
Article:
Jackson, C.E. orcid.org/0000-0002-4971-144X, Green, N.H., English, W.R. et al. (1 more author) (2024) The use of microphysiological systems to model metastatic cancer. Biofabrication, 16 (3). 032002. ISSN 1758-5082

https://doi.org/10.1088/1758-5090/ad3b70

TOPICAL REVIEW • OPEN ACCESS

The use of microphysiological systems to model metastatic cancer

IOP Publishing Biofabrication 16 (2024) 032002 https://doi.org/10.1088/1758-5090/ad3b70

Biofabrication

OPEN ACCESS

RECEIVED
13 November 2023

REVISED
29 February 2024

ACCEPTED FOR PUBLICATION
5 April 2024

PUBLISHED
18 April 2024

TOPICAL REVIEW

The use of microphysiological systems to model metastatic cancer

Caitlin E Jackson[1,2], **Nicola H Green**[1,2], **William R English**[3] **and Frederik Claeyssens**[1,2,*]

[1] Materials Science and Engineering, The Kroto Research Institute, University of Sheffield, Sheffield S3 7HQ, United Kingdom
[2] Insigneo Institute for In Silico Medicine, The Pam Liversidge Building, University of Sheffield, Sheffield S1 3JD, United Kingdom
[3] Norwich Medical School, University of East Anglia, Norwich NR3 7TJ, United Kingdom
[*] Author to whom any correspondence should be addressed.

E-mail: f.claeyssens@sheffield.ac.uk

Keywords: cancer, metastasis, micro physiological systems, lab-on-a-chip, microfluidic

Abstract

Cancer is one of the leading causes of death in the 21st century, with metastasis of cancer attributing to 90% of cancer-related deaths. Therefore, to improve patient outcomes there is a need for better preclinical models to increase the success of translating oncological therapies into the clinic. Current traditional static *in vitro* models lack a perfusable network which is critical to overcome the diffusional mass transfer limit to provide a mechanism for the exchange of essential nutrients and waste removal, and increase their physiological relevance. Furthermore, these models typically lack cellular heterogeneity and key components of the immune system and tumour microenvironment. This review explores rapidly developing strategies utilising perfusable microphysiological systems (MPS) for investigating cancer cell metastasis. In this review we initially outline the mechanisms of cancer metastasis, highlighting key steps and identifying the current gaps in our understanding of the metastatic cascade, exploring MPS focused on investigating the individual steps of the metastatic cascade before detailing the latest MPS which can investigate multiple components of the cascade. This review then focuses on the factors which can affect the performance of an MPS designed for cancer applications with a final discussion summarising the challenges and future directions for the use of MPS for cancer models.

1. Introduction

Cancer is one of the leading causes of death worldwide, presenting as an ever current, ongoing challenge and one of the most significant barriers to increasing life expectancy in the 21st century [1, 2]. Cancer has a huge social and economic burden on modern society, with an estimated 1 in 3 people to be diagnosed with cancer within their lifetime [3]. To overcome the multitude of challenges cancer poses, many different disciplines of research have come together worldwide to further develop techniques for prevention, diagnosis and treatment.

Moreover, a significant challenge faced by cancer researchers in this field is the need for appropriate platforms to test potential therapies and to improve the ease and efficiency of drug development. For studies of metastasis, the current standard is to use *in vivo* animal models, predominantly mice. These provide biophysical conditions comparable to human tissue and can replicate all aspects of the metastatic cascade or select steps. For example, metastasis can be studied from primary tumours to the secondary, metastatic site through implantation of cancer cells, tumour tissue or the use of genetically engineered mouse models or via transit through the circulation to secondary sites by intravenous or intracardiac injection [4–6]. However, there are high cost, time and ethical implications associated with animal studies. Furthermore, there is a very low success rate (8% on average) of translation to clinical cancer trials from animal studies [7]. One of the most significant problems to overcome in the use of *in vivo* models is their limitations in the replication of heterogeneity of cancer in the clinical setting. Even a single cancer type, defined histologically in combination with molecular markers, will show significant levels of inter patient and intra tumoral molecular heterogeneity that has a significant impact on response to therapy and propensity to metastasise in an individual patient [8]. This is driving changes in the approach to cancer drug discovery to develop model systems that

can rapidly assess the impact of cancer heterogeneity on drug response and ultimately improve the probability of successful translation into the clinical setting.

Microphysiological systems (MPS) are rapidly developing to model a range of both healthy and diseased tissues, including cancer and more specifically the metastatic cascade. There are a vast number of models being developed to focus on many aspects of cancer [9–13], including cancer type, progression, and different steps of the metastatic cascade [14–18].

This review introduces MPS, cancer metastasis and the metastatic cascade before continuing to discuss each step of the metastatic cascade in depth, highlighting the gaps in the current knowledge and understanding of the cascade. Furthermore, within this we explore MPS which have been developed to focus on investigating the individual steps of the metastatic cascade. Further discussion moves into detailing the latest MPS which can investigate multiple components of the cascade, summarising the key strengths and weaknesses of each design. Factors which can affect the performance of an MPS are then discussed. Finally, this review highlights the crucial design requirements that must be met and challenges that must be overcome to better recapitulate *in vivo* conditions within *in vitro* models to ensure wider adoption of MPS.

2. MPS

Cells *in vivo* have a three-dimensional (3D) geometry, supported by a complex extracellular matrix (ECM). However, cells *in vitro* are commonly cultured in two-dimensional (2D) monolayers. Whilst 2D assays provide quick, easily repeatable and simple models, they lack the complexity to recapitulate *in vivo* microenvironments. The lack of cell-cell and cell-matrix signalling pathways reduces physiochemical cues, resulting in a negative effect on cell identity and behaviour, and further impacting cell growth and function [19, 20]. However, *in vitro* models that can perform to a similar standard to *in vivo* models result in reductions in cost, time and ethical challenges that are normally associated with *in vivo* models. MPS, also known as complex *in vitro* models, lab-on-a-chip or organ-on-a-chip models, are rapidly developing to mimic human physiology and disease [21, 22]. MPS models can study cell-cell interactions and cell-ECM interactions within a 3D microenvironment with an improved semblance of *in vivo* biophysical and chemical properties. There are many different designs for MPS which can be divided into 3 themes; single chip designs, well-plate designs and connected chamber designs (figure 1). 3D models can also be divided based on the culture conditions into 3 subclasses: suspension cultures or non-adherent plates, cultures in gel-like medium and cultures on scaffolds [23]. Many 3D models commonly use scaffolds (natural or synthetic) combined with human cells to support the growth and expansion of new tissue structures [24]. In addition, many include the use of growth factors or biophysical conditions to further enhance cell growth within the scaffold and integration. Cells, scaffolds and growth factors/growth stimulating conditions are often referred to as the tissue engineering (TE) triad [25].

3. Cancer and metastatic cells

Metastasis is the detachment, migration and colonisation of tumour cells from the bulk of the primary mass to a secondary site, either in the surrounding or distant tissue from the primary site [26]. Due to such a large association with cancer mortalities, it is important to gain a deeper understanding of cancer metastasis and identify potential drug targets to reduce it.

Metastatic cells originate from mutated cells (tumour cells). Cellular mutations may occur spontaneously during mitosis due to instability in base pairs or DNA replication errors. Alternatively, exposure to environmental factors may induce mutations, such as ultraviolet light or chemical carcinogens [27–29]. Most mutated cells undergo apoptosis (pre-programmed cell death). However, in a small number of cases, apoptosis is suppressed and the mutated cells proliferate at an unregulated, increased rate, forming a neoplasm which may develop into a tumorous mass [30]. In addition, the process is further accelerated if stromal cells are affected by the mutation. This is due to increased expression of growth factors within the tumour, such as transforming growth factor, and vascular endothelial growth factor (VEGF) [31]. Increased VEGF supports the formation of vascular networks, further supporting and accelerating the growth of a tumour by providing a source of oxygen and nutrients [32].

Often within cell culture there is a tendency for the blanket use of growth factors across cell systems, however this is not fully recapitulative of the *in vivo* environment in which growth factors are discreetly secreted by cells due to specific stimulus. Meng *et al* developed a model in which the release of vascular endothelial and epidermal growth factors could be reliably triggered via laser irradiation [33], giving greater control and regulation of the concentration of growth factors and thus the directional stimulation of angiogenesis within the system. The model consists of a central vessel within a fibroblast-laden hydrogel matrix which also contained a tumour cell droplet and 3D bioprinted stimuli-responsive microcapsules containing the growth factors. The model used epidermal growth factor (EGF) and VEGF to stimulate cancer cell migration and angiogenic sprouting respectively. At 9–12 d cancer cells could be observed within the main vessel, demonstrating cancer cell

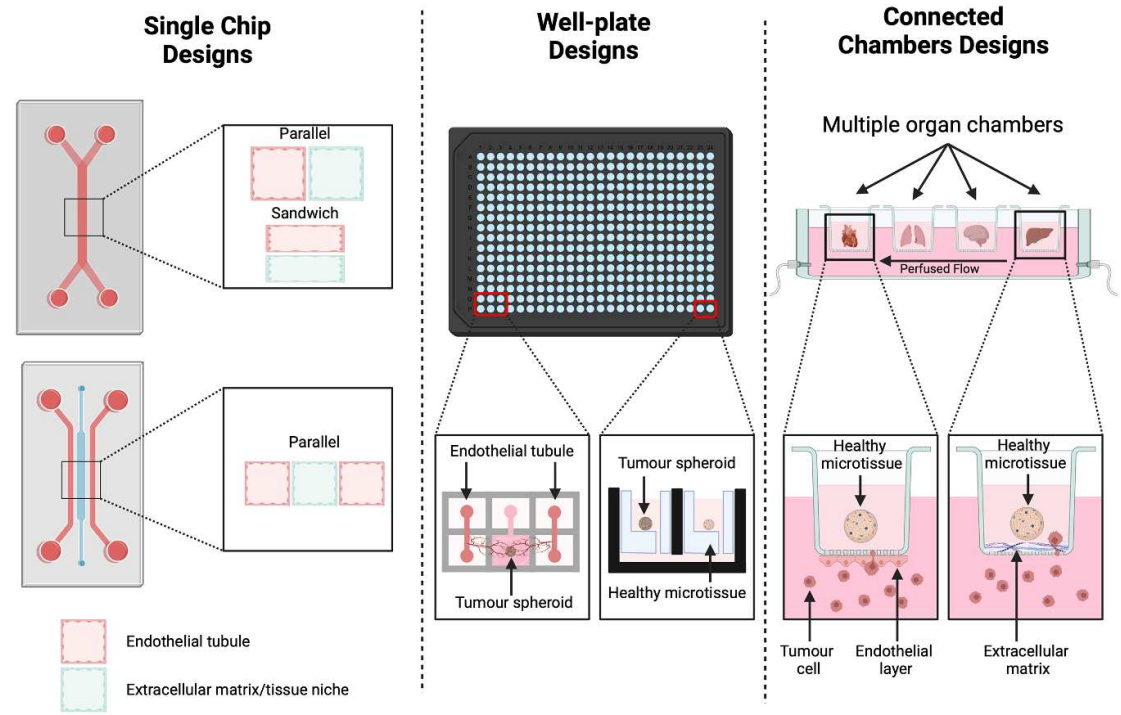

Figure 1. Simplified diagrams demonstrating examples of microphysiological system designs within the 3 different themes; single chip designs, well-plate designs and connected chambers designs. Single chip designs consist of a single experimental replicate on each chip, recapitulating one tissue microenvironment. Well-plate designs consist of multiple chips on a standard 384 well-plate, with each chip consisting of 2–6 interconnected wells depending on the specific MPS design. Often these two designs consist of 2–3 parallel channels, in which at least one channel is lined with endothelial cells to create an endothelial tubule adjacent to the tissue niche. Connected chamber designs consist of $\geqslant$2 chambers, designed to each represent different tissue microenvironments connected via fluid flow, these designs often incorporate an endothelial and/or ECM barrier between the chamber and perfused flow. Created with BioRender.com.

intravasation had occurred. Furthermore, these cells could be observed travelling within the fluid flow as circulating tumour cells (CTCs) and as such could be collected in chambers and analysed. Whilst the concentration of growth factors could be reliably controlled, this artificial release of growth factors does not recapitulate the *in vivo* mechanisms for the controlled release of growth factors by the cells in the tumour microenvironment (TME).

Metastatic cells demonstrate high levels of heterogeneity within cell populations [34]. There are 3 mechanisms described in literature to explain the high levels of heterogeneity within metastatic populations: (i) clonal evolution, (ii) the cancer stem-like cells (CSC) model and (iii) the cancer stem-like cell plasticity model [35]. Clonal evolution describes a mechanism which follows the basic principle of Darwin's evolutionary theory, in which there are genetic and/or epigenetic changes in individual cells within populations which result in natural selection of the 'fittest' clone [36]. The model suggests there are numerous occurrences of mutations with successive clonal dominance, otherwise known as selective sweeps, in which the dominant clone is the one which can best survive and thrive within the specific TME [37]. The CSC model describes a sub-population of cancer cells which possess stem-like properties and functions [38], including self-renewing abilities that can initiate and maintain long-term tumour growth [35]. The CSC plasticity model proposes that clonal evolution and the CSC model are not mutually exclusive [39]. It is suggested that due to the high plasticity of cancer cells they are capable of transitioning between non-CSC and CSC states depending on the specific chemical and mechanical cues from the TME [40].

4. The metastatic cascade

Metastasis of primary tumours occurs via three routes: blood vessels, lymphatic vessels, and serosal surfaces [41]. In addition, cancer can be divided into three categories based on their cell lineage: carcinomas (epithelial), sarcomas (mesenchymal) and leukaemias and lymphomas (blood and lymph tissue respectively) [42], each of which metastasise via different routes. Most cancers (90%) are carcinomas, which commonly metastasise via lymphatic vessels. Sarcomas, bone and soft tissue malignancies metastasise via blood vessels and few malignancies metastasise via serosal surfaces, i.e. the outer lining of organs and body cavities of the abdomen and chest [43].

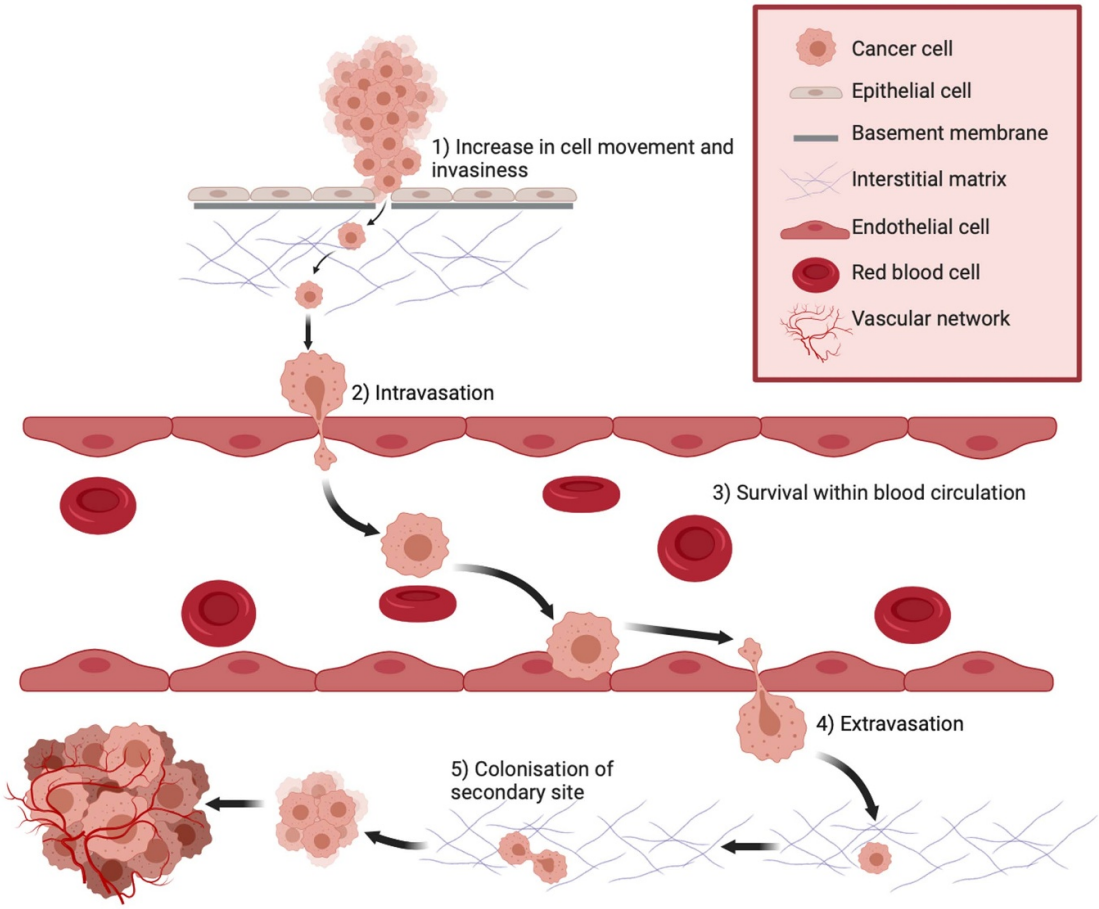

Figure 2. Cascade of steps outlining the process of cancer cell metastasis. Created with BioRender.com.

The process of metastasis is not yet fully understood. However, it has been defined by a cascade of steps: loss of cellular adhesion, increase in cell movement and invasiveness, intravasation (entry into the circulatory system), extravasation (exit from the circulatory system) and colonisation at a secondary site (figure 2) [30, 44].

4.1. Metastatic cell invasion and migration

Metastatic cell invasion follows the disaggregation of metastatic cells from the primary tumour via the breakdown of cellular adhesions [45]. Within carcinomas the epithelial cells must first undergo epithelial-mesenchymal transition (EMT) before the process of invasion can occur [46, 47]. Undergoing this process leads to a loss of the main epithelial cell adhesion mediator, E-cadherin [45–47]. Complementary to this, Perl *et al* reported evidence of a causal link between the loss of the E-cadherin adhesion mediator and the progression from benign adenoma to malignant carcinoma *in vivo* [48]. Furthermore, through a collagen invasion assay, Frixen *et al* found that E-cadherin expressing cells were largely non-invasive compared to E-cadherin deficient cells [49]. It is important to note that EMT does not always lead towards a fully mesenchymal phenotype and may only involve a partial transition [35]. It is thought sarcomas go through a similar process through development of the metastable phenotype, suggesting underlying mechanisms may be conserved amongst solid tumour types [50]. Aiello *et al* show how partial EMT phenotypes promote collective tumour cell migration and the formation of CTC clusters [51]. Furthermore, due to the high plasticity of cancer cells, EMT is a reversible process and as such allows them to switch between proliferative to invasive phenotypes when necessary [52]. The progression of EMT, whether it be partial or full, impacts tumour growth and metastasis differently depending on the tumour type and stage of tumour progression [35].

During invasion metastatic cells penetrate through the basal lamina of surrounding tissue via adhesion to and digestion of the ECM components, including collagen (type IV) and laminin [44, 53]. ECM degradation is mediated by actin-rich membrane protrusions formed by metastatic cells, known as invadopodia [54, 55]. These protrusions mediate ECM degradation by localised proteolytic activity of matrix metalloproteinases (MMPs) [54]. Liotta *et al*

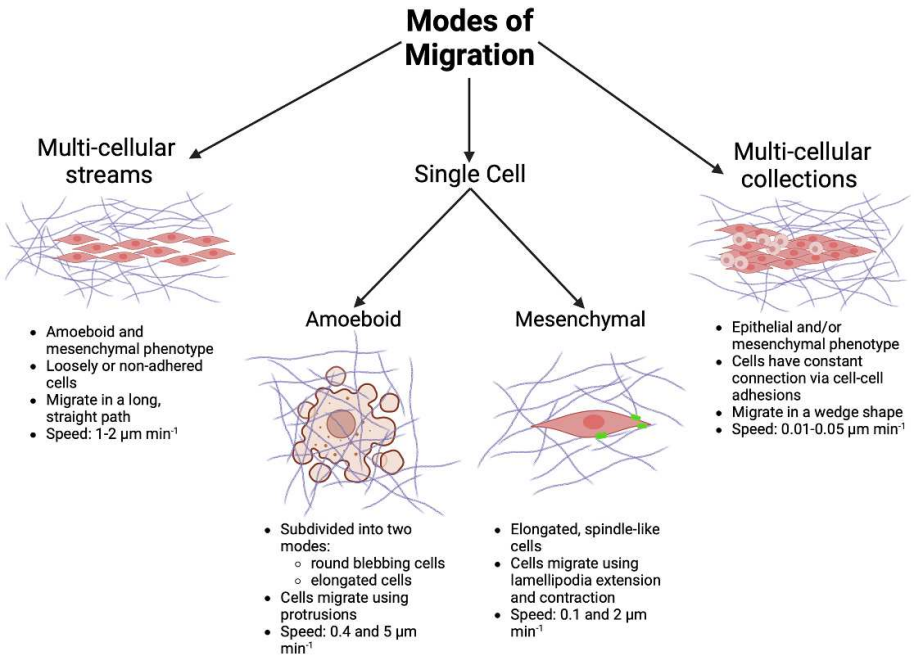

Figure 3. Metastatic cells modes of migration. Single-cell amoeboid migration: blebby, spherical protrusions used to push and squeeze through the ECM. Single-cell mesenchymal migration: Spindle-like cells using strong integrin-mediated adhesions (green) to adhere and migrate through the ECM. Multi-cellular stream migration: loosely- or non-adhered cells migrating in the same direction. Collective migration: epithelial and/or mesenchymal phenotypes migrate in the same direction with constant cell-cell contact. Created with BioRender.com.

report a positive correlation between the activity of type IV collagenase (a member of the MMP family) and metastatic cell invasion [56]. Following partial degradation of the ECM, metastatic cells form pseudopodia in response to the expression of epidermal growth factor from tumour associated macrophages [55]. Pseudopodia attach to collagen fibrils in the ECM and facilitate the movement of metastatic cells through the ECM, whilst also allowing the metastatic cells to squeeze between other cells present in the surrounding tissue [57]. Interestingly, Shankar *et al* reported a link between pseudopodia dynamics and EMT [46]. The authors further suggested targeting the molecules crucial for the formation of pseudopodia to potentially reverse EMT, inducing mesenchymal-epithelial transition. Thus, inhibiting the potential of malignant cells to metastasise.

Metastatic cells have developed multiple strategies to migrate through the ECM to invade towards the blood and lymphatic vessels, which can be classified into three migratory modes: single cells, loosely attached cell streams and well-organised multicellular collections (figure 3) [58, 59]. The mode of migration is dependent on the cell phenotype and the surrounding ECM [58]. Single cell migration is further divided into amoeboid (rapid single cell crawling) and mesenchymal cell phenotype migration [59]. Cells which migrate via amoeboid migration have a rounded or ellipsoid morphology, lack mature focal adhesions and constantly change shape during motion [59, 60]. Amoeboid migration can again be subdivided into two modes of migration. The first is rounded cells which form blebs (small spherical protrusions) using cytoplasmic pressure, that exert a pushing motion to squeeze and migrate through the ECM [60–62]. This mode does not involve any adhesion or pulling on the surrounding substrate, leaving the ECM intact. The second mode occurs in elongated amoeboid cells which produce actin-rich filopodia that weakly adhere to the surrounding substrate via actin-protein binding, resulting in a gliding motion [60, 63]. Amoeboid migration utilises the use of protrusions instead of the attachment and contraction of lamellipodia to the ECM. Thus, this is the fastest mode of migration, with speeds between 0.4 and 5 μm min^{-1} [64]. In addition, Denais *et al* demonstrated the ability of amoeboid cells to undergo nuclear envelope rupture and self-repair to facilitate migration through tight interstitial spaces within dense ECM [65].

Mesenchymal migration is commonly observed in connective-tissue tumours [66]. Single cell mesenchymal migration is characterised by elongated, spindle-like cell morphologies with cells utilising lamellipodia to form strong integrin-mediated adhesions to the ECM [66, 67]. This migration mode is not a smooth continuous process, the leading edge of the cell moves via the lamellipodia extensions followed by contraction of the trailing edge [68, 69]. Focal contacts form and turnover within 10–120 min, thus resulting in a slow migration speed, ranging from 0.1–2 μm min^{-1} [66].

Multi-cellular streams are often formed of mesenchymal or amoeboid phenotypes, which are either loosely- or non-adhered cells, migrating along the same pathway [70]. It is often observed that cell streams have longer, straighter pathways than single cell migration and typically migrate at a speed of 1–2 μm min^{-1} [64]. Patsialou *et al* reported a significant correlation between multicellular streaming and metastatic cell intravasation and increased levels of CTCs in the blood within primary human breast tumours [71].

The final mode, collective migration, is formed of epithelial and/or mesenchymal phenotypes [64]. All the cells migrate in the same direction in constant contact with the neighbouring cell due to the retention of cell-cell adhesions [70]. The cell collections may migrate in a wedge shape with a singular leader cell or in a broader, irregular shape with a multi-cellular leading row [72]. In addition, the phenotypes may differ between the leading and following cells. Collective migration is the slowest mode of migration with a typical speed ranging from 0.01–0.05 μm min^{-1} [64].

4.2. Intravasation

Following migration and invasion of the metastatic cells through the ECM, the metastatic cells enter circulation via 2 pathways. Hematogenous intravasation (via the blood vessels) is generally the most common pathway for entry into the circulatory system. Lymphatic intravasation also occurs, however, the lymphatic system eventually drains into venous circulation [73, 74]. The mechanism for metastatic cells to undergo hematogenous and lymphatic intravasation differs due to the vessels' structural differences [74]. Blood vessels have tight endothelial junctions compared to lymphatic vessels. Thus, lymphatic vessels are defined as 'leaky' in comparison, reducing the barriers for intravasation [73].

There are three modes of migration that metastatic cells use to migrate from the ECM into the lumen of vessels and vice versa during intra- and extravasation. Firstly, cells can migrate via paracellular migration, in which the metastatic cells migrate between two endothelial cells, disrupting the inter-endothelial cell-cell junctions by extending invadopodia through the endothelium at the junctions. In addition, there are two further modes of migration through the endothelium: transcellular migration, where metastatic cells migrate through the endothelium cells, and mosaic process, where metastatic cells become a part of the endothelial layer for a short time (up to 24 h) [75].

Silvestri *et al* developed a simple MPS to study tumour-vessel interactions and breast cancer intravasation that was capable of observing mosaic vessel formation [76]. The model consists of a central microvessel within a collagen gel. Within the model they observed cancer cell-vessel interactions, including mosaic vessel formation, observing the cells physically displace the endothelial cells in the vessel wall to migrate through into the vessel lumen in clusters. Linville *et al* continued to develop the model to study the tumour-vessel interactions in the blood tumour barrier [77]. Brain microvascular endothelial-like cells were used to create the central vessel within a combined collagen and Matrigel hydrogel seeded with breast cancer spheroids and macrophages. Whilst this model was able to study tumour-vessel interactions, it was also capable of investigating the effects of immune cells on these interactions, recapitulating the significant effect of macrophages on brain metastases *in vivo*.

Whilst Silvestri *et al* observed breast cancer cell intravasation via mosaic vessel formation, Jing *et al* present a two-chamber laminated MPS design which observed breast cancer cell via the paracellular mode [78]. Furthermore, using the same model they observed liver cancer cells utilised transcellular intravasation. These studies highlight the fluidity of cancer cells to intravasate via the most suitable mode according to the conditions within the TME and vessels they are intravasating into, further emphasising the need of appropriate MPS to study metastasis.

Both models used simplistic single vessel designs to investigate intravasation, however, this is not recapitulative of the *in vivo* environment, which is composed of a complex network of vessels. Nagaraju *et al* developed a model to study intravasation composed of three concentric channels separated by microposts in which MDA-MB-231 breast cancer cells had to migrate through a collagen stromal layer before intravasating into the outer vasculature [79]. The vascular component of the model comprised a spontaneously assembled network of HUVECs which more closely resembled capillaries *in vivo*. The invasion of the cancer cells into the stromal layer increased in the presence of the vascular network, in response the cancer cells induced morphological changes to the vascular network, resulting in thinner and more permeable vessels. This model was successful in observing responses similar to those seen *in vivo* and could provide real-time analysis of single cell intravasation.

4.3. CTCs

Once in circulation, metastatic cells are known as CTCs, of which less than 0.01% survive. This is due to applied shear stress from the blood circulation and the presence of immune cells, particularly natural killer cells [74, 80]. However, there is an increased chance of survival in lymphatic vessels due to reduced shear stress, because of the significantly reduced flow in lympathic vessels [73, 81]. Metastatic cells can employ protective methods once in circulation. In 1968, Gasic *et al* first reported one such

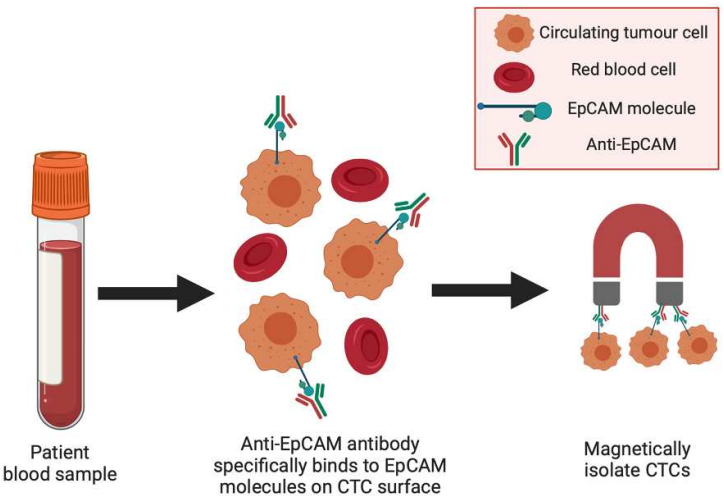

Figure 4. Simplified process diagram of the CellSearch CTC capture system. Created with BioRender.com.

method of protection, the formation of tumour cell-platelet microaggregates which physically shielded the metastatic cells from the shear stress and immune cells [82]. Egan *et al* have since demonstrated this phenomenon, reporting a decrease in shear-induced membrane damage of ovarian cancer cells in the presence of platelets [83].

In recent years it has also been suggested that the presence of neutrophils could play an active role in CTC extravasation [84, 85]. Yang *et al* show how neutrophil extracellular traps (NETs) are present in the liver metastasis of breast and colon cancer [86]. Furthermore, the DNA component in these NETs acts as a chemoattractant to CTCs, helping facilitate cancer metastasis. Cancer cell plasticity can also play a significant role in CTC survival in response to capillary-induced restriction [85]. Nuclear deformation is a critical process that can enable CTCs to successfully transit through capillary beds towards optimal sites of extravasation [87]. Yamauchi *et al* showed how fibrosarcoma cells are capable of elongating their normal length 4 fold and increasing the length of their nuclei 1.6 fold to allow survival and transit through capillaries [88]. Furthermore, evidence has been found to show that CTCs can regulate cell stiffness and thus deform to migrate through confined spaces [89]. Such extensive cell deformations can cause the CTCs to undergo hybrid EMT in response to the mechanical stimuli [90].

Given their importance in metastasis, the isolation of CTCs is critical for the study of the metastatic ability of cancer cells and are key indicators in a patient's blood for the presence of metastatic tumours [91]. A variety of methods follow similar aims to target, isolate, count and characterise CTCs. CellSearch is an FDA-approved lab-on-a-chip device that is used as a prognostic tool for breast, prostate and colorectal cancer [92]. The device uses antibodies that can specifically identify and bind to epithelial cell adhesion molecule (EpCAM) expressing CTCs, which are further magnetically isolated, stained and counted (figure 4) [93]. Allard *et al* presented an initial study using CellSearch, investigating the efficacy of identifying CTCs to further identify the presence of metastatic carcinomas [94]. The study found the presence of CTCs was extremely rare in healthy patients and patients with non-malignant diseases (1/344 patients 2 CTCs/7.5 ml of blood). However, for patients with metastatic carcinomas there was a wide range of frequencies of CTCs. Rao *et al* reported a 10-fold decrease of EpCAM expression in CTCs compared to tumour tissue from the primary and secondary sites [95], suggesting that the expression of EpCAM is dependent upon the local microenvironment. Reduced expression in CTCs may lead to reduced accuracy within the CellSearch device. CellSearch amplifies the magnetic load per antigen using avidin/biotin chemistry in order to reduce the effect of reduced EpCAM expression in CTCs [93].

ScreenCell and Rarecell are antibody-free devices which feature track-etched polycarbonate filters to sieve and isolate the CTCs due to their large size [96]. Mu *et al* demonstrated successful, simple and effective isolation of CTCs and CTC-clusters using ScreenCell [97]. The authors further reported isolation of single cells for genetic characterisation by the combination of ScreenCell filtration with the DEPArray system. The DEPArray system consists of a digital sorter combining microfluidics and microelectronics to isolate single CTCs for further analysis [98]. Filtration methods overcome the challenges associated with reduced EpCAM expression and can detect whole cell populations which could be missed by CellSearch. This includes populations that have undergone EMT; these cells lose the expression of epithelial markers through

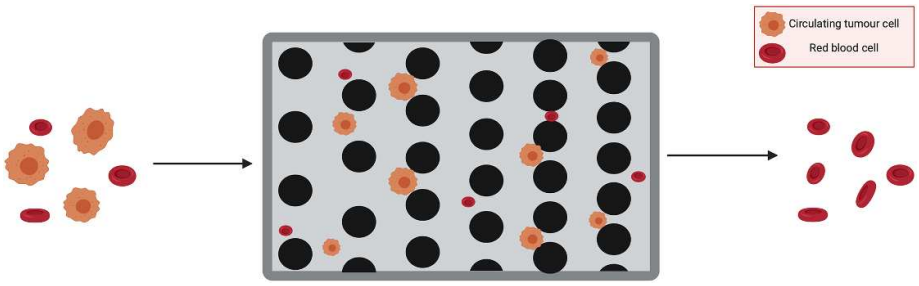

Figure 5. Simplified process diagram of lateral displacement filtration in which CTCs are captured within the device between the micropillars whilst red blood cells pass through freely. Created with BioRender.com.

the EMT process [96]. Conversely, filtration methods may miss cells which are smaller than 12 μm in diameter whereas CellSearch detects cells between 4–18 μm [99].

Microfluidic deterministic lateral displacement (DLD) is an alternative filtration method for the capture and isolation of CTCs [100]. The method comprises blood flow through an array of microposts, where each row of posts is laterally offset from the previous row and post separation reduces in each successive row, simulating *in vivo* bifurcations [101]. Cells below a critical diameter pass through the array whilst CTCs are arrested within the device and thus are separated from the sample (figure 5). Loutherback *et al* demonstrated the ability of the DLD array to isolate CTCs from a blood sample with >85% efficiency, with no effect on cell viability [102]. Furthermore, Au *et al* reported 99% efficiency by using a two-stage DLD device [103]. The first stage captures larger CTC clusters via 'standard DLD' whilst the second stage uses asymmetric posts with height restrictions to capture smaller clusters. Although this two-stage process increases the capture efficiency compared to 'standard' DLD, cell viability is reduced due to physical damage to the cells, reported at 87% at its lowest.

Whilst these devices are efficient in capturing CTCs for further analysis and investigation, they do not consider the interaction and behaviour of CTCs within the TME. Toh *et al* developed an MPS using a microchannel divided into three smaller channels via micro-pillars [104]. Cancer cells were seeded and cultured into the central channel, forming tumour aggregates. A 3D collagen barrier, resembling the dense basement membrane of the ECM, was formed around these tumour aggregates. The migration and invasion of tumour cells from the aggregates through the collagen membrane to the outer channels via chemo-attractant stimulation was observed in real time. This method allows for the study of cancer cell migration at high spatial and temporal resolution whilst better mimicking the 3D TME than the previously mentioned CTC devices.

Additionally, it is often observed that CTCs transit through and then arrest within capillaries. Chesnais *et al* developed a fully perfusable vascularised model which was matrix-free [105], that was capable of studying vascular remodelling of capillaries over two weeks. Whilst this model is not flexible to the inclusion of 3D tissues, it could be used successfully as a cancer model to investigate circulating tumour cell transit and/or arrest within capillaries.

These models above use standardised vessels architectures, however, a model by Wu *et al* has been developed which can reproduce various microenvironments, taking into account vascular surface properties and vessel geometry hemodynamic effects on CTCs [106]. The model could identify mechanophysiological conditions which increase CTC adhesion, such as vessels with hemodynamic disturbances.

4.4. Extravasation

Following CTC survival, cells must undergo extravasation. Whilst it is not as well defined as intravasation, it involves cellular adhesion to the endothelium, penetration of the cell through the endothelial barrier and trans-endothelial migration. This process is facilitated by platelets and results in metastatic cells reaching the underlying tissue [107, 108].

Crippa *et al* developed a microphysiological system to model the early metastatic niche to investigate breast cancer cell extravasation and the impact of platelets on the system [109]. The model was able to monitor the upregulation of cancer cell transendothelial migration due to the presence of platelets and neutrophils. Moreover, it was observed that the inclusion of platelets led to an increased expression of EMT markers and thus the use of a clinically approved antiplatelet drug led to reduced expression of EMT markers as observed *in vivo*. This model shows promise in providing a platform in which extravasation can be monitored and assessed whilst importantly incorporating blood cell types which, as demonstrated in this study, can significantly affect the efficiency of cancer cell metastasis. The development of these models demonstrates the importance of including additional cell types within microfluidic models to

better recapitulate the TME to successfully model events of the metastatic cascade.

In addition, Chen *et al* developed a model capable of high-level imaging and real-time analysis for extravasation [110]. The model is composed of three hydrogel channels within a PDMS chip, in which human microvascular networks are formed over 4–5 d. Human umbilical vein endothelial cells and lung fibroblasts were used to establish vascular and TMEs in the channels respectively. The device successfully monitored the extravasation of breast cancer cells (MDA-MB-231) from a perfused flow within the vascular channel into the ECM compartment. Utilising standard confocal techniques, the model could differentiate between the tumour cell migrating via paracellular migration versus transcellular migration. Furthermore, extracellular proteins, such as F-actin, VE cadherin and focal adhesion proteins could be observed. To achieve such high-resolution imaging this model confines the vascular network to one plane, and therefore does not wholly recapitulate the characteristics of a thick 3D tissue. Furthermore, the authors note the model is not capable of fully representing the *in vivo* microenvironment, as a range of additional cell populations, such as stromal cells and immune cells would be required. However, the model has been developed with a capacity of up to 36 devices per experiment, increasing its throughput capabilities, which is a rising demand to advance the use of MPS within preclinical studies.

Mei *et al* presented a study which successfully reduced the ability of MDA-MB-231 breast cancer cells to extravasate into a bone microenvironment composed of osteocyte-like MLO-Y4 cells [111]. By integrating stimulatory bone fluid flow to stimulate the osteocytes, there was a significant reduction in extravasation of the breast cancer cells into the bone microenvironment. This work supports the suggestion of exercise as a cancer intervention strategy however further work is required to understand the biomechanical mechanism in order to develop a therapeutic target strategy.

Following extravasation, migration of metastatic cells towards the secondary site occurs via similar methods described previously for invasion [112]. The metastatic cells rapidly proliferate at the secondary site, initially forming a micro-metastasis (0.2–2 mm) [113, 114]. Following further proliferation and angiogenesis the colony develops into a macro-metastasis (>2 mm) [113]. Unfortunately, the success of treatment after the establishment of a metastatic colony in a secondary site is greatly reduced. With significantly decreased responses to drug treatments, surgery is often the only viable treatment. Furthermore, the presence of CTCs in the circulatory system may lead to the formation of colonies in multiple sites, thus resulting in a poorer patient prognosis.

5. Organ-specific metastasis

It has been shown that many types of cancer frequently metastasise towards specific target organs within the body. For example, breast cancer metastasises towards the brain, liver, lung and bone [115–117], colorectal cancer towards the liver and lungs [118] and lung cancer to the brain, bone, liver, lymph nodes and adrenal glands [115, 119, 120]. There have been advances towards creating MPS which can integrate multiple tissue microenvironments representative of different organ sites, with tissue-specific functions, these designs are often referred to as organs-on-chips [21].

Berisini *et al* demonstrated the use of a tri-culture system, composed of 3 media channels, to observe the effect of an osteo-cell conditioned microenvironment on the extravasation of metastatic breast cancer cells (MDA-MB-231) [121]. The breast cancer cells transmigrated through an endothelial layer into the osteo-cell conditioned regions, resulting in the formation of micrometastases within these regions. Additionally, the model was able to identify key molecular pathways for the process of extravasation involving breast cancer cell surface receptor CXCR2 and bone secreted chemokine CXCL5.

An alternative device design has been developed by Skardal *et al,* composed of two chambers, independently housing gut and liver constructs, connected by fluid flow in series, investigating metastasis of colorectal cancer [122]. Each construct is composed of representative host tissue cells, suspended within hyaluronic acid-based hydrogels. The liver construct uses HepG2 cells whilst the gut construct contains INT-407 cells and colon carcinoma cells (HCT-116) which formed tumour aggregates. Observations found the tumour aggregates grew before undergoing dissemination from the gut construct and migrating to the liver construct via the circulation system. However, whilst this does allow modelling of two independent organ constructs, the model lacks a full recapitulation of *in vivo*-like function due to the simplistic cell encapsulation within the hydrogel and a lack of endothelial barriers to model extravasation and intravasation.

Xu *et al* also demonstrated the use of a multi-organ-on-a-chip platform to investigate the metastasis of lung cancer to three target organs [123]. The PDMS chip was composed of an upstream lung compartment separated via PDMS microporous membranes from three downstream brain, bone and liver compartments. The study observed the formation of a tumour mass and increased invasive capacity following EMT. Furthermore, the model was able to investigate the effect of cancer cell metastasis within the cell populations in the downstream compartments, observing the overexpression of specific proteins: CXCR4, RANKL and AFP. Whilst PDMS is a

good candidate for MPS due to its transparent, gas permeable and biocompatible properties, it also has disadvantages [124, 125]. A key issue of PDMS in MPS is the absorption of small molecules onto the surface of the PDMS, which can have a profound effect on the outcome of drug screening studies, as highlighted by Toepke and Beebe [126].

Firatligil-Yildirir *et al* utilised two organ-on-chip platforms in combination, IC Chip and EX-Chip, to study invasion/chemotaxis and extravasation respectively [127]. The study investigated the invasion and extravasation of breast cancer cells towards tissue specific microenvironments, including lung (WI-38), liver (BRL-3A) and breast (MCF-10A). The results from the IC Chip show that MDA-MB-231 breast cancer cells had a higher preference to invade to the liver and lung microenvironments than the breast microenvironment. Moreover, in the EX-Chip the MDA-MB-231 cells extravasated more into the lung microenvironment than the liver and breast microenvironments.

6. Important factors to consider for the development of MPS for cancer applications

6.1. Constitution of the TME

The TME plays an integral role in maintaining normal cell function and behaviour and can have a significant impact on cancer development [128, 129]. The TME is composed of tumour cells, stromal cells, such as fibroblasts, endothelial cells and infiltrated immune cells [130]. Regier *et al* demonstrated the difference in gene expression between mono-, bi- and tri-culture within an MPS system [131]. The study highlighted that the co-culture of three cell types more strongly alters cell type-, time- and complexity-dependent gene expression than models that are limited to only two cell types. In addition, it showed how varying the complexity of microenvironments within MPS models affects the response of tumour and stromal cells. Thus, expanding past conventional bi-cultures towards heterotypic cultures is crucial. This has been demonstrated by Bradney *et al* who observed differences in the invasive behaviour of pancreatic ductal adenocarcinoma when cultured in a heterogeneous environment [132]. The study was able to observe complex interactions between the heterogeneous cancer populations resulting in an increase in the aggressive and invasive nature of the cancer cells.

Furthermore, Gadde *et al* used a simplistic microfluidic model to investigate the initial steps of breast cancer cell intravasation from an ECM into a singular central vessel [133]. The model used inflammatory breast cancer cells to successfully stimulate and model *in vivo* events of sprouting of the endothelium of the vessel encircling clusters of the cells. However, this model lacked additional stromal or immune cell types. Therefore, Gadde *et al* further developed this model to include tumour associated macrophages [134]. After the inclusion of these macrophages there was an increase of ECM porosity, increased vascular sprouting and enhanced permeability of the endothelium. Importantly, the addition of the tumour associated macrophages led to the successful intravasation of the breast cancer cells into the vessels.

As briefly mentioned, the TME plays a key role in the EMT. The TME provides signals to cancer cells and due the high plasticity of cancer cells, these signals can result in full, partial or reversal of the EMT [35]. Furthermore, the TME is involved in therapy-induced plasticity, in which cancer-associated fibroblasts (CAFs), a stromal cell population in the TME, have been well-documented in promoting therapy resistance [130]. Factors produced by CAFs can activate stem-like associated pathways promoting a shift in tumour cells to CSCs, correlating with poor patient prognosis [38, 135, 136]. Erdogan *et al* developed a parallel, 2 chamber MPS and observed how fibronectin assembled by CAFs mediates CAF-tumour cell interactions and directional migration of tumour cells [137].

A significant element of the TME is the ECM. The ECM is comprised of multiple components: proteins, glycoproteins, proteoglycans and polysaccharides, which form both the basement membrane and the interstitial matrix (figure 6) [129, 138]. Many studies utilise hydrogels composed solely of fibrin, Matrigel or collagen, however these gels do not solely reconstitute the full ECM. To improve this, Agrawal *et al* investigated the use of a combined fibrin, Matrigel and collagen gel to improve cellular interactions and cross talk [139]. The study found the combination of gels led to improved vascularisation of HT-29 colorectal tumour spheroids with intravasation events of the cancer cells observed in the combined gel, identifying strands of cancer cells asymmetrically growing and infiltrating from the tumour spheroid to the nearest microvasculature.

Collagen is the most abundant protein in the human body and collagen fibres are the most substantial component of the ECM, ranging from 50–500 nm in diameter [140]. In addition, it has been increasingly reported to have a significant impact on tumour progression [129, 141–144].

Provenzano *et al* reported dense, highly linear and oriented collagen type I fibres aligned perpendicular and radially to tumorous breast tissue [141]. Furthermore, invasive metastatic cells in direct contact with the collagen fibres were observed migrating across the fibres via amoeboid migration. By comparing the collagen alignment in tumorous breast tissue to normal breast tissue, this study provides evidence that strongly indicates the ability of cancer cells to trigger collagen fibril reorganisation into a radial alignment, aiding metastatic cell invasion. Moreover,

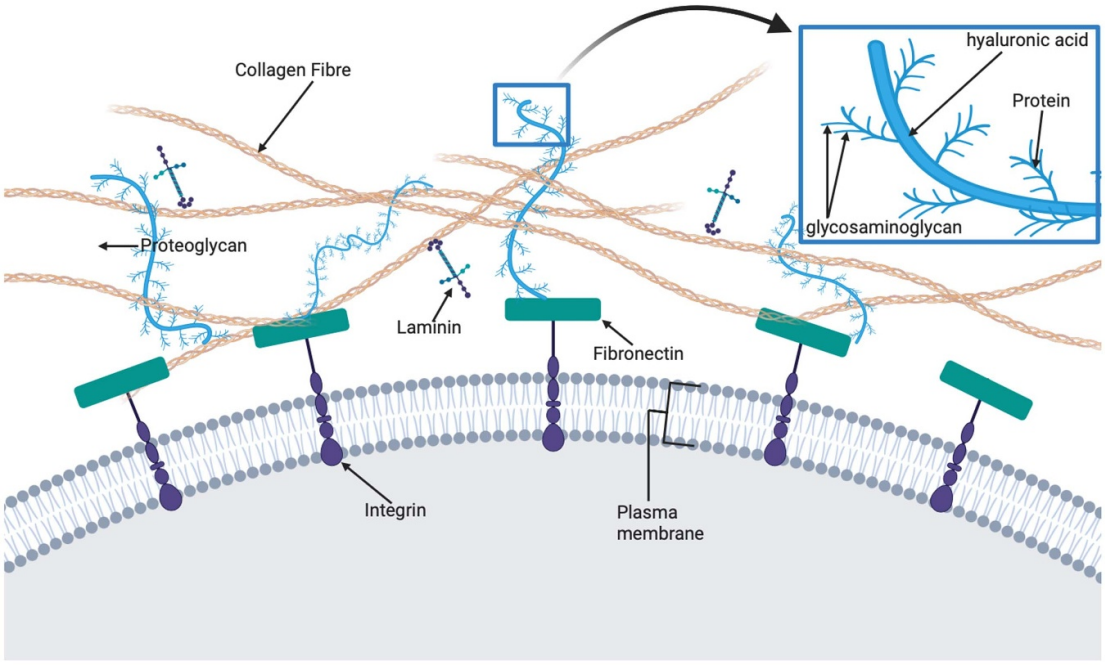

Figure 6. Extracellular matrix and cell membrane, demonstrating the position and structure of the extracellular matrix components. Created with BioRender.com.

Ramaswamy *et al* described the increased expression of genes encoding type I collagens (COL1A1 and COL1A2) within tumours with metastatic potential [145]. The reorganisation and increased formation of collagen type I and III results in the gradual stiffening of the ECM [129, 146]. This leads to increased tension in the tissue which can affect cell differentiation and gene expression. Potentially, this then promotes increased tumour growth and metastatic potential, resulting in a poorer prognosis for patients [129, 145, 147].

Pathak and Kumar developed an MPS to study the independent impacts of ECM stiffness and pore size on cancer cell migration [148]. The device contains microfluidic channels of varying widths (10–40 μm) and stiffnesses (0.4–120 kPa) formed via polymerisation and gelation of polyacrylamide hydrogels. The authors reported a decrease in glioma cell migration speed with increasing pore size at a fixed stiffness. However, at a fixed pore size the relationship between migration speed and stiffness varied. This suggests that pore size can significantly affect migration speed and also influences the way matrix stiffness controls migration speed.

Whilst cancer cells can alter a tissue stiffness there are many tissues which also constantly undergo changes in stiffness due to mechanical forces on the tissue, such as the intestines. Strelez *et al* successfully utilised the Emulate system and demonstrated the ability to tune the TME to assess the invasive capabilities of colorectal cancer [149]. To improve the physiological relevance to the TME within the intestine, peristalsis-like mechanical forces were applied across the endothelial:epithelial interface, consistently altering the stiffness across the ECM. Via applying these forces alongside the co-culture of the tumour cells with fibroblasts the study was able to quantify key differences in the invasion of aggressive and non-aggressive colorectal cancer cell lines. However, this chip is fabricated using PDMS and requires specialised equipment to operate. This need for specialised equipment reduces the accessibility of the model to many laboratories and researchers and thus reduces the uptake of models capable of recapitulating these complex mechanical forces.

6.2. Vascularisation

The successful use of TE constructs in clinical applications is limited due to poor vascularisation within the tissue [150]. The constructs are restricted to 100–200 μm tissue thickness due to diffusional mass transfer limitations. Constructs with a thickness greater than 200 μm fail due to ischemia and cell death within the tissue [151]. Vascularisation of *in vitro* models provides access to nutrients and removal of waste for cells within the bulk of the constructs. Therefore, by improving vascularisation culture times can be extended and the culture conditions more accurately simulate *in vivo* conditions. Moreover, vascularisation is key in facilitating the metastasis of tumour cells and significantly influences the efficacy of anti-cancer treatments [152]. Consequently, there is a large emphasis on promoting vasculogenesis within models to improve the physiological relevancy,

accuracy and increase the information gained from *in vitro* models over a longer period. There are 3 distinct designs to fabricate vascularisation within MPS: endothelial barrier, template-based vasculature and self-assembled networks [153].

Kameda *et al* developed a self-assembled network model which utilised removable membranes to enable direct contact between tissue and an on-chip vascular bed [154]. This model allows for vascular bed formation without the need of angiogenic factors released from the tissue. Thus, the model can be utilised to study the interaction of vasculature with any 3D tissue, independent of whether it secretes angiogenic factors. However, the model is cultured under static conditions and thus lacks active perfusion of nutrients through the vascular network. These self-assembled networks can provide vascular networks which are more physiologically relevant and can provide insight into how the presence of metastatic cells can alter the vascular characteristics, such as poorly developed luminal structures and immaturities in the vasculature. Similar findings of vessel thinning and increased vessel permeability due to the presence of MDA-MB-231 breast cancer cells have been reported by Nagaraju *et al* in a similar self-assembled network model [79].

SynVivo have developed an alternative template based vascular 3D tumour model that uses scans of vascular networks to generate a chip that incorporates geometrically and spatially relevant vascular beds with additional space for tissue or tumour sections, consisting of microchannels separating independent compartments [155]. Pradhan *et al* utilised this model to culture breast cancer cells and stromal fibroblasts in adjoining tissue compartments separated by an interstitial space containing pores to mimic leaky vasculature *in vivo*. The study was able to investigate the efficacy of anti-cancer drugs under dynamic flow conditions. Other template-based designs use a microneedle or rod to create simple single channel designed, an approach used by many of the MPS models that have been mentioned earlier in this review [33, 76, 77, 133, 134].

Endothelial barrier models do not wholly recapitulate the 3D environment and are termed as pseudo-3D [153]. Therefore, they are limited to replicating the endothelial lining within a vessel and not a whole vessel construct. However, this does provide a clear interface for analysis at the endothelial barrier as demonstrated by Zervantonakis *et al* [156].

6.3. Hypoxia

Hypoxia is a key feature in the TME, in which there is disparity between the oxygen delivered to a tissue and the consumption of oxygen within the tissue, and is most commonly associated with solid tumours [157]. Hypoxic conditions have a significant effect on the behaviour of cancer cells and can mediate the effects of cancer treatments such as, chemotherapy, radiotherapy and immunotherapy; thus hypoxia is associated with poor patient prognosis [158]. Furthermore, hypoxia has been identified to induce EMT and CSC features, such as acquiring a mesenchymal phenotype, loss of differentiation, tumorigenesis and increased resistance to therapy [159]. This phenomenon occurs due to hypoxia-inducible factor signalling in response to low oxygen conditions. MPS that allow the development of hypoxic regions, or in which the partial pressure of oxygen (pO_2) can be varied are important.

Song *et al* utilised a self-organised 3D microvascular model to investigate the effect of hypoxia on tumourigenic (MCF-7 and MDA-MB-231) and non-tumourigenic (MCF-10A) breast cancer cell lines [160]. Initial observations identified that hypoxic conditions (1% O_2) induced apoptosis of the MCF-10A cell line. The same conditions for 5 d significantly reduced the proliferation of MCF-7 and MCF-10A cells but did not have a significant impact on the proliferation of MDA-MB-231 cells. This study highlights how the response to hypoxia depends on the malignancy of the cell line, where MCF-7, which is a non-invasive breast cancer cell line, had a higher sensitivity to hypoxic conditions than the MDA-MB-231 invasive breast cancer cell line. Therefore, the degree of hypoxia within an MPS needs to be aligned with the invasive potential of the chosen cancer cell line to better recapitulate the *in vivo* environment and behaviour of the specific cancer cells.

Whilst this model was able to study hypoxic conditions, the control conditions of the oxygenated environment were 21% O_2 [161, 162]. The physiological pO_2 ranges in the body from 14% in the arterial blood, 4%–14% in the lung, liver and kidneys and 0.5%–7% in less irrigated organs and tissues. Similar to this model, the majority of models use 20% O_2 concentration, representing hyperoxic, non-physiological conditions. For the continuing development of MPS it is essential that the physiological pO_2 levels of the tissue of interest, both healthy and diseased, are used to better recapitulate the TME.

6.4. Fluid flow

Fluid flow is another factor that can affect the response of cells to the microenvironment. Cancer cells experience fluid flow, either blood or interstitial, throughout all steps in the metastatic cascade [163]. Hajal *et al* demonstrate the important role of physiological luminal, trans-endothelial and interstitial fluid flows during intravasation and extravasation on the local metastatic potential of tumour cells [164]. The study utilised a human microvasculature fluidic model and observed that physiological luminal flow promoted extravasation potential of tumour cells. Models such as this could be utilised to develop and investigate new therapeutic strategies

that target specific cancer sites based on the specific fluid flow rate.

Polacheck *et al* present an MPS which could observe the response of breast cancer cells, within a 3D collagen type I matrix, after exposure to high interstitial flows [165]. The model is composed of two media channels on either side of a central channel containing MDA-MB-231 cells embedded within a collagen type I hydrogel. A flow field was produced through the collagen gel by creating a higher fluid pressure in one channel than the other. The model identified key molecular biophysical mechanisms that lead to protrusion formation on the upstream side of the cell when exposed to high rates of interstitial flow (4.6 μm s^{-1}). This biophysical mechanism is just one example of a potential therapeutic target.

Further effects of interstitial fluid flow on the mode of cell migration were observed within a model developed by Huang *et al* [166]. The device was composed of three parallel cell channels separated by polydimethylsiloxane ridges with a fluid flow (2 μm s^{-1}) channel perpendicular to the cell channels. The model showed that the cells exhibited both amoeboid and mesenchymal migration modes. However, the influence of interstitial flow promoted the migration of the cells towards amoeboid mode. It was suggested this was due to the interstitial flow washing away fibronectin, an important molecule in cell adhesion, vital for mesenchymal migration. Further observation showed that the addition of exogenous fibronectin promoted cell migration via the mesenchymal mode. Additionally, the model demonstrated that increasing interstitial flow increases the migration speed but decreases unidirectional migration.

7. Current challenges faced in the development of MPS

Broadly, there is a need to encourage industry to progress from the use of MPS for internal project decisions to the use of MPS for regulatory progression. Following the Modernization Act 2.0, signed in December 2022, the FDA no longer requires animal testing for the progression of drug candidates to clinical trials [167]. However, no MPS data have been used within regulatory applications to progress to clinical trials [168]. To ensure further adoption of MPS it is crucial to overcome key challenges currently associated with the use of these models. However, there are many challenges that are stalling the development, uptake and adoption of MPS.

The throughput of many models is low, with single chip designs increasing the number of experiments needed to collect a full dataset. There are ways to improve model throughput, one such method is via MPS which are designed with multiple chips on a standard well plate. These designs increase throughput whilst also improving the integration of the model with current standardised imaging techniques since most microscopy equipment is capable of imaging a standard well plate. Mimetas platforms provide microfluidic MPS within standardised 384-well plates that can be easily imaged on existing imaging systems and require little extra specialised equipment to utilise the platform. Lanz *et al* utilised the Mimetas platform to demonstrate the use of an MPS for therapy selection for triple negative breast cancer [169]. The model improved 3D cell culture viability via constant media perfusion and showed successful compatibility with dissected tumour tissue. Furthermore, the study highlighted the potential for MPS systems to be used within personalised medicines for drug selection and the prediction of patient response. However, there is a compromise between model complexity and throughput, thus the complexity of these well plate multi-chip design models are often scaled back in comparison to the single chip designs. There is currently no viable solution that can accommodate high complexity models at high throughput.

Furthermore, an associated challenge due to low throughput is often the lack of reliability of the model across experiments. Oliver *et al* presented a combined approach using artificial intelligence (AI) alongside an MPS to provide a more robust technique to produce a more reliable, clinically applicable model [170]. The blood brain niche MPS in combination with AI can identify the extravasation potential of cancer cells via minute differences in cell phenotype. The use of AI allows for the continual improvement and training of the model as they expand the number of patient samples used in the future. This method could be applied to many MPS and could provide a solution to improving the reliability of these models.

Another challenge of MPS is the cost and time implications of developing, fabricating and optimising each new design. Optimisation and testing are required at each step of the model development process, thus creating a bottleneck in the advancement of MPS. The lack of integrated automation systems is another factor that limits the use of MPS within clinical studies. The number of staff-hours that are required to set-up and maintain the number of complex *in vitro* models to capture a complete data set for clinical studies is not feasible and thus automation is required for at least the maintenance of models to achieve this goal. It is a common complication across MPS, as technologies, such as automation and fabrication techniques, are striving to keep up with the advances in models, thus industry is rapidly learning how to repurpose existing technology to help advance MPS.

There are also additional challenges when developing an MPS to study the metastatic cascade. The metastatic cascade is a highly complex systemic process, which currently cannot be recapitulated within one device. This would require a multi-organ model

which could integrate all the stages of the cascade, organ specific stromal cells, an immune response and allow for sample collection and analysis at each point in the system. Furthermore, this system would require the optimisation of a cell culture medium for the entire system. Alternatively, the system would require each microenvironment to be compartmentalised with tight control of each one, regulating both physical and biological parameters, such as shear stress, ECM stiffness, porosity, pH, temperature and oxygen.

Furthermore, the use of MPS for oncology drug development and therapeutic strategies is further hindered by the lack of validation of the platforms. Many devices are presented as proof of concept with demonstrations of the potential use and applications of the model. A large factor that impacts the validation of these models is that they lack the whole-body perspective, thus potentially harmful off-target effects of therapeutics are not likely to be discovered. Furthermore, initially cancer grows in a primary location however, following dissemination through the metastatic cascade, cancer is a systemic disease, therefore a therapeutic strategy that may work within a model studying a primary tumour is unlikely to have a therapeutic effect on cells at a secondary site as these cells often exhibit different characteristics to the primary tumour. Careful selection of the MPS model used for a particular assay, together with a clear understanding of the limitations of the model is therefore essential.

Whilst there are still many associated challenges with MPS, there are several driving forces to encourage the adoption of MPS. The current cost and time for drug discovery and development is high and both could be reduced through the judicious incorporation of robust and reproducible models into the drug discovery pipeline. Furthermore, the introduction of the 3Rs principles (replacement, reduction, and refinement) concerning animal research is a critical social and regulatory pressure promoting the development and use of MPS.

8. Future direction of MPS

Whilst cancer remains to be one of the most important global healthcare challenges, not only because of high mortality rates but also in reduction of health expectancy for patients, there are still a number of questions surrounding the underlying mechanisms of the metastatic cascade and the optimal strategies to inhibit the progression of metastatic cancer.

This review highlights the current advances in the development of MPS for the investigation of cancer metastasis, outlining the models which have shown potential in exploring multiple stages of the cascade with multiple 'organ sites'. Current designs of MPS, as presented in this review, mainly focus on the use of simplistic compartmentalised systems. There is a wealth of information and knowledge gained from these systems regarding a vast array of factors that influence the cancer microenvironment and thus the behaviour of cancer. It is crucial to utilise this wealth of knowledge to further drive and inform future MPS model designs towards heterotypic cultures forming complex microenvironments. Furthermore, better recapitulation of the TME can be achieved by focusing on more fully representing the ECM, tuning the mechanical properties, incorporating CSCs, stromal cells and immune cells, and using physiological levels of pO_2. However, few models have been further developed to focus on the heterogeneity of the cancer cