Microfluidics meets 3D cancer cell migration

Mehta, Pranav; Rahman, Zaid; ten Dijke, Peter; Boukany, Pouyan E.

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Review

Microfluidics meets 3D cancer cell migration

Pranav Mehta,[1,2,3] Zaid Rahman,[2,3] Peter ten Dijke,[1,*] and Pouyan E. Boukany[2,*]

An early step of metastasis requires a complex and coordinated migration of invasive tumor cells into the surrounding tumor microenvironment (TME), which contains extracellular matrix (ECM). It is being appreciated that 3D matrix-based microfluidic models have an advantage over conventional *in vitro* and animal models to study tumor progression events. Recent microfluidic models have enabled recapitulation of key mechanobiological features present within the TME to investigate collective cancer cell migration and invasion. Microfluidics also allows for functional interrogation and therapeutic manipulation of specific steps to study the dynamic aspects of tumor progression. In this review, we focus on recent developments in cancer cell migration and how microfluidic strategies have evolved to address the physiological complexities of the TME to visualize migration modes adapted by various tumor cells.

Highlights

Microfluidic cancer cell migration models enable an integrated assessment of how cell–cell and cell–matrix interaction, matrix stiffness, interstitial and shear flow, hypoxia and cytokine, and metabolic gradients affect dynamic cancer cell migration.

While many *in vitro* static cell migration models fail to mimic the complexities and biomechanical features of the tumor microenvironment (TME), incorporation of microfluidics allows us to investigate cancer cell migration through the TME and invasion under well-controlled pathophysiological conditions associated with tumor progression events.

Tumor cells can elicit distinct migratory behavior in the form of single or collective cell migration modes in response to extracellular biochemical and biophysical cues.

Microfluidic modeling of cell invasion

Metastasis, the spread of cancer cells away from the primary solid tumor and into the body's healthy tissues, eventually leading to the formation of secondary tumors causing organ failure, is responsible for over 90% of cancer-related deaths [1]. Metastasis requires the complex and coordinated migration of invasive tumor cells into the surrounding TME containing ECM. The TME plays a critical role in the early stages of metastasis, dictating cancer cell motility, invasion, and spread into neighboring tissues [2]. Key extracellular determinants of cancer cell migration include the biophysical and biochemical cues provided by the ECM, interstitial flow (IF), and cancer cell–TME interactions, including the interplay with cancer-associated fibroblasts (CAFs).

Conventional tumor models previously used to study these cell motility mechanisms lie on either end of the spectrum: on one end, macroscopic *in vitro* models are easy and simple, but poorly recapitulate the local TME characteristics, including biomechanical forces, IF, fluid shear stress, and ECM remodeling. On the other end, animal models can mimic the pathophysiological complexity of tumors, but they are expensive, time-consuming, and still have a degree of error due to the innate differences between animal and human physiology. The use of microfluidics in 2D and 3D models has become an attractive alternative to overcome these challenges. Going a step further, microfluidics can more closely mimic the TME by incorporating various physiologically relevant biophysical and biological cues with precise spatiotemporal control (Figure 1, Key figure) [3,4]. Microfluidic devices also permit real-time imaging of cellular dynamics, enabling them to probe biophysical, biochemical, and (epi)genetic activities present in healthy and diseased multicellular tissues [5]. The field of microfluidics continues to extend the development of tumor models ranging from tumor cancer spheroids to cancer-on-a-chip models with relevant TME and (patho)physiological conditions [6,7].

Microfluidic platforms have been used for the study of the metastatic microenvironment, tumor–stroma interactions, and the TME in cancer metastasis and therapy [8–11], cancer extravasation in response to biophysical and chemical cues [12,13], single-cancer-cell separation, detection, and migration [14], biophysical studies of cancer cells at the single-cell level [15], modeling cancer

[1]Department of Cell and Chemical Biology and Oncode Institute, Leiden University Medical Center, Leiden, The Netherlands
[2]Department of Chemical Engineering, Delft University of Technology, Van der Maasweg 9, 2629 HZ, Delft, The Netherlands
[3]These authors contributed equally to the work

*Correspondence:
P.ten_Dijke@lumc.nl (P. ten Dijke) and p.e.boukany@tudelft.nl (P.E. Boukany).

Key figure

Schematic of primary tumor (lung or breast) metastasis to different parts of the body and application of microfluidics to study tumor invasion

