it has been associated with tumor progression, evasion of apoptosis, resistance to chemotherapy, and poor prognosis.[86] The influence of stromal cells on the invasiveness of cancer cells was also investigated in a recent study by Bischel *et al.*[87] In this contribution, they described the integration of a microfluidic co-culture of C4-2B prostate cancer and MC-3T3-E1 bone marrow stromal cells, with a multi-photon imaging component (Fig. 4Ci). They used this device to investigate the mechanisms by which soluble signals from the microenvironment were able to influence prostate cancer cell invasion (Fig. 4Cii). Similar to the results described by Yu *et al.*, the work presented by Bischel *et al.* demonstrated that the cross-talk between tumor and stromal cells led to the promotion of cancer cell invasion. In particular, this enhancement in the protrusive phenotype of prostate cancer cells, was shown to be mediated by the increased activity of the polyamine catabolic enzyme APAO (Fig. 4Ciii).

Tumorspheres have also been integrated in microengineered models of tumor invasion. Recently, Choi *et al.* developed a biomimetic microsystem that recapitulated the 3D structural organization of breast ductal carcinoma *in situ*[88] (Fig. 4Di). The co-culture was comprised of MCF10 breast tumorspheres in association with human epithelial cells from the mammary duct, as well as human fibroblasts embedded in a biomimetic scaffold. This culture system was then integrated into a compartmentalized microfluidic system that mimicked the native microarchitecture of the gland (Fig. 4Dii). They investigated the application of their microdevice as a drug screening platform, using the widely used chemotherapeutic agent paclitaxel. Their results showed that although the diameter of the spheroids remained unaltered, the administration of the drug prevented invasion into the surrounding stroma (Fig. 4Diii).

The approaches described in this section demonstrate the application of microengineered systems to generate physiological models of cancer invasion, which recapitulate the functional and structural interactions of cancer cells with different cell types and soluble signals. However, cancer cell invasion of the ECM is also promoted by local physical forces in the microenvironment, such as the increased interstitial fluid pressure (IFP). IFP is brought about by the collapse of lymphatic vessels due to increased mechanical compression from solid tumors, and has been shown to influence the

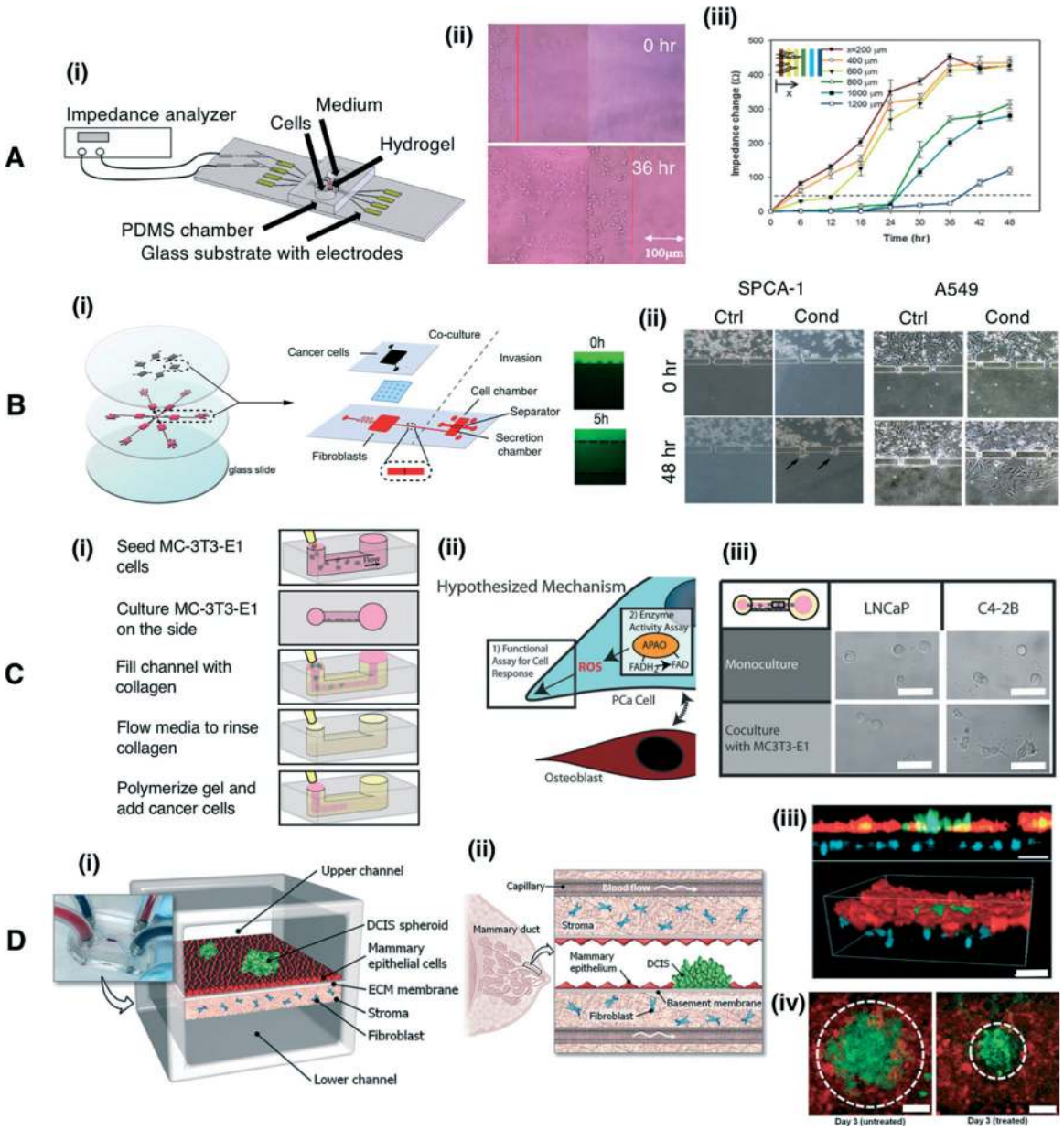


**Fig. 4** Models of cancer cell invasion. A. Microfluidic device for impedimetric monitoring of cell invasion. (i) Schematic of the experimental setup for the invasion assay. The device was composed of a glass substrate embedded with 8 straight electrodes, and a poly(dimethylsiloxane) (PDMS) layer with 2 reservoirs connected through a methylcellulose hydrogel; (ii) successive microscopic images of cell invasion, the red lines represent the leading edges of cells at the corresponding time points; (iii) impedimetric monitoring of cell invasion under IL6 signaling, each series represent the reading recorded once cells reached each electrode, positioned at the indicated distance (dash line: threshold) (reproduced with permission from ref. 84). B. Microfluidic device to study CAF-mediated breast cancer cell invasion. (i) Schematic of the integrated microfluidic device for non-contact cell co-culture and invasion. The device contained six chip units, with co-cultures of cancer and stromal cells to study cell invasion across a gradient of cell-basement membrane extract. The panel also shows a representative image of the concentration gradient engineered across the biomimetic ECM after 5 h; (ii) representative images of the effect of control and CAF-conditioned growth media on SPCA-1 and A549 lung cancer cell migration (reproduced with permission from ref. 85). C. Microfluidic device to study the influence of the bone microenvironment on prostate cancer cell invasion. (i) The schematic shows the process to generate the co-cultures of MC-3t3-E1 osteoblasts and prostate cancer cells for the invasion assays; (ii) the schematic shows hypothesized mechanism by which osteoblasts interact with prostate cancer cells through the increased activity of ROS-producing APAO; (iii) representative image of cells that developed protrusions into the collagen I hydrogel within the device. Scale bars: 50 μm (reproduced with permission from ref. 87). D. Microfluidic biomimetic model of breast cancer invasion. (i) Schematic of the microarchitecture of the breast cancer-on-a-chip microdevice. Both culture chambers are separated through a membrane that mimics the native basement membrane of the gland. Tumor spheroids are seeded in the epithelial layer in the upper chamber, while the stromal layer embedded with fibroblasts is created on the opposite side of the membrane; (ii) illustration of the tissular architecture of breast ductal carcinoma *in situ* (DCIS), within the mammary duct; (iii) fluorescence imaging showing cross-sectional and 3D rendered views of DCIS spheroids (green) bound to the mammary epithelial cells (red). Spheroids grow in association with human mammary fibroblasts (cyan) in the stromal layer. Scale bars: 100 μm; (iv) fluorescence micrographs of DCIS spheroids without (left) and with (right) paclitaxel treatment. Scale bars: 100 μm (reproduced with permission from ref. 88).

 

migratory behavior of cancer cells in 3D culture.[89] Different approaches, such as the one recently reported by Piotrowski-Daspit *et al.*, have demonstrated that IFP leads to pressure-induced changes in genes that modulate cell invasion and motility.[90] Hence, microengineered invasion models that incorporate the influence of physical forces, could potentially reveal molecular pathways or cellular responses that might not be observed under static conditions.

### 5.2. Models of cancer cell trans-endothelial migration

Another critical event in the metastatic cascade occurs after migratory cells invade the local stroma, and intra- or extravasate the systemic circulation in a process referred to as trans-endothelial migration. Cancers of epithelial origin, also referred to as carcinomas, are the most common type of cancer observed in the clinic. The majority of human carcinomas disseminate from the primary tumor into the interstitial space, and eventually intravasate into lymphatic vessels where they spread to distant sites in the body.[91] However, the cellular mechanisms by which cancer cells interact with the lymphatic endothelium are still not fully understood. Recently, Pisano *et al.* engineered a microfluidic system that recreated the different types of physiological fluid forces occurring at the tumor microenvironment (Fig. 5Ai). They used this device in conjunction with image analysis to quantify cell invasion and transmigration of MDA-MB-231 mammary adenocarcinoma cells[92] (Fig. 5Aii). Their results demonstrated that luminal and transmural flows promote invasion and transmigration of MDA-MB-231 cells across the ECM and the biomimetic lymphatic endothelium (Fig. 5Aiii). The different geometries integrated into this platform allowed mimicking of the different compartments in lymphatic capillaries, as well as their corresponding biomechanical cues. Moreover, the integration of a standard invasion assay within a controlled microfluidic system allowed the quantification of tumor cell transmigration rates and dynamics, as well as their rate of detachment from the endothelium. Hence, this study not only provides new insight into the modulation of tumor migration by fluid forces in the lymphatic microenvironment, but it also presents a novel *in vitro* tool to study tumor mechanobiology.

Cellular heterogeneity is a key characteristic of cancer cells within the tumor mass that leads to subgroups of cells having distinct growth advantages, and different migration and metastatic capabilities.[93] Conventional *in vitro* migration assays study invading cells at the population level, and do not allow the recovery of migrating viable cells for analysis. This could potentially mask intrinsic differences among individual cells, which would limit their application as screening platforms. To address this issue, Chen *et al.* engineered a single-cell microfluidic platform to study the migration of individual MDA-MB-231 breast cancer cells, upon a hepatocyte growth factor (HGF)-chemotactic gradient (Fig. 5Bi). HGF is a multi-functional cytokine that is involved in embryogenesis, organogenesis, adult tissue regeneration, and carcinogenesis.[94] The approach presented by Chen *et al.* allowed the post-migration collection and analysis of cells with varying degrees of invasiveness[95] (Fig. 5Bii). Furthermore, they incorporated choke points into the migration channels with dimensions similar to lymphatic capillaries *in vivo*, which helped mimic the intravasation of tumor cells to the lymph nodes (Fig. 5Biii). Isolation and propagation of highly chemotactic and non-chemotactic cells revealed that the migratory mesenchymal phenotype was heritable (Fig. 5Biv), and that these cells overexpressed known migration and metastasis-associated genes. In particular, this approach demonstrates the remarkable potential of microengineered systems as highly selective screening platforms that can be used to assay and isolate individual cells, based on clinically relevant features of heterogeneous cancer populations.

The entry of tumor cells into the bloodstream is an important route for cellular metastasis to distant organs.[96] Hence, several microfluidic models that simulate 3D functional vascular networks have been engineered to study cancer cell migration into the blood vessels.[97] Wang *et al.* engineered a 3D microsystem to model the transvascular migration of hepatocellular carcinoma HCCLM9 cells, through biomimetic and tunable blood vessels that mimicked the native vasculature and hemodynamic conditions[98] (Fig. 5Ci). The engineered device consisted of transparent, elastic and porous cellulose-based microtubes, lined with a 3D endothelium and implanted into a biomimetic 3D collagen matrix. Their results demonstrated that endothelial cells were able to migrate across the artificial blood vessel wall, and into the surrounding collagen matrix (Fig. 5Cii). In addition, they demonstrated that HCCLM9 cells could successfully transverse the biomimetic vasculature wall under HGF stimulation (Fig. 5Ciii). The approach presented by Wang *et al.* demonstrates the potential of microengineered systems to recapitulate physiological structures and parameters *in vitro*, such as blood vessels and vascular hemodynamics, respectively. Moreover, the successful reconstruction of a vascularized microenvironment with transvascular migration under biochemical signals, has strong implications for basic and translational research on tumor–vascular interactions.

After metastatic cells enter the systemic circulation they must survive several stresses, which include hemodynamic shear forces, immune surveillance, lack of substratum, and entrapment in capillary beds.[99] After this, metastatic cells should be able to extravasate the circulation into the metastatic site, and re-initiate tumor growth. Extravasation is a critical event that constitutes an attractive target for diagnostic and therapeutic intervention and yet, the precise mechanisms that regulate this process remain unclear.[100] In addition, despite efforts to model organotypic tumor microenvironments, the mechanisms that drive the organ-specificity of cancer metastases remain largely unresolved. In a recent contribution, Jeon *et al.* engineered an organ-specific microfluidic model to study breast cancer cell extravasation, within a perfusable microvascularized bone-mimicking microenvironment[101] (Fig. 5Di). They used this device to generate different biomimetic microenvironments,

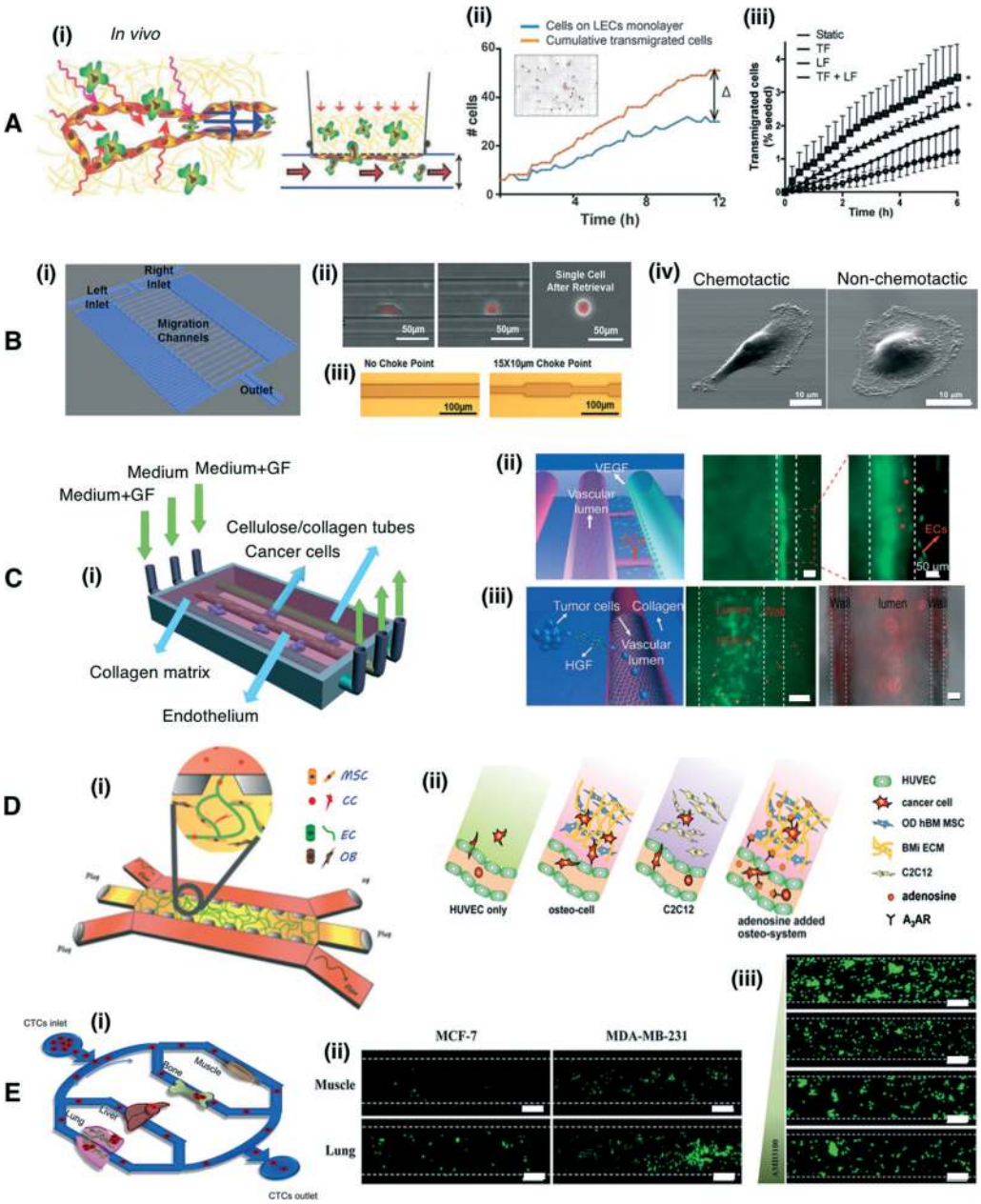


**Fig. 5** Models of cancer cell trans-endothelial migration. A. Microfluidic model of the tumor–lymphatic interface in cancer cell migration. (i) Comparative schematic of interstitial (pink arrows), transmural (red arrows), and luminal (blue arrows) flows exerted on cancer cells (green) during invasion across the interstitial–lymphatic interface; (ii) tumor cell transmigration rates across lymphatic epithelial cells (LECS) were calculated from the appearance and disappearance of cells on the membrane (inner panel), $\Delta$ reflects the number of cells leaving the membrane; (iii) dynamics of invasion and intravasation of MDA-MB-231 cells under luminal (LF) and transmural (TF) flows (reproduced with permission from ref. 92). B. Microfluidic device for single cell migration. (i) Schematic of the device. Cells are seeded at the left side of the device and migrate through the channels towards the right side following a chemoattractant gradient; (ii) representative images of the single-cell migration assay in response to HGF; (iii) variation in the geometry of migration channels with the addition of choke points; (iv) scanning electron microscope images of a non-chemotactic *vs.* highly chemotactic cell after retrieval (reproduced with permission from ref. 95). C. Microfluidic artificial blood vessel implanted tumor transvascular migration model. (i) Schematic of the artificial transvascular migration model. Cellulose/collagen artificial blood vessels are implanted in a collagen matrix and seeded with endothelial cells to simulate the vasculature. Tumor cells are seeded in the collagen matrix to mimic transvascular migration; (ii) schematic and fluorescence imaging of endothelial cell (EC) migration in response to VEGF; (iii) schematic and fluorescence imaging of intravasation of HCCLM9 cells (red), in response to HGF. Scale bars: 50 μm (reproduced with permission from ref. 98). D. Microfluidic organotypic extravasation assay. (i) Schematic of the device. Two lateral channels enable the addition of cancer cells, soluble factors, and the generation of flow across the biomimetic vasculature in the gel channel. (EC: endothelial cells, MSC: mesenchymal stem cells, OB: osteoblast-differentiated cells, and CC: cancer cells); (ii) schematic of the different vascular microenvironments that were investigated. (OD hBM MSC: osteoblast-differentiated MSCs, BMI ECM: bone matrix, $A_3$AR: adenosine bound to the $A_3$ receptor) (reproduced with permission from ref. 101). E. Microfluidic assay for organ-specific metastasis. (i) Schematic of the invasion assay. Circulating tumor cells (CTCs) were pumped over multiple artificial organs, including lung, liver, bone, and muscle cells; (ii) fluorescence imaging of the adhesion of MCF7 and MDA-MB-231 cells to the endothelium. Scale bar: 200 μm; (iii) fluorescence imaging of metastatic inhibition by different concentrations of AMD3100. Scale bars: 200 μm (reproduced with permission from ref. 102).

 

which they used to investigate the effect of antagonists of the $A_3$ adenosine receptor on extravasation, as well as the anti-metastatic role of adenosine[101] (Fig. 5Dii). In particular, the approach presented by Jeon *et al.* constitutes a functional heterocellular culture system, in which human breast cancer cells are able to attach to and metastasize across a vascular network. Thus, this platform is a remarkable example of how microengineered systems can be used to mimic the pro- or anti-metastatic signatures of different microenvironments, in order to study cancer cells in controlled, organ-specific, physiological conditions.

More recently, Kong *et al.* developed a microfluidic model of multiple-organ invasion of CTCs. This platform allowed the study of the metastatic spread of human MCF7 and MDA-MB-231 breast adenocarcinoma, and ACC-M cystic adenocarcinoma cells to primary mouse cultures established from different organs[102] (Fig. 5Ei). Using this approach, they demonstrated that the three tested tumor cell lines exhibited a significantly higher tropism to lung, liver, and bone marrow tissues, when compared to muscle (Fig. 5Eii). Interestingly, this behavior was consistent with results observed in experiments using nude mice models, as well as the reported clinical behavior of these cancers. Moreover, they used their device to perform comparative studies of the ability of different concentrations of the CXCR4-antagonist AMD3100 to prevent metastasis, both *in vitro* and *in vivo*. Their results demonstrated that AMD3100 was able to prevent metastasis to the lung by preventing the adhesion of CTCs to the endothelium *in vivo*, a process which was efficiently mimicked by the microfluidic model *in vitro* (Fig. 5Eiii). Interestingly, the similarities in the results obtained with the microfluidic system and those obtained *in vivo*, indicate that these type of approaches could eventually be used in preclinical tests to assess the ability of anti-cancer compounds to prevent organ-specific metastasis.

### 5.3. Models of tumor angiogenesis

After metastatic cells escape the systemic circulation and invade into the parenchyma of the target organ, they either remain dormant or proliferate and develop into micrometastasis. As metastatic tumors grow, they undergo a process known as angiogenic switch, which refers to the progression from a non-angiogenic state towards the active growth of new blood vessels promoted by the vascular endothelial growth factor (VEGF).[103] The conventional notion of angiogenesis stipulates that tumor cells induce vessel growth in collaboration with the neighboring stroma. However, as additional mechanisms of tumor vascularization have been identified, the specific pathways that lead to the formation of the tumor vasculature are not fully understood.[104] Chung *et al.* engineered one of the first platforms to quantify cell migration and capillary morphogenesis, using a 3D collagen scaffold that integrated biochemical and biomechanical stimuli[105] (Fig. 6Ai). This system was used to monitor tumor cell-induced endothelial cell migration and angiogenesis in real-time (Fig. 6Aii). More recently, Lee *et al.* used a similar approach to quantify tumor cell angiogenesis, by further integrating the ability to monitor transendothelial migration[106] (Fig. 6Bi). Microvessel formation in this device was achieved through vasculogenic stimulation of endothelial cells by fibroblasts. This resulted in smooth and continuous vessel boundaries that closely mimicked *in vivo* conditions (Fig. 6Bii). After microvessel formation, U87MG glioblastoma cells were introduced into the perivascular region, where they secreted angiogenic factors that induced the formation of angiogenic sprouts. In this contribution, Lee *et al.* explored the implementation of this device as both, a cancer angiogenesis assay to test the anti-VEGF effect of the drug bevacizumab, as well as a cancer intravasation assay to test the pro-angiogenic effect of TNF-$\alpha$ (Fig. 6Biii). Moreover, this platform could also be used to study cancer cell or leukocyte extravasation, mechanotransduction of endothelial cells to the intraluminal flow, as well as for selective retrieval of invading cancer cells for analysis. In particular, this approach demonstrates how normal physiological processes can be reproduced *in vitro*, in order to engineer biomimetic tissues that can be used for experimentation. In turn, the generation of physiological structures that closely resemble human tissues will also help develop more accurate preclinical models of human disease.

In a recent study, Theberge *et al.* engineered a microfluidic system to study the effects of soluble factor signaling on endothelial tubule formation.[107] Using this system, they investigated the effect of incorporating human macrophages to co-cultures of endothelial cells and fibroblasts, in an arrangement that enabled soluble factor communication between them (Fig. 6Ci). This is relevant because macrophages are important mediators of angiogenesis, and act as a cellular link that spatially and temporally connects angiogenesis with lymphangiogenesis.[108] Using this system, Theberge *et al.* showed that the macrophage-secreted factor MMP12 was able to suppress microtubule formation and angiogenesis (Fig. 6Cii and Ciii). More importantly, this approach was sensitive enough to resolve the dose-dependent nature of the anti-angiogenic action of MMP12, a response which was completely missed in more simplified assays.

The platforms described in this section could be used for fundamental studies of the mechanisms that modulate physiological angiogenesis, as well as screening platforms to test potential anti-angiogenic compounds. In particular, the implementation of microengineered systems for drug screening would be greatly favored by their enhanced physiological relevance and accuracy, when compared to conventional *in vitro* approaches. However, key challenges to current systems would first need to be addressed, which are mainly related to the precise modulation of mechanical and biochemical factors to achieve physiologically-relevant conditions, (ii) enhancing the biomimicry of the engineered microvasculature, and (iii) integrating the role of intraluminal and interstitial flows in the microcirculation.

### 5.4. Models of the physical features of the microenvironment

In addition to cellular and biochemical cues, the actual physical interactions of tumor cells with their neighboring tissues

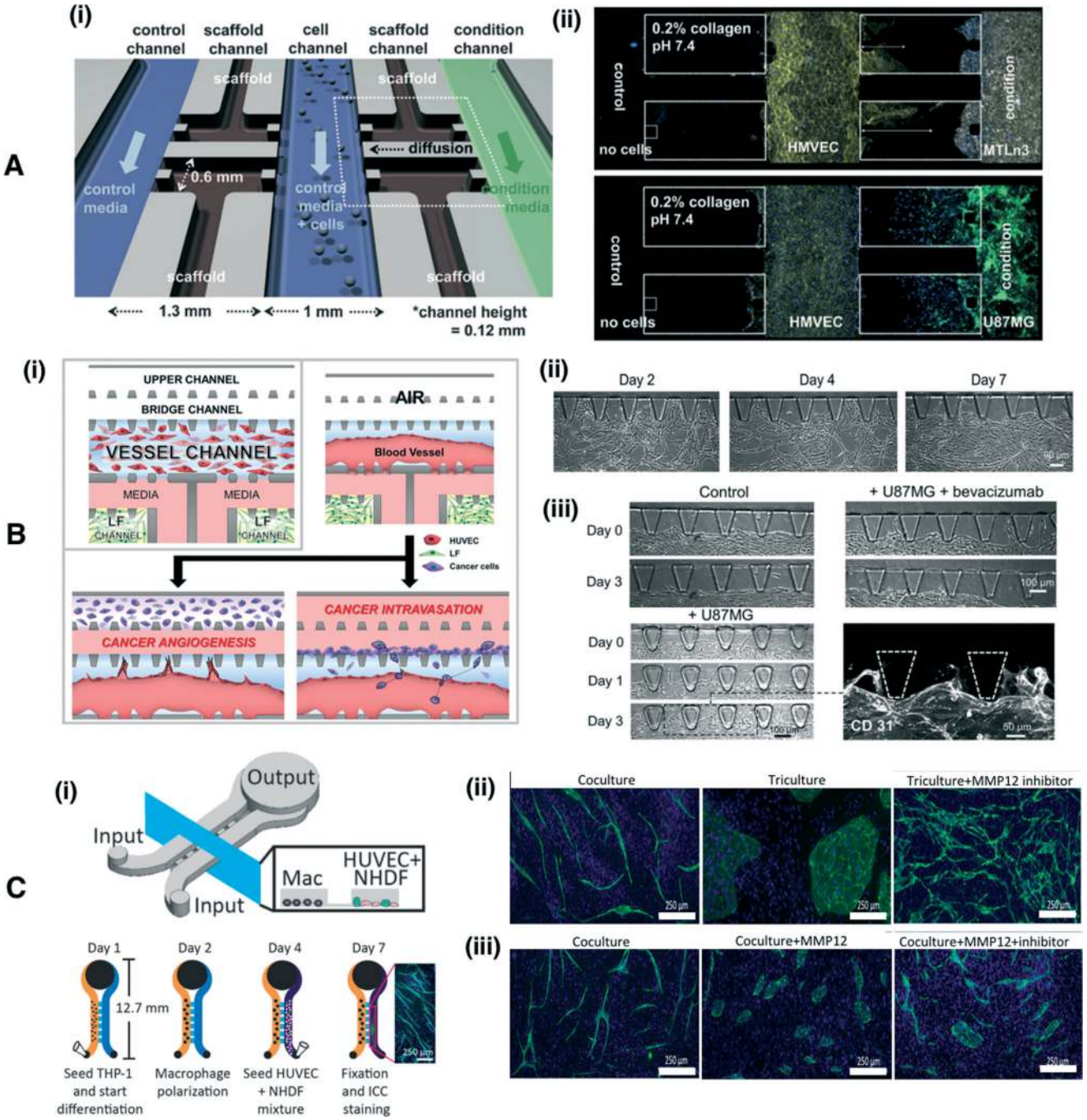


**Fig. 6** Models of tumor angiogenesis. A. Microfluidic device for capillary morphogenesis. (i) The schematic shows the set-up for the cell migration assay. The arrangement of the device enables the direct comparison of endothelial cell migration (middle), triggered by chemotactic signals from two side channels with different experimental conditions. The three channels are separated by a collagen scaffold (brown channels); (ii) representative images of HMVEC cell migration, in response to the chemo-attractant effect of MTLn3 breast cancer and U87MG glioblastoma cells in co-culture (reproduced with permission from ref. 105). B. Microfluidic model of angiogenesis and intravasation. (i) Design scheme and experimental procedures: (top left) schematic drawing of the microfluidic chip, (top right) microvessel formation, (bottom left) cancer angiogenesis assay, (bottom right) cancer intravasation assay (LF: lung fibroblasts); (ii) time-lapse micrograph of microvessel formation. Endothelial cells scattered in the vessel channel fuse together with a patent, perfusable lumen; (iii) micrograph of the microvessel wall after injection of U87MG cancer cells, and cancer cells + bevacizumab (reproduced with permission from ref. 106). C. Microfluidic model of biomolecular angiogenesis. (i) Schematic showing the two culture channels for seeding macrophages, and the HUVEC + normal human dermal fibroblasts (NHDF) mixture, which are connected by a series of communication channels to allow soluble factor signaling. The schematic also shows the device workflow for seeding and differentiation of THP-1 monocyte cell line to macrophages, polarization, seeding of HUVEC-NHDF mixture, and immunocytochemistry analysis. (Green: CD31, blue: DAPI); (ii) endothelial cells form tubules in co-culture with fibroblasts. Tri-culture with macrophages prevents tubule formation, which is rescued by the addition of an MMP12 inhibitor. (iii) In co-cultures of endothelial cells and fibroblasts, tube formation is decreased by the addition of exogenous MMP12, and partially rescued by the addition of an MMP12 inhibitor (reproduced with permission from ref. 107).

 

are key determinants of the metastatic process.[109] Throughout the entry, circulation and escape of metastatic cells from the circulation, they undergo deformation and hemodynamic forces that affect their viability, as well as their ability to establish metastatic tumors.[109] However, the relationships between hemodynamic forces and cancer cell viability, proliferation, motility, deformation, and interaction with the endothelium are not fully understood. To investigate these aspects of cancer metastasis, Huang *et al.* engineered a microfluidic extravasation model that mimicked the mechanical and biochemical microenvironmental cues from the vasculature[110] (Fig. 7Ai). They used this device to study the individual and synergistic effects of mechanical (*i.e.*, fluid shear stress and cyclic stretch) and biochemical stimuli (*i.e.*, TNF-α signaling) on the behavior of cervical adenocarcinoma HeLa cells. Their results demonstrated that mechanical strain could promote TRAIL-induced apoptosis in cancer cells, and influence their adhesion to the endothelium (Fig. 7Aii). The incorporation of TNF-α further promoted tumor cell adhesion to the endothelium, which was weakened by the action of this cytokine (Fig. 7Aiii). In addition, they also investigated the potential of this system as a screening platform to evaluate the antioxidant effect of platinum nanoparticles (Pt-NPs).[111] Their results demonstrated that Pt-NPs effectively recovered the integrity of the biomimetic endothelium, and decreased the number and spreading area of adhered tumor cells. The work presented by Huang *et al.* exemplifies the incorporation of multiple physicochemical stimuli into a single device that can be used to model physiological features of human tumors. However, the low-throughput of this approach could limit its application as a platform for screening large libraries of candidate anti-metastatic compounds. To address this issue, Spencer *et al.* recently developed a high-throughput mechanofluidic system to study cancer cell adhesion to the endothelium, the ECM, and platelets when subjected to physiological levels of shear stress (Fig. 7Bi).[112] Using this platform, they screened the effect on cancer cell adhesion of various anti-inflammatory compounds, as well as integrin and kinase inhibitors. Their results demonstrated that inhibitors of the FLT-3 and AKT pathways were able to selectively block cancer cell adhesion, while also exerting minimum effect in the adhesion of leukocytes to endothelial cells. In a follow-up work, they further interfaced their mechanofluidic platform with cell-substrate impedance sensing, which enabled the label-free evaluation of cell adhesion under flow with high-throughput and in real-time (Fig. 7Bii).[113] Using this improved version of their platform, they screened the effect of small molecule inhibitors on the adhesion kinetics of breast cancer cells. Taken together, the results presented by Spencer *et al.* demonstrated that there are marked discrepancies in the data obtained in static conditions, when compared to cell adhesion assays performed under physiological shear stress (Fig. 7Biii). Moreover, their screening process revealed that some compounds might promote cancer cell adhesion even if they also inhibit their proliferation. This property demonstrates the remarkable utility of microengineered devices as predictive models to screen out compounds with undesirable properties, prior to exhaustive and expensive pre-clinical studies.

Metastatic cells also adapt their migratory mechanisms across the basement membrane and ECM in response to their exposure to varying levels of physical confinement.[114] Nuclear deformation often limits migration across confined environments, due to the particularly large size and stiffness of cellular organelle.[115] In order to study the physical limitations of cancer cell translocation, Malboudi *et al.* engineered a microfluidic device to evaluate the ability of MDA-MB-231 breast carcinoma cells to alter their shape when migrating across gaps of different sizes[116] (Fig. 7Ci). The device consisted of an open chamber connected to a main channel through perpendicular microchannels of different widths (Fig. 7Cii). Cancer cell translocation and migration across the microchannels were triggered by a serum-induced chemotactic gradient, and the process could be visualized in real-time (Fig. 7Ciii). Their results showed that the physical limit for constriction and migration of MDA-MB-231 cells in a confined setting was reached at the 7 μm width microchannels. The approach presented by Malboudi *et al.* could be further used to study the morphological changes in the cytoskeleton and the nucleus that occur during migration across a chemotactic gradient. More recently, Liu *et al.* also engineered a microfluidic system to evaluate the ability of cancer cells to migrate through micro-constrictions, effectively mimicking cell perfusion through the circulatory system during metastasis[117] (Fig. 7Di). In this study, Liu *et al.* described a novel cellular parameter called "transportability", which is determined by cell stiffness and the frictional properties of the cell surface. They demonstrated the ability of their device to achieve precise, high-throughput separation of different breast cancer cell lines, based on their size and transportability (Fig. 7Dii). Furthermore, they found that treatment with the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) could enhance the transportability of cancer cells (Fig. 7Diii), a response that also correlated with the up-regulation of CSC markers (Fig. 7Div). This approach could potentially be used to isolate and study populations of cells based on their mechanical phenotype, as well as to study the correlation with their biophysical and invasive properties and expression of relevant biomarkers.

In addition to the physical properties of the cell membrane and the nucleus, the stiffness of the ECM also provides mechanical cues that are involved in cancer metastasis. In a recent study, Garcia *et al.* engineered a microfluidic system that could generate stable, linear and diffusive biochemical gradients in polyacrylamide hydrogels, which possessed an additional perpendicular stiffness gradient[118] (Fig. 7Ei). Using the non-cancerous MDCK canine cell line as a model, they validated their approach by investigating the cross-talk between a biochemical HGF-derived gradient and a physical stiffness gradient. Their results revealed that cell scattering was directly dependent on the synergy between the two gradients (Fig. 7Eii and Eiii). Previous works have already studied the biophysical interactions between tumor

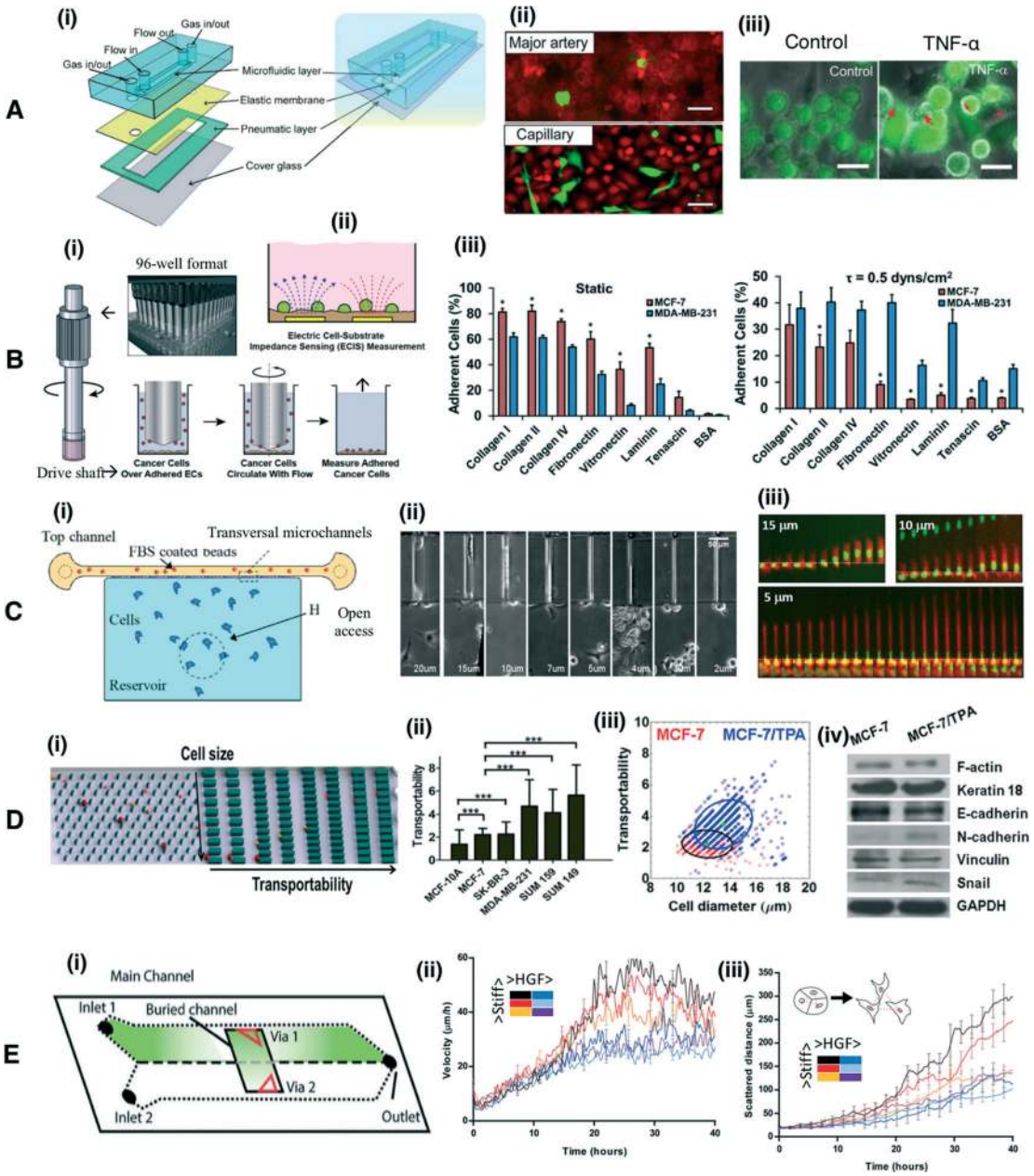


**Fig. 7** Models of the physical features of the microenvironment. A. Microfluidic model of the physicochemical cues from the vascular microenvironment. (i) The layered composition of the device enabled the deformation of the seeded cells in the elastic membrane, when vacuum is applied to the system; (ii) fluorescence imaging of the adhesion of the tumor cells (HeLa, green) on the endothelial monolayer (HUVEC, orange) under physiologically-relevant mechanical conditions; (iii) tumor cells–endothelium interaction in a TNF-α conditioned environment. Fluorescence imaging of tumor cells adhered to the endothelial monolayer, and their morphological changes in the presence of TNF-α. Scale bars: 20 μm (reproduced with permission from ref. 110). B. Microengineered mechanofluidic platform for cell adhesion under flow. (i) The design of the device was based on a 96-shaft gearbox, coupled to a standard 96-well culture plate. The panel also shows the overall process for detachment assays; (ii) schematic of the label-free adhesion measurement system; (iii) adhesion of strongly metastatic MDA-MB-231 and moderately metastatic MCF-7 cells, under static (left) and flow (right) conditions (reproduced with permission from ref. 112 and 113). C. Microfluidic device for cancer cell deformation. (i) The design consisted of an open chamber connected to a main channel through perpendicular microchannels of different widths. Cells in the reservoir are allowed to migrate through the microchannels in response to FBS; (ii) representative phase contrast images of migration, which shows that cells in the device are able to project into the microchannels independently of their width. (iii) Representative kymographs of individual translocating cells (reproduced with permission from ref. 116). D. Microfluidic device for transportability. (i) The device consisted of a deterministic lateral displacement microarray (left), and a trapping barrier microarray (right); (ii) quantification of the average transportability of six breast cancer cell lines; (iii) transportability *versus* cell diameter is plotted for MCF-7 and TPA-induced MCF-7 cells. Black and blue circles indicate the 80% confidence interval centered at the mean depicted by a green dot; (iv) western blot analysis of biomarker expression in MCF-7 and TPA-induced MCF-7 cells (reproduced with permission from ref. 77). E. Microfluidic model of biochemical and substrate stiffness gradients. (i) The chemical and mechanical gradients in the device were generated within a buried channel, which was located beneath a Y-shaped channel perpendicular to the angle of the flow; time evolution of (ii) cell velocity and (iii) scattered distance for the MDCK cell migration assay. The direction of the HGF and stiffness gradients for each of the 6 regions formed in the device are shown in the inner panels (reproduced with permission from ref. 118).

 

cells and the ECM. For example, Pathak *et al.* engineered a platform based on microfabricated polyacrylamide channels, with defined wall stiffness and geometry.[119] Using this system, they were able to determine that cells migrating through narrow channels moved faster than those in wide channels and 2D surfaces, due to an enhanced polarization of cell–ECM traction forces. The approach presented by Garcia *et al.* is the first to integrate substrates with tunable stiffness, as well as the spatial distribution of soluble biochemical factors. However, further iterations of this approach should explore the incorporation of human cancer cell lines or primary tumor cells in order to enhance its physiological relevance.

Integrative platforms that incorporate stimuli form soluble biochemical signals and physical substrate cues, can be used to generate more sophisticated microenvironments with enhanced biomimicry. For example, one approach reported previously by Huang *et al.*, described the patterning of different cell-laden hydrogels to generate continuous interfaces, as well as chemically and mechanically tailored 3D cellular niches.[120] More recent studies are beginning to explore the generation of responsive matrixes, through native deposition and assembly of ECM proteins by human cells cultured under hydrodynamic and biochemical stimulation.[121] Next-generation mechanotransduction platforms could be particularly useful to study more dynamic phenomena, such as the ECM remodeling carried out by migratory cells. For example, a recent contribution by Rahman *et al.* focused on cell migration through ECM "microtracks", which are tube-like features that are formed through the proteolytic degradation of the ECM.[122] Using this platform, they determined that the focal adhesion molecule vinculin played a critical role in maintaining unidirectional migration of MDA-MB-231 cells through microtracks. However, this approach did not take into account the chemoattractant effect of biochemical or oxygen gradients on the directionality of cancer cell migration. Thus, future works should continue to address the synergy between the physical features of the ECM, and the physicochemical gradients present in the tumor microenvironment.

### 5.5. Models of tumor hypoxia

Another aspect of tumorigenesis that is independent of cellular or biochemical factors is the insufficient tissue oxygenation at the tumor site, also referred to as hypoxia. Solid tumors exhibit hypoxic regions due to the rapid growth of cells, whose oxygen and nutrient demands exceed those supplied by the associated vasculature. Furthermore, the overexpression of hypoxia inducible factors such as HIF-1 promotes the expression of a series of hypoxia-inducible genes, which leads to cancer cell invasion and metastasis.[123] To investigate the influence of hypoxic conditions in the promotion of metastasis, Acosta *et al.* engineered a microfluidic invasion assay that integrated a co-culture of cancer and endothelial cells in a collagen matrix, with a system

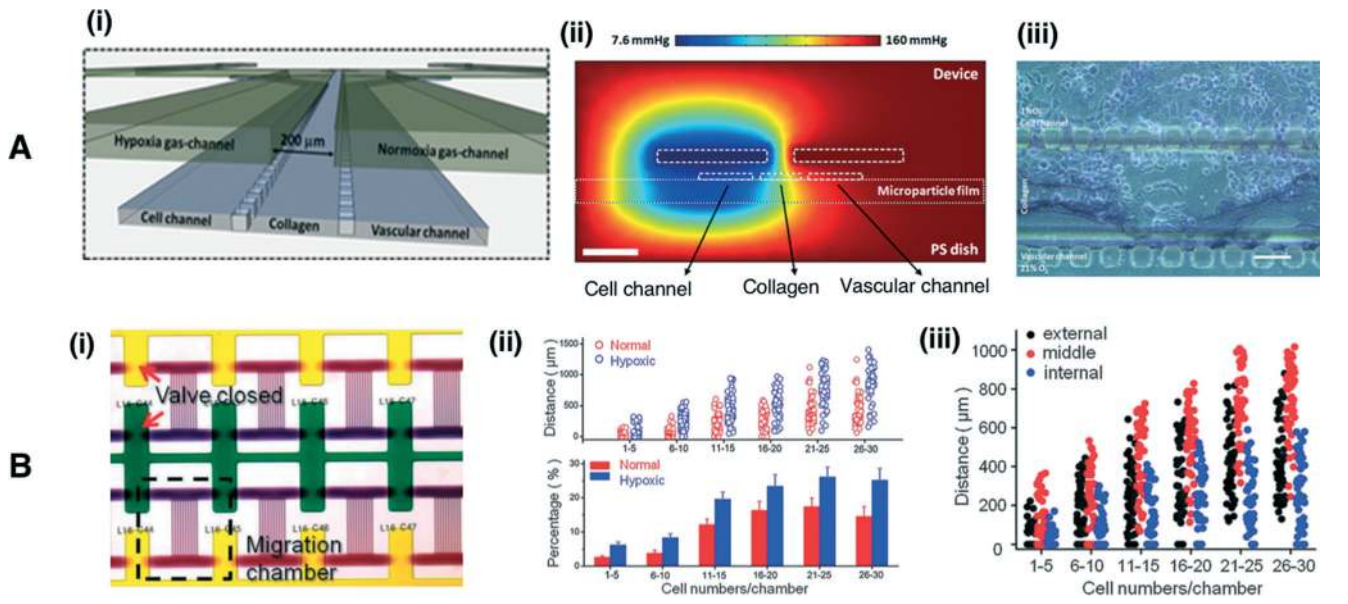


Fig. 8 Models of tumor hypoxia. A. Microfluidic device of chronic and intermittent hypoxia. (i) The schematic shows a cross-section of the device indicating the cell and vascular channels connected through a collagen matrix, as well as the gas channels used for delivery of the hypoxia and normoxia gas mixtures; (ii) map of oxygen concentrations at steady-state within cross-section of the microfluidic device. The oxygen content in the hypoxia and normoxia channels is 1% and 21%, respectively. Scale bar: 400 μm; (iii) phase contrast image of PANC-1 cells within the microfluidic device during culture under the $O_2$ gradient. Scale bar: 100 μm (reproduced with permission from ref. 124). B. High-throughput microfluidic device for hypoxia-driven invasion. (i) Bright-field micrograph shows the arrangement of the device micro-chambers, as well as the established chemotactic gradient. Each chamber has an upper chemoattractant reservoir that is connected to a cell culture chamber through parallel migration microchannels; (ii) the graphs show the percentage of migration and migrated distance (migratory potential) of SUM-159 cells, under normoxic and hypoxic environments; (iii) comparison between the migrated distance and the percentage of migratory cells from samples corresponding to different depths within the tumor (reproduced with permission from ref. 127).

for temporal and spatial oxygen control (Fig. 8Ai). Using this device, they were able to establish long-term (up to 8 days) cultures of PANC-1 pancreatic adenocarcinoma cells, in a collagen scaffold with oxygenation gradients consistent with chronic and intermittent hypoxia[124] (Fig. 8Aii). They investigated the potential of their device to study cancer cell invasion in response to different oxygen gradients. Invasion of PANC-1 cells across the biomimetic collagen barrier occurred in response to variations in the oxygen partial pressure, from 21% (ambient) to 1% (hypoxia) (Fig. 8Aiii). The approach developed by Acosta *et al.* enabled the long-term culture of cells in spatial oxygen gradients and dynamically changing hypoxic microenvironments. Thus, this approach has remarkable potential to study not only tumor growth, but also normal developmental processes that are influenced by hypoxia,[125] as well as other non-malignant diseases.[126]

Hypoxia has also been involved in the promotion of EMT and metastasis through its synergistic action with tumor acidosis. Recently, Zhang *et al.* engineered a high-throughput microfluidic platform with a controlled oxygen microenvironment, and used it to study mesenchymal-mode migration of SUM-159 breast carcinoma cells under hypoxia[127] (Fig. 8Bi). Their results demonstrated that the distance migrated, and the percentage of migratory SUM-159 cells was consistently greater under hypoxic conditions (Fig. 8Bii). They also investigated the potential of the engineered device as a screening platform, using the small-molecule compounds linifanib and 227013, as well as the HIF-1$\alpha$ inhibitor 2-MeOE$_2$. Using this approach, they were able to identify phenotypic differences in primary cancer cells isolated from different areas of the tumor, mainly with respect to their viability and migratory velocity (Fig. 8Biii). In particular, the approach presented by Zhang *et al.* could be used to assay the migratory potential of samples with particularly low cell numbers under tunable oxygenation conditions. Moreover, the high-throughput of this platform enables its implementation as a screening platform to evaluate the efficacy of anti-metastatic compounds.

## 6. Future perspectives

Although several applications of microengineered systems have been described in the context of cancer research, the dynamism and plasticity of these platforms hold remarkable potential for anti-metastatic drug research and development. Metastatic cells engage in heterotypic interactions with different tissue-specific microenvironments, as they migrate from the primary tumor site to a target organ. Hence, therapeutic intervention at any of the different stages of the metastatic cascade holds significant promise for cancer patients with, or at risk of metastatic disease. The multi-parametric nature of the metastatic process, together with the remarkable heterogeneity of human cancers demonstrate the need for high-throughput screening platforms for drug discovery. Nevertheless, the translation of microengineered assay systems to high-throughput testing presents additional challenges, which include: (i) the miniaturization and automation of platform components, (ii) straight-forward interfacing with standard probing and scale-up technologies, (iii) the precise integration of large numbers of cells into complex configurations, and (iv) the lack of versatile cellular scaffolds and device materials.[128] Theoretically, the volume for each assay unit in microfluidic cell-based screening systems could be scaled down to the submicroliter level, which would translate to assays based on hundreds or even single cells.[129] However, one major drawback of such approach would be that the inclusion of too few cells might impair cellular microarchitecture, which in turn would reduce the predictive power and physiological relevance of the assay. Moreover, next-generation microengineered platforms could also allow the modular integration of different organs-on-chips arrays, to account for the cross-talk between different tissues *in vivo* and their contribution to disease progression. Nevertheless, future progress will not solely rely on the capacity to manufacture and scale-up physiologically relevant assays, but also on the adaptation and development of readout techniques and probing methodologies. In summary, the industrial scaling of cell-based systems with such degrees of complexity like the ones described in this review still presents many technical and operational compromises, and several technological challenges remain to be solved. Thus, despite the high-throughput capabilities of some of these devices, they are still unlikely to be widely extrapolated into the actual clinical context at their current level of development. However, the enhanced physiological relevance of these platforms could be used today to bridge the gap between conventional *in vitro* and *in vivo* models, in order to produce more accurate data and screen out non-effective candidate compounds prior to extensive animal and human trials.[130]

One of the most critical areas of opportunity for microengineered cell-based assays is the incorporation of human primary tumor cells. This strategy would allow the generation of more clinically relevant assays that are able to account for inter-patient and inter-tumor heterogeneity. Primary and established cell cultures could also be further tailored through site-specific genome targeting, in order to evaluate the effect of sequence-specific modifications on cancer cell phenotypes, as well as screening of therapeutic efficacy in biomimetic environments.[131] The engineering and incorporation of smart biomaterials will also help enhance the biomimicry of 3D cultures for long-term modeling and assay of tissues with increased levels of sophistication, as well as develop durable prophylactic and therapeutic anti-cancer agents.[132] Next-generation microengineered devices should be used to investigate complex fundamental aspects of tumor malignancy, such as the microenvironmental promotion of tumor dormancy and regression. In addition, the engineering of patient-specific metastasis-on-a-chip platforms could one day eliminate the need for animal models, while also enabling the opportune design of tailored and molecularly-targeted therapies. Ultimately, incorporating multiple organ- and tumor-on-chip approaches will provide unprecedented insight into the interplay between normal and

 

cancer tissues, which underlie the development and progression of metastasis.

## 7. Concluding remarks

State of the art microengineered systems allow the precise and dynamic control of microenvironmental parameters that are critical in the establishment of the clinical behavior of metastatic tumors. These parameters include soluble and physical cues from the tumor associated stroma, heterocellular interactions of migratory cancer cells with surrounding tissues, physical stresses inflicted on CTCs during transendothelial migration, and physicochemical gradients that direct cell motility and invasion. Microengineered devices allow the real-time and non-invasive monitoring of cell-based assays with tissue- and organ-level complexity. Their implementation in several areas of fundamental and translational cancer research provides the unique opportunity to dissect and recreate remarkably complex physiological processes, with a level of human-relevance and precision that cannot be achieved by conventional methods. As cancer therapeutics begin to shift to personalized and molecularly-targeted approaches, the robustness and predictive power of microengineered devices will undoubtedly impact the way in which the preclinical evaluation of novel compounds is conducted.

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