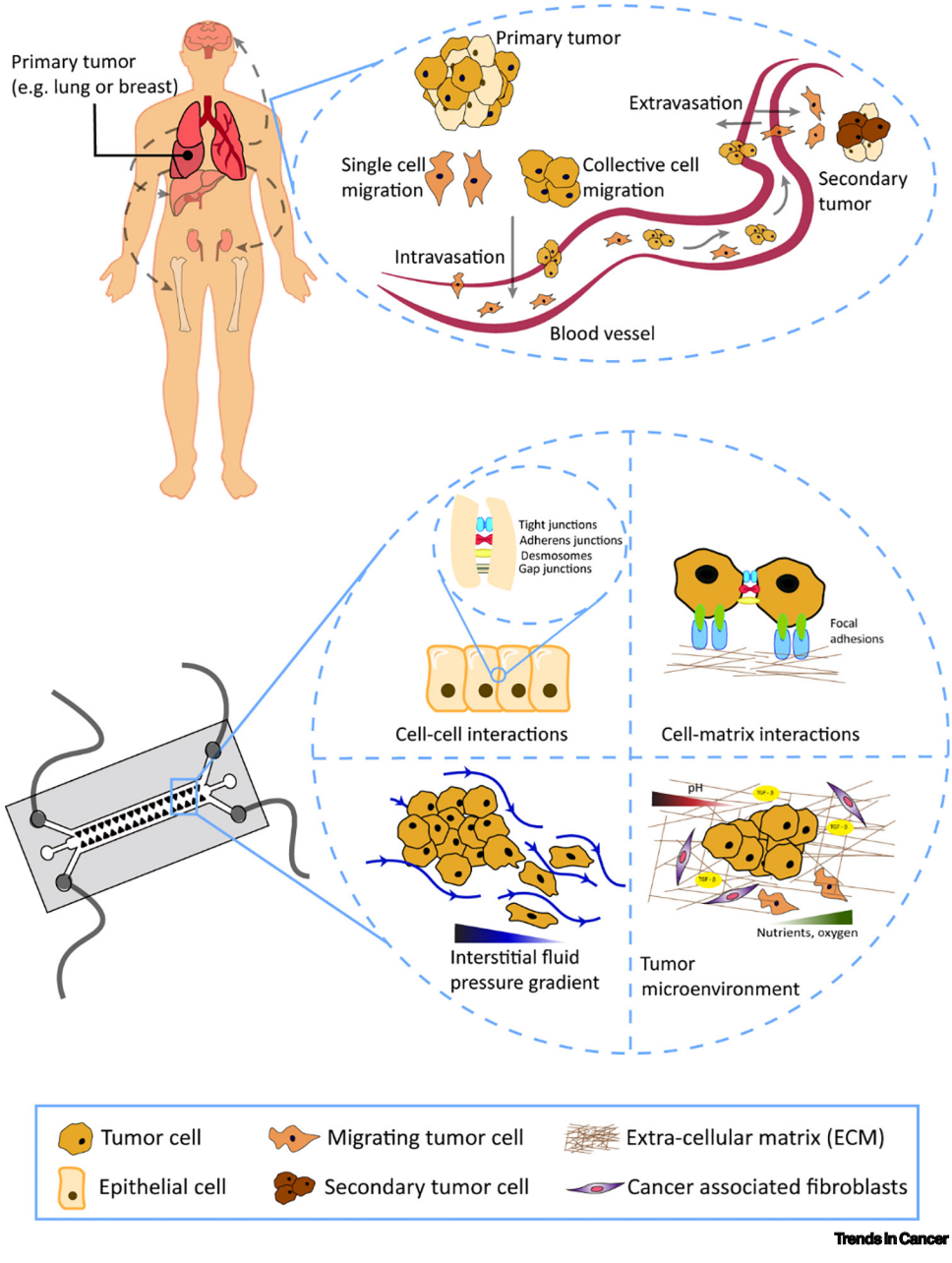

Figure 1. Top: invasive cancer cells metastasize from a primary solid tumor (such as the lung or breast) to different parts of the body such as the brain, liver, adrenal glands, and bones. Primary tumors disseminate into a single or cluster of migrating tumor cells with adaptive migration morphology. These disseminated tumor cells can then enter the blood vessel, known as

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immune interaction [16], and development of tumor/organ-on-a-chip in personalized cancer therapy [17,18]. However, a critical perspective on microfluidic approaches for mechanistic studies of cancer cell migration into surrounding TME, for example, how cancer cells adapt their migratory behavior through different TME, is still missing. Here, we discuss how cancer cells adapt their migration strategies through different and dynamically changing TMEs, and how novel microfluidics platforms have evolved to unravel the mechanisms of collective cancer cell migration through a tunable TME. We discuss how microfluidics is an effective tool to mimic the metastatic microenvironment for mechanistic investigations (Figure 1). We then discuss the underlying migration mechanisms adopted by cancer cells in a physiologically relevant TME model. We focus on lung and breast cancer cells as they are well studied but remain two of the most diagnosed cancers worldwide in terms of incidence and mortality due to the complexity of the TME [1,2]. Next, we highlight the role of tumor heterogeneity, ECM, interstitial and shear flow as biomechanical stimuli, CAFs, and the cytokine, transforming growth factor beta (TGF-β) as relevant biochemical cues to emphasize tumor invasion properties. Finally, we discuss challenges and new directions in applying 3D matrix-based microfluidic devices in personalized medicine and cancer therapy.

Microfluidics in collective cell migration

Both biophysical and cellular cues alter tumor cell responses (from migration modes to the level of invasiveness) inside the dynamic TME, resulting in the dissemination of cancer cells from the primary tumor. 3D *in vitro* tumor models such as heterogeneous or coculture multicellular spheroid models are widely used to investigate cancer cell migration and invasion [10]. Static (no-flow) 3D cell culture models have been used to examine cell migration through semipermeable membranes or to study the biological response to specific chemoattractants or drugs [13]. Moreover, a programmable and multifunctional 3D cancer cell invasion platform has been recently developed that dynamically releases mature TGF-β to induce invasive migration of cancer cells by assembling functionalized micro-buckets and a tunable ECM that emulates a more complex TME during cancer invasion [19]. A major advantage of using 3D microfluidic platforms is that different cell types can be cocultured long term in a controlled microenvironment, thus more closely resembling physiology *in vivo*. Through real-time visualization and the ability to study specific biological cues, 3D microfluidic platforms allow the characterization of individual cellular responses, providing insight into intercellular communication. For example, 3D matrix-based microfluidic models incorporated the use of endothelial cell (EC)-covered microchannels and spatiotemporally controlled ECM compartments consisting of stromal cells, tumor-associated macrophages (TAMs), CAFs, and immune cells to recreate intratumor heterogeneity [13,20,21]. Other 3D microfluidic models explored the use of patient-derived cells [9,22], response of natural killer (NK) or immune cells [23], and drug delivery for drug screening applications [24,25]. Researchers have also been able to reconstitute *ex vivo* a human tumor ecosystem by coculturing

intravasation. Tumor cells travel through the blood vessel and exit at a distant location, known as extravasation. The tumor cells that survive and adapt to the new surrounding microenvironment have the potential to form a secondary tumor (e.g., in the brain). This chain of events is known as the metastatic cascade, where external factors such as biochemical and biomechanical cues influence the migratory behavior of cells from the primary tumor . Bottom: schematic of a microfluidic device highlighting the important applications used to study various critical biological phenomena [ranging from cell/cell and cell/extracellular matrix (ECM) interactions to interstitial flow within tumor microenvironment (TME)] associated with the metastatic cascade. Microfluidics gives us the advantage of real-time imaging to study cell–cell and cell–matrix interactions by quantifying biophysical properties such as changes in levels of adhesion proteins. A microfluidic device also provides desirable control to include external forces such as shear stress or interstitial flow, usually referred to as promigratory factors that influence cancer cell migration behavior. Last, recent developments in microfluidic models have focused on building a physiologically relevant TME that can provide a comprehensive understanding of the migratory behavior of specific cancer cell types.

four distinct cell populations (cancer, immune, endothelial, and fibroblasts) in a microfluidic device in 3D. Using this platform to screen for drug interactions and mechanisms, researchers elucidated cancer–immune–CAF cell interactions, illustrating the long-time scale (several hours) signaling cascade between cancer and immune cells and CAFs that antagonized the effects of the drug.

3D microfluidic models create a more effective and powerful platform to track intermediate steps to differentiate between single and collective cell migration mechanisms in cancer models with a dynamic interplay of multiple (dynamically phenotypically changing) cell types [26] (Box 1). Various microfluidic models have been developed to investigate metastasis-related phenomena, such as cancer cell migration, epithelial-mesenchymal transition (EMT), cell invasion, intravasation, and extravasation. In the following sections, we take a closer look at the microfluidic models developed for lung and breast cancers highlighting microfluidic modeling parameters and improved biological outcomes. Furthermore, these (3D) microfluidic devices have been adapted to investigate the impact of TME complexity on a wide variety of tumor invasions. An overview of 3D microfluidic models for various cancer types modeled for different tumor progression events and with chemotherapeutic applications is provided in Table 1.

Microfluidic models incorporating biophysical and biochemical cues

Biophysical cues, including IF, fluid pressure, and ECM stiffness, play a central role in directing cell movement, migration, and invasion inside the TME. For tumor cells to extravasate into circulation and distinct tissues, they interact by altering their mechanical properties to adjust to the physical changes of the ECM. The ECM architecture goes through constant remodeling, additionally influenced by interstitial fluid flow (IFF) and interstitial fluid pressure (IFP). Tumor cells respond to these biophysical cues by mechanotransduction pathways, which in a dynamically controlled manner, result in cell migration and invasion. For example, increased ECM stiffness activates the EPHA2/LYN complex, thereby triggering EMT in breast cancer [27]. Increasing collagen density in the ECM diminishes collective cell migration in breast cancer cells [28]. Matrix porosity can also

Box 1. Migration dynamics and epithelial-mesenchymal plasticity (EMP)

Cell migration can be broadly classified into two modes, individual or single-cell migration and collective cell migration. Morphologically and functionally, single-cell migration can be further classified into amoeboidal and mesenchymal movement. Amoeboid single migrating cells often display structures referred to as plasma membrane blebs, defined by a bulky and rounded morphology, and these blebs are an extension of the plasma membrane caused by local disruption of the membrane actin cortex interactions, high intracellular hydrostatic pressure, and enhanced contractility [66,67]. Single cells displaying a mesenchymal migration mode, moreover, display actin-rich protrusions and rely on the proteolytic degradation of the surrounding ECM [68,69].

Collective migration occurs when cells maintain their intercellular junctions and thus migrate jointly as an integrated group. The collective morphology and dynamics of cohesive motility are strongly dependent on the type and stability of their intercellular junctions and extracellular tissue conditions [67]. Collective migration in cancer is characterized by the expression of homophilic cell–cell junctions and epithelial morphologies within the migrating cluster [70]. Protrusive cells at the leading edge, termed leader cells, are connected to the rest of the cluster and have been shown to coordinate multicellular sheet/strand migration [71]. Collectively migrating cancer cells display structural ECM remodeling, further promoting migration and metastasis [72]. Cellular plasticity, the ability to repress or activate specific gene expression and modulate the activity of gene products to allow for optimal migration mechanics or survival, is crucial for collective cell migration. This plasticity allows migrating cancer cells to integrate various mechanical and chemical cues around them and adjust their direction, speed, and mode of migration [67].

Epithelial cancer cells can undergo a switch to a mesenchymal phenotype, a process termed epithelial–to–mesenchymal transition (EMT) [73]. New studies show that EMT is a dynamic plastic process, where cells can acquire different hybrid intermediate states called partial EM states (p-EMT), a process referred to EMP [74,75]. In such states, cells retain various epithelial features while also expressing EMT markers such as N-cadherin and vimentin intermediate filaments [67]. Cells undergoing complete EMT (c-EMT) transcriptionally suppress the epithelial markers such as the transmembrane protein E-cadherin [74].

Table 1. 3D microfluidic models to study cancer cell migration and anticancer therapeutic approaches in different tumor types

Cancer type	Microfluidic model highlights	Biological observations/outcome	Limitations	Refs
Breast cancer				
Type: tumor invasion Cells: SUM-159 breast cancer cells and CAF coculture	3D microfluidic based organotypic model. Focuses on tumor invasion by stromal activation. Coculture of SUM-159 and patient-derived fibroblasts to model patient-specific TME. Allows for tumor–stroma crosstalk.	RNA-seq is applied to profile the transcriptome of breast cancer cells with CAFs to delve deeper into molecular mechanisms in tumor–stroma bidirectional crosstalk. Breast cancer invasion enhanced in the presence of CAFs, is mediated by the expression of glycoprotein nonmetastatic B (GPNMB) in breast cancer cells.	Patient-derived CAFs heterogeneously express varying levels of specific biomarkers. Two out of three patient-derived CAFs showed tumor-promoting behavior. No defined single or set of biomarkers was defined owing to CAF heterogeneity.	[83]
Type: hypoxia-driven tumor migration Cells: HUVECs, MCF-10A, MDA-MB-231, MCF-7 and normal human lung fibroblasts (NHLFs)	Microfluidic model consisting of vascular networks to quantify breast cancer extravasation when exposed to different oxygen concentrations. Device with three-gel channels, alternated with media channels. An advantage in the study of different cell lines and conditions of normoxia and varying hypoxia levels in short-term cultures.	Increase in hypoxia inducible factor (HIF)-1α protein levels in hypoxic conditions were linked to elevated levels of metastatic potential. The increase in aggressive phenotype under hypoxic conditions is independent of the malignancy of different cell lines.	Effect of hypoxia on cell proliferation and viability can be different in cells lines of varying malignancy based on short-term or long-term exposure to hypoxic conditions. More studies are needed to understand the signaling pathways responsible for hypoxia-induced increased metastatic potential.	[84]
Type: tumor extravasation Cells: MDA-MB-231 breast cancer cells, monocytes	A 3D vascularized microfluidic model that provides high-resolution visualization of intravascular migration, transmigration, and differentiation of monocytes through human vasculature. Characterization of tumor cell extravasation in the presence of monocytes. Physiological differences between inflammatory and patrolling monocyte extravasation patterns were measured by the platform. Potential to study effects of monocytes on tumor progression.	Undifferentiated monocytes present with MDA-MB-231 cancer cells in intraluminal vasculature reduced tumor cell extravasation. Heterogeneity of cancer cell in extravasation mediated by monocyte type was observed. This difference was attributed to the role of myosin IIA in monocyte cell motility.	Future studies should examine media conditioned by cells in 3D compared with 2D system conditioned medium. More studies need to be performed on monocyte homeostasis and extravasation cascade in response to flow. Incorporation of other immune cells.	[85]
Lung cancer				
Type: tumor migration Cells: H1299 lung adenocarcinoma cancer cells	A 3D matrix-based microfluidic device with mixed hydrogel (collagen, Matrigel) compositions to investigate impact on migration. Quantitative image analysis to measure H1299 lung cancer migration in different hydrogel (ECM) compositions.	Hydrogel composition mimicking the TME. A disorganized basement membrane at the front of cancer invasion was mimicked using an ECM containing hydrogel. An increase in migration speed in collagen-Matrigel hydrogel compared with collagen only was observed because of increased stiffness and pore size. Hydrogel composition was identified as an important determinant for cell migration.	Inconsistency with results on migration speed and morphology with increasing matrix stiffness and pore sizes. Difficult to identify the exact composition of *in vivo* ECM based on specific cancer types and their location.	[86]
Type: tumor migration and anticancer therapy response Cells: A549 lung cancer cells, miR-497 exosomes.	A 3D microfluidic device in which A549 lung cancer cells and HUVECs are cocultured that is capable of delivery and controlled regulation of miRNAs via exosomes to explore therapeutic potential.	Significant inhibition of A549 cell migration and reduced sprouting of HUVECs was observed in the presence of miR-497 exosomes.	The model does not consider the inclusion of additional cell types such as CAFs and/or immune cells and hypoxic gradients.	[87]

(continued on next page)

Table 1. (continued)

Cancer type	Microfluidic model highlights	Biological observations/outcome	Limitations	Refs
	The model permitted the study of the role of VEGF concentration gradient on angiogenic sprouting. miRNA therapeutics combined with microfluidics for a predictive, low-cost development tool for targeted cancer therapy.	Exosomes containing miR-497 suppressed tumor growth and expression of associated genes.	The role of ECM composition in the lung TME and the mechanical strain on the lung remains uncharacterized.	
Pancreatic ductal adenocarcinoma (PDAC)				
Type: heterogeneous tumor invasion Cells: pancreatic cell (PCC) line	A complex and heterogeneous PDAC model was developed by embedding a duct of genetically engineered PCCs surrounded by collagen matrix. Precisely controlled intratumoral heterogeneity was engineered to enable heterogeneous invasion characteristics as a response to TGF-β1 on EMT and local invasion.	Confirmation of TGF-β1 promoting EMT and local invasion in different PCC lines. Study revealed complex interaction between different cancer cells making them more aggressive and invasive. Model can be used to study interaction between heterogeneous patient-derived cancer and stromal cells.	The microfluidic model falls short to incorporate CAFs and other ECM components as a part of pancreatic TME.	[88]
Type: tumor progression Cells: PANC-1 pancreatic cells and pancreatic stellate cells	Microfluidic models using pancreatic stellate cells (PSCs) to study their role in cancer progression and drug resistance. Coculture of pancreatic tumor spheroids (made of PANC-1 cells) with PSCs in 3D collagen matrix to mimic *in vivo* TME to visualize EMT and quantify chemoresistance.	Expression of EMT markers such as vimentin, TIMP1, interleukin (IL)-8, and TGF-β was higher in cocultured PANC-1 spheroids compared with monocultures. PANC-1 cells also showed greater cell motility. EMT leads to drug resistance, mediated by the TME components such as stromal cells and their interactions with cancer cells.	Contradicting results when using different CAFs targeting strategies raises an issue of intratumoral CAF heterogeneity.	[89]
Prostate cancer				
Type: chemotherapeutic response Cells: DU 145 prostate cancer cells	Microfluidic platform representing a 3D TME for rapid evaluation of chemotherapeutic drugs on different cancer cells. Multiple chambers equipped with perfusion channels allows screening of multiple drugs to determine the right combination in a patient-specific manner. Integration of electrical sensing modality with microfluidics to measure electrical (impedance) response of a cancer cell upon exposure through dynamic drug delivery in a 3D matrix environment.	The proposed platform enabled the differentiation between drug susceptible, drug tolerant/resistant cancer cells in less than 12 hours. Impedance measurement of cells seeded in gel provided information on cell growth, morphology, and cell density. Potential to study patient-derived samples for personalized medicine in a rapid, low-cost approach for real-time drug screening analysis.	Polydimethylsiloxane (PDMS)-based microfluidic devices have the disadvantage of being porous to molecules less than 500 Da. These molecules can be absorbed creating a difference in the concentration of drugs.	[90]
Melanoma				
Type: tumor migration Cells: fibroblast and keratinocyte	Equipped with air walls to pattern cells without using conventional hydrogel barriers. Consists of circular chambers with narrow connections. Ability to coculture melanoma cells with keratinocytes and dermal fibroblasts. Air walls dissolve progressively and allows cellular crosstalk and migration. Optical metabolic imaging provided further insights into different metabolic features of different cell lines used.	The presence of dermal fibroblasts and keratinocytes led to changes in melanoma cell morphology and growth pattern. The proposed analysis demonstrated upregulation of multiple secreted factors involved in tumor progression.	The chemokine secretion analysis method does not explicitly identify the cell type responsible for secreting the chemokines. Cellular crosstalk can involve multiple mechanisms such as exosomes, RNAs, or mechanical forces that were not investigated.	[91]

promote single to collective transitions such as cell jamming, the switch between the two being determined by cellular adhesion to the ECM and the extent of actomyosin contractility of the cytoskeleton [28,29]. Confinement of cells in tissues can also cause structural changes in cells. Cells deform into small unstable blebs so they can squeeze through the confinement [30,31]. This change can cause damage to the nuclear envelope, leading to DNA damage and aberrant epigenetic regulation [32]. As such, confinement and changes in matrix porosity can lead to mesenchymal-to-amoeboid transition (MAT) [31,33].

Collagen fiber alignment in conjunction with matrix stiffness regulates cell migration characteristics, such as speed and persistence, and it also affects their ability to migrate collectively [34]. This property is relevant for cell migratory behavior in a 3D matrix environment compared with cells that prefer directional migration on 2D stiffer substrates. Traditional 3D *in vitro* models, such as Boyden Chambers, and heterogeneous tumor spheroid invasion assays compared with *in vivo* models are inexpensive, require less expertise to operate, and can better recapitulate cell–cell/cell–ECM interaction when embedded in hydrogel matrices [35]. Cell migration behavior observed using 3D cell culture and 2D microfluidic models have provided insights into more guided cell trajectory in response to a biochemical signal or to analyze cellular response by mimicking vascular networks using micro-confinement geometries [36,37]. In conventional 2D/3D culture models, cancer cells are frequently grown on top or within synthetic ECM as monolayers. However, they fail to capture the behavior of cell–cell and cell–matrix interactions present in the 3D cellular architecture of a tumor tissue [38]. These classical *in vitro* culture models lack the ability to include fluid flow or shear stress that drives tissue deformation and transport across vascular endothelium, all observed in an *in vivo* TME [4]. Due to these limitations, these models fall short in recreating relevant physical cues, chemokine signals, or hypoxic gradients present in the TME.

Microfluidic models can incorporate biochemical and biomechanical factors by replicating tumor stroma in a controlled microenvironment. Recently, a microfluidic assay was employed to study the role of collagen densities and the biochemical signal, TGF-β, on the migratory behavior of H1299 spheroids [established from a non-small cell lung cancer cell line (NSCLC)] (Figure 2A) [39]. This programmable platform provides controllable conditions to correlate migrating mechanisms from single to collective migration with varying degrees of micro-confinement. Increasing collagen density inhibited single cell migration and promoted clustered migration patterns, whilst TGF-β stimulation promoted cell detachment and single cell migration. Furthermore, migrating cells in high-density collagen matrices showed an increase in strand-like collective motility on encountering TGF-β (Figure 2Aii and iii)]) [39]. Jamming transitions under static conditions have also demonstrated that high tissue density or increased confinement can regulate collective migration by driving EMT in epithelial cancer cells via mechanotransduction pathways [28,39]. When large cell populations encountered enriched matrices, they moved as multicellular sheets or strands, forming weak cell–cell junctions due to EMT. Moreover, the matrix was proteolytically degraded to create migration tracks. It has been proposed that cooperation between homotypic cancer cells increases migration efficiency and directional persistence and minimizes the energy costs needed compared with individual cell migration, and improves cancer cell survival when invading into neighboring tissues [28,39,40].

The influence of mechanical confinement and substrate topology are important factors in determining the role of tumor heterogeneity in migratory behavior during cancer cell invasion and progression [37,41,42] (Box 2). To investigate such complex tumor–stroma interactions, a novel 3D bio-microfluidic platform was designed to implement microchamber arrays that replicate the hollow mammary glands that are a part of the tumor microstructures [43]. MCF-10A (normal breast epithelium) cell cultures in the microchamber arrays resulted in the formation of

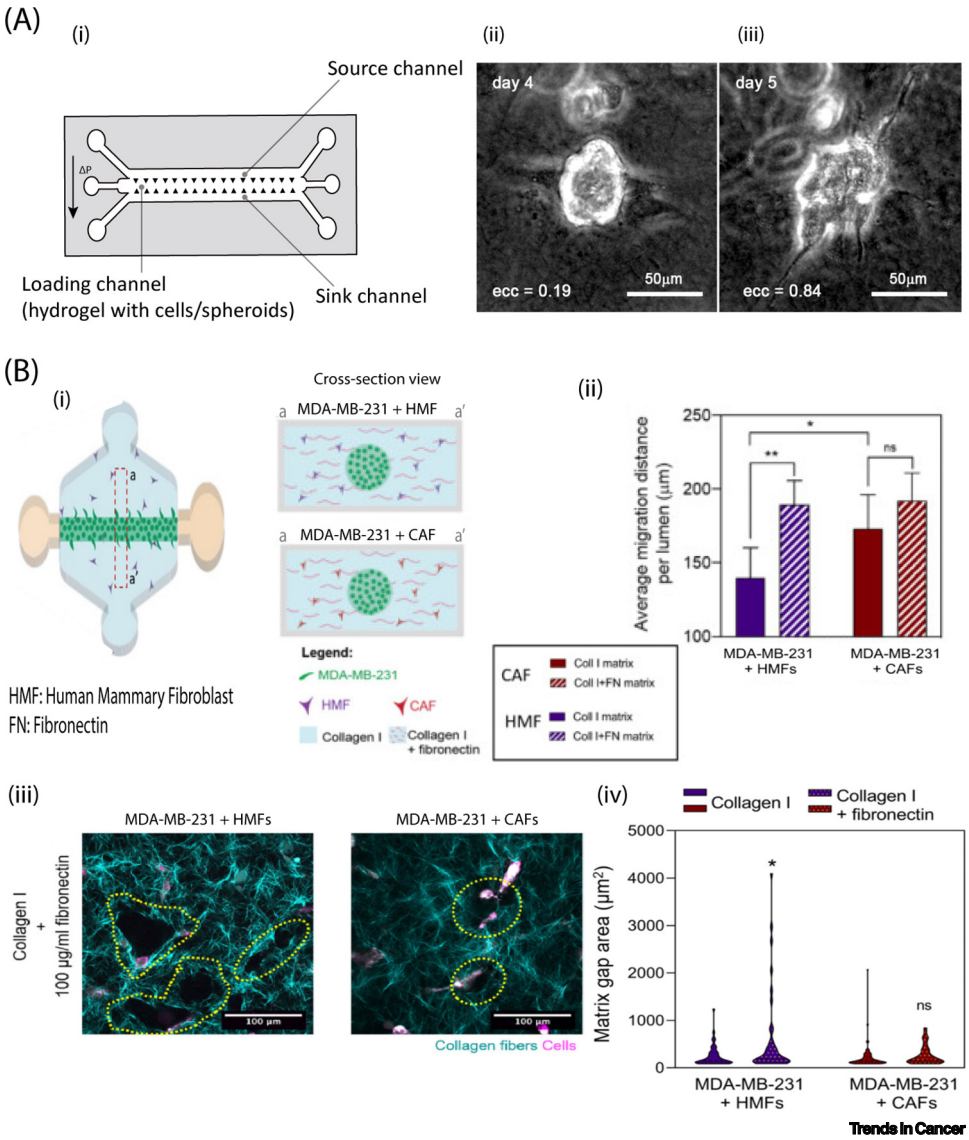

Figure 2. 3D matrix-based microfluidic models with physiologically relevant tumor microenvironment (TME) features. (Ai) A microfluidic device with a three-channel geometry. The source and the sink channel ensure proper hydration and study the impact of interstitial flow (IF) on cell migration. This model is very commonly used to study the effect of different cytokines on cell behavior. (Aii) Bright field image of transforming growth factor beta (TGF-β)-treated H1299 cells in a cluster-like morphology after 4 days, (Aiii) same cluster with strand-like morphology after 5 days [39]. (Bi) A 3D matrix-based microfluidic coculture model to recapitulate the TME with incorporation of fibronectin in collagen matrix and human mammary fibroblasts (HMFs) or cancer-associated fibroblasts (CAFs), (Bii) quantitative analysis of the average migration distance of metastatic cells (MDA-MB-231) from the edge of the lumen after 48 hours when cultured in the presence of HMFs or CAFs and in the presence of collagen without or with fibronectin. (Biii) Second harmonic generation (SHG) imaging of collagen fibers depicted in cyan and cells in magenta. Degradation of the collagen matrix (matrix gap area) in the presence of HMFs and CAFs highlighted by yellow broken line [63]. (Biv) Quantification of matrix gap areas for the conditions in (Biii) using violin plot representation [63]. (Aii) and (B) are reprinted and adapted from [39,63] licensed under CC by 4.0. * denotes a significant difference for $P \leq 0.05$ and ** for $P \leq 0.01$.

cellular aggregates that covered the entire microchamber area. These aggregates with tight cellular adhesions led to the formation of lumen-like structures mimicking epithelial cells that surround the basement membrane. Cocultures of MCF-10A with MDA-MB-231 (a highly invasive triple-negative

Box 2. CAFs and ECs in the TME

Cancer is a highly heterogeneous disease caused by multiple distinct (epi)genetic alterations in epithelial cells [76]. During cell proliferation, intratumoral heterogeneity can arise due to genetic, epigenetic, or proteomic changes in different cells present within a tumor [76]. In addition, cell-extrinsic factors, such as the local varying TME, hypoxia in the tumor core, residing and infiltrating immune cells, ECs, and CAFs, can contribute to intratumoral heterogeneity [76]. In addition to cancer cells, TME cells display heterogeneity in function, behavior, and origin. For example, the term 'CAFs' describes all activated fibroblasts found within the TME, regardless of their location, phenotype, or function [58]. Recent data show distinct CAF subtypes and subpopulations within the tumor that appear as cancer progresses over time [57,77]. These CAF subtypes have unique roles within the TME and are plastic, and one subtype can differentiate into another subtype [57,58]. Forming heterophilic cadherin junctions with cancer cells to lead collective invasion, CAFs can also intricately deposit fibronectin to remodel and produce a fibronectin-rich ECM to promote migration [61]. Intratumoral heterogeneity and signaling cascades between cells (including tumor cells, immune cells, and CAFs) are exacerbated by the presence of growth-induced stresses, IFP, and flow. This further activates mechanotransduction signaling pathways within the TME and promotes intercellular crosstalk between CAFs, immune cells, and tumor cells by creating chemokine gradients [78,79].

Hypoxia is prevalent in the tumor core and is a key driver of angiogenesis, one of the most vital stages of cancer progression [80]. In adults, ECs are quiescent and have low proliferation rates; however, cancer tumors can induce angiogenesis to sustain themselves, grow and eventually metastasize [81]. In cancer cells, the activation of oncogenes and mutations in tumor suppressor genes cause increased expression of VEGF which in turn activates ECs through paracrine signaling and stimulates their migration, proliferation, and ultimately results in angiogenesis [81]. There exists a high degree of heterogeneity within ECs obtained from the TME [81,82]. Combining microfluidics with hydrogel scaffolds to incorporate CAFs or ECs with cancer cells provides a unique platform to investigate cell migration characteristics, the role of CAFs/ECs within the TME, and other stages of the metastatic cascade such as tumor dissemination, intravasation, and extravasation.

breast cancer cell line), however, inhibited cluster formation [43]. The inability of MCF-10A cells to form tight cell adhesions was attributed to secretion of matrix-metalloproteases (MMPs) by MDA-MB-231 cells. MMPs mediated the cleavage of E-cadherin on MCF-10A cells, which resulted in poor cell–cell adhesion that prevented cluster formation. The microfluidic channels allow for gradients of biochemical signals, that is, a range of concentrations of MMP inhibitors induced dose-dependent effects on MCF-10A cluster formation. The model, equipped with microchambers surrounded by collagen hydrogel, mimicked the TME and showed the importance of surrounding cell structures (i.e., epithelial layer) and basement membrane. The microfluidic model recapitulated the characteristic property of lumen-like structures around the basement membrane that prevented metastatic cells from intravasating into deeper ECM areas.

Microfluidic models incorporating hydrodynamic flow

Cells can sense IF and fluid shear stress (or pressure) and change cellular behavior [44]. Fluid flow and shear stress dynamically influence activation or inhibition of a series of mechanosensitive molecules, such as insulin growth factor (IGF)-2, vascular endothelial growth factor (VEGF), Rho-associated kinase (ROCK), and caveolin (CAV)-1. IF and shear flow are crucial migratory factors for cancer cell invasion [45,46]. Flow can trigger increased migration of cells via activation of surface receptors, such as the chemokine receptor CXCR4 and CCR7 [47]. Autologous chemotaxis, induced by a transcellular chemokine gradient, is responsible for driving directional cell migration in the presence of IF [48]. The most significant effect of IF, elevated IFP and solid stresses, leads to constricted and leaky vasculature, which impacts on drug delivery and the collapse of these vessels, which eventually causes hypoxia. These biophysical factors are important in inducing tumor migration events, which are not addressed in conventional *in vitro* culture models. The first step is to introduce biomimetic and natural matrices to recapture the native architecture of the ECM. To address physical stresses in the TME, a biomimetic ECM must be equipped with perfusion (or relevant IF rates). In recent years, the development of 3D matrix-based microfluidic devices as 3D *in vitro* cell migration platform addresses the limitations to integrate biophysical cues. Such studies have highlighted the role of 3D microfluidics and the importance of mechanical forces from ECM remodeling and IF in cancer cell migration events [45,49,50].

Microfluidic models can generate flow rates and shear stress mimicking those found within tumor tissues. For example, increased interstitial and shear flow can lead to morphological and epigenetic changes that can result in a more aggressive behavior of breast cancer cells [45,49]. Recent microfluidic models have highlighted the importance of flow-induced hydrodynamic shear stress on EMT in A549 (lung epithelial adenocarcinoma) cells cultured in a 3D microfluidic platform for several days [51]. Striking phenotypic changes were observed in A549 cells within the device when stimulated with shear flow compared with static cultures (no flow). Shear flow and IFP in solid tumors have been implicated in reducing the efficacy of cancer therapeutics [52]. Indeed, when the effect of flow on the efficacy of drugs on A549 cells was investigated, distinct cytotoxic effects were observed compared with static models, highlighting the role of flow in response to drugs [51]. A downregulation of E-cadherin and an increase in vimentin and N-cadherin expression was observed, which is indicative for EMT, a mesenchymal cell phenotype that is frequently associated with therapy resistance [51]. This dynamic microfluidic model serves as a drug screening tool for different cancer types.

IF-induced downregulation of cadherins has also been implicated in increased tumor invasion as shown in a heterogeneous spheroid model consisting of MDA-MB-231 and MCF10A cells embedded in a collagen type I matrix (Figure 3B) [49]. In no-flow experiments, the heterogeneous spheroid does not show any disintegration, and only peripheral MDA-MB-231 cells migrate (Figure 3Bii and iii). When IF was applied, the reduced E-cadherin expression on MCF-10A cells led to the dissociation of MDA-MB-231 cells from the spheroid core (Figure 3Bii and iv). Designed to operate under a flow rate of 2 μm/s, lower than the elevated IF rates observed in animal models (up to 9 μm/s) or human patients (up to 55 μm/s), this platform highlights the importance of biophysical parameters in the TME [49]. This effect is a characteristic of morphological change that occurs if cells switch from a mesenchymal to an amoeboid migration mode. In cells, structural rearrangements are determined by time-dependent adhesion interactions and long-range hydrodynamic interactions along with actin remodeling. In the presence of flow, amoeboid motility is triggered by the lack of molecules such as the fibronectin, which fails to form long-lived adhesions with collagen fibers [53] (Box 1).

Shear flow is a key regulator of cancer cell intravasation. Cancer cells preferentially intravasate in areas of low shear flow as high shear flows can destroy the cells [54,55]. A microfluidic platform was developed to investigate the mechanism of intravasation and the way in which cells detect shear flow. Cells migrated inside longitudinal microchannels with an orthogonal channel such that cells would encounter an active fluid flow to mimic intravasation [55]. The shear stress sensed by cells due to fluid flow activated the transient receptor potential melastatin 7 (TRPM7), which promoted the influx of extracellular calcium ions and led to the reversal of migration direction via RhoA/Myosin-II and CDC42 pathways [55]. This study shows that cancer cells with high TRPM7 expression are not able to intravasate into blood vessels, thereby reducing the probability of metastasis [55]. Interstitial and shear flow studies using microfluidic models have improved our understanding of the migratory behavior of cells in the presence of flow and cellular plasticity as influenced by mechanical stimuli.

Microfluidic models incorporating cell–cell interactions

As cells migrate collectively into healthy tissues, the dynamic reciprocity between the secreted cytokines and their cell surface receptors governs cell migration. Intercellular signaling cascades between tumor cells and other non-cancerous cells (CAFs, stromal cells, and immune cells) further contribute to tumor progression events [2,56]. CAFs are frequently one of the most prominent and active components in the TME. While their origins remain obscure, recent data suggest that distinct subpopulations of CAFs exist within the tumor and their roles evolve and change as

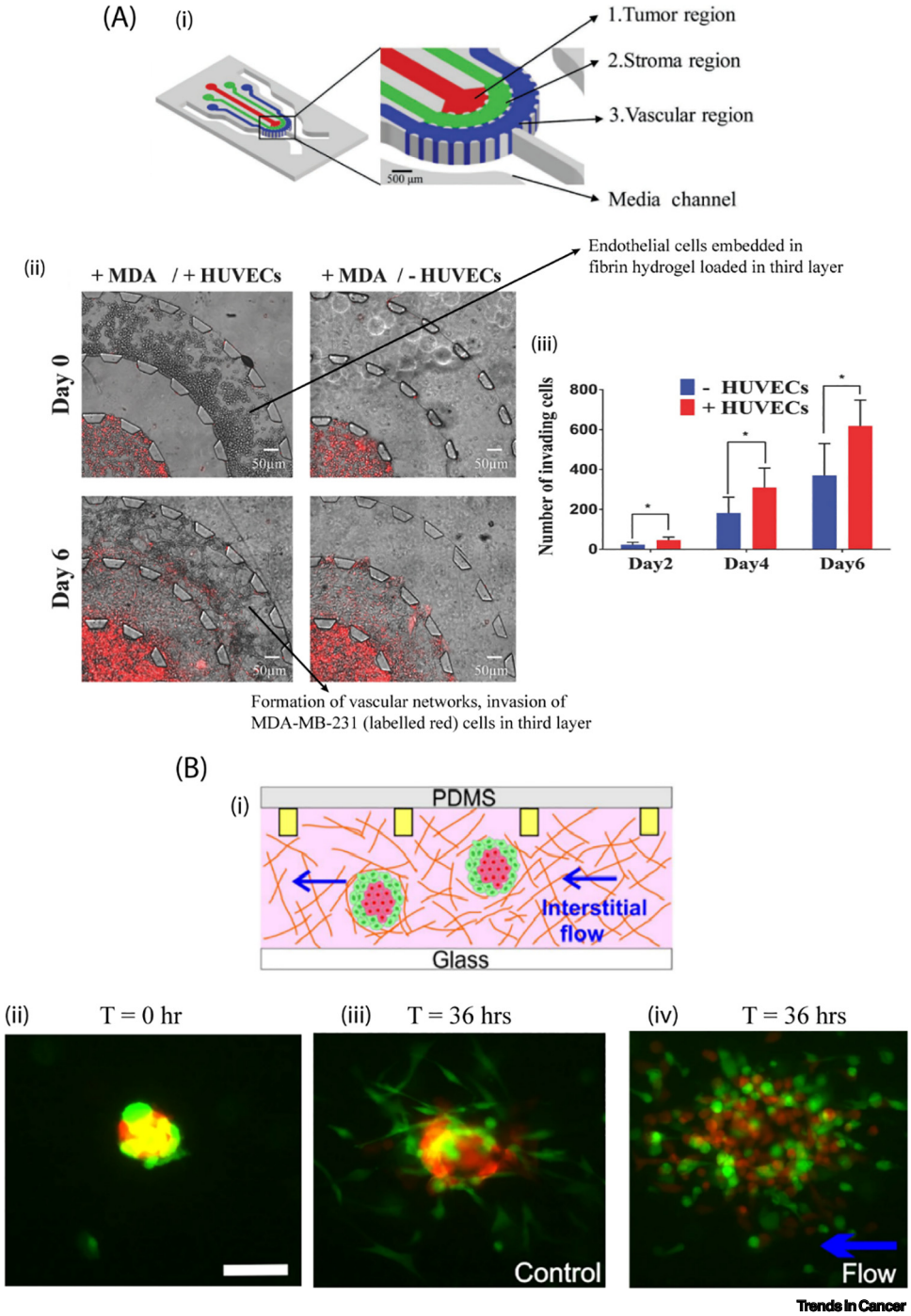

Figure 3. 3D microfluidic models to study and investigate invasion and intravasation events. (Ai) Schematic of multilayered tumor–stroma microfluidic model. The microfluidic model consists of three layers: 1. tumor region: consisting of MDA-MB-231 cells embedded in collagen hydrogel, 2. stromal region: to introduce tumor–stroma crosstalk, 3. vascular region: endothelial cells embedded in fibrin to produce vascular networks. (Aii) MDA-MB-231 breast cancer cells (red fluorescence) invade the stromal region from the tumor region with simultaneous formation of vascular networks by the end of day 6. (Aiii) Quantitative analysis of MDA-MB-231 breast cancer cells invading into the tumor stromal region in the

(Figure legend continued at the bottom of the next page.)

the disease progresses [57,58]. CAFs, in some cancers, have been shown to lead to collective migration from the front by forming heterophilic junctions with cancer cells [59–61]. Through secreted cytokines, epigenetic reprogramming, and signaling cascades within the TME, CAFs have been implicated in ECM remodeling, the creation of a hypoxic microenvironment, and the alteration of the metabolic state of tumor cells, resulting in an acidic microenvironment [28,60–62]. Tumor angiogenesis is key to cancer progression and metastasis. The creation of a hypoxic TME causes the secretion of VEGF, a key angiogenic factor. VEGF activates ECs through paracrine signaling and stimulates cell migration and proliferation of ECs, eventually resulting in the induction of angiogenesis.

3D microfluidic models have been developed to recapitulate TME heterogeneity by introducing CAFs as ECM remodeling components that secrete MMPs. Stromal cells play a key role in the dynamic nature of the TME with an active influence on remodeling, which controls physical properties such as stiffness, viscoelasticity, and pore size of ECM [34]. For example, a 3D matrix-based microfluidic platform was developed to colocalize MDA-MB-231 embedded in fibronectin-rich collagen matrix as a lumen-like geometry with fibroblasts to recreate a 3D tumor-stroma model (Figure 2B). The microfluidic model incorporates human mammary fibroblasts (HMFs) and CAFs, thereby assessing the impact of cancer-stromal crosstalk on the migratory behavior of MDA-MB-231 cells [63]. In the first set of studies, fibronectin-rich matrix showed a qualitative increase in the number of cells migrating, regardless of the type of fibroblast present (Figure 2Bii). A second set of studies were performed to observe the effect of HMFs and CAFs on the surrounding matrix degradation due to the secretion of MMPs shown in Figure 2Biii and iv. Matrix degradation was quantified based on the gaps formed that are indicative of structural deformation as assessed by fluorescence imaging. In a TME consisting of fibronectin-rich collagen embedded with HMFs, invasive human breast cancer (MDA-MB-231) cells migrated faster and for longer distances. The fibronectin-rich matrix signals HMFs to produce and secrete MMPs, which are responsible for ECM degradation [63]. These results indicate the influence of biochemical factors inside the TME (i.e., fibroblasts and ECM protein compositions) that directly induces matrix degradation, which results in a biomechanical response. Therefore, microfluidics, with its extensive modeling parameters, allows the incorporation of different cancer cell types and other TME-related biological components to recreate key components of a tumor model. The model provides a platform to study the effect of different chemokines and a combination of matrix materials for a comprehensive understanding of certain metastatic events.

A model developed to simultaneously study cell invasion and intravasation integrates a 3D multilayered microfluidic platform in the tumor stroma with MDA-MB-231 cancer cells embedded in a collagen hydrogel. In this model, MDA-MB-231 cells had increased migratory behavior, and they invaded the stromal region in the presence of vascular networks, which mimicked *in vivo*-like capillaries on day 0 compared with day 6 (Figure 3Aii) [64]. The unique advantage of this model is the spatial control on the 3D tumor, stroma, and the vascular network region that allows for the formation of *in vivo*-like capillaries by human umbilical vein

presence and absence of human umbilical vein endothelial cells (HUVECs) [64]. * indicates a significant difference for $P < 0.05$. (Bi) cross-sectional view: channel filled with collagen hydrogel embedded with spheroids of cocultured MCF-10A normal breast cells and MDA-MB-231 breast cancer cells. (Bii–iv) (enlarged) fluorescence images of MDA-MB-231 cells (in green), MCF-10A (in red), scale bar 100 μm. (Biii) Control experiments (no-flow) do not promote spheroid dissociation, only peripheral MDA-MB-231 cells invaded outwards. (Biv) after application of 36 hours of interstitial flow, tumor dissociation is induced and is demonstrated by the disintegration of the spheroid core [49]. (A) is reproduced from [64] with permission from © 2018 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (B) is reprinted and adapted from [49] licensed under CC by 4.0. Abbreviation: PDMS, polydimethylsiloxane.

endothelial cells (HUVECs) embedded in relevant biomimetic ECM matrices. The presence of HUVECs led to the enhanced invasion of MDA-MB-231 cells and the degree of invasiveness was quantified as shown in Figure 3Aiii. The microfluidic design serves as a novel platform to study interactions and biological mechanisms between cells and their TME for invasion and intravasation studies. While the collagen and fibrin matrices used in the study are physiologically relevant, the adaptability of microfluidics enables the incorporation of patient-derived primary tumor cells and decellularized ECM to increase the accuracy of 3D matrix-based microfluidic models [9].

Concluding remarks

3D matrix-based microfluidic models allow for more precise physiological representation and recreation of the TME, enabling us to determine the dominant mechanism of cancer cell migration events under conditions mimicking more closely the aspects of *in vivo* situation. By combining relevant physicochemical parameters in a tumor–stroma model, the extent of specific roles of ECM, tissue heterogeneity, chemical signals, biomolecules, and mechanical forces can be explored. In recent years, microfluidic models were able to critically interrogate the influence of biochemical and biomechanical factors in tumor progression events. In addition, the advantage of real-time imaging makes this platform highly suitable to visualize dynamic processes and distinguish between single or collective migratory behavior displayed by tumor cells. The ability to select synthetic biomaterials that can be tuned for properties such as stiffness and pore size and to promote cell–matrix interactions gives an added advantage in decoupling interdependent factors for an in-depth revelation to interpret cell migration mechanisms.

This review focuses on recent advances in microfluidic models to study the TME and the migration mechanisms of cancer cells (mostly breast and lung cancer) involved in tumor metastasis events, such as dissemination and invasion. Based on the type of tumor and influencing factors, including CAFs, ECM, TGF-β, and IF, the modes of cancer cell migration differ from each other. These emerging results demonstrate the importance of microfluidic models to determine preclinical therapeutic responses. By consolidating complex features of TME inside microfluidics, we can implement crucial physical and chemical cues (associated with different cancer types) that may enable more relevant drug screening platforms than could be achieved using static models. In addition, it has been confirmed that the increase of vascular density leading to enhanced mechanical forces during tumor growth can drastically diminish the delivery of drugs and oxygen supply at the inner region of the tumor [65].

There exists an opportunity to replace animal models preceding clinical trials for drug screening and anticancer therapeutics in the next decade by establishing specific quality controls for such 3D *in vitro* models. To fully identify the potential of 3D-microfluidic approaches in cancer metastasis modeling and clinical trials, several key challenges and issues must be confronted (see Outstanding questions). To achieve this vision, microfluidics needs to undergo considerable upgrades by integrating it with cutting-edge and complementary research tools such as 3D bioprinting, engineered tissue constructs, artificial intelligence, and computational modeling. This integration will create next-generation robust microfluidic models with a vision for personalized therapies by using patient-derived cells and mimicking patients' *in vivo* TME conditions. In the future, 3D microfluidic models will become more refined and equipped with automation functionalities and artificial intelligence algorithms to automatically identify crucial factors in tumor progression events, improving our understanding of cancer invasion and discovering a new class of drugs for cancer treatment. Therefore, microfluidics will become an ideal analytical tool to systematically investigate these complex and coupled features. Moreover, various types and combination of drugs can be tested to select the most suitable therapeutics for each cancer patient.

Outstanding questions

How can we adopt microfluidic models further to replicate nearly all of the complexities of the TME?

Will the incorporation of microfluidics in multicellular 3D models enable us to recapitulate the multiple steps of the metastatic process, from initial invasion into adjacent tissue, intravasation, survival in circulation, and extravasation to outgrowth in distant tissue?

What next steps are needed for microfluidic models to be utilized in routine clinical studies for drug screening and personalized cancer therapy?

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Declaration of interests

No interests are declared.

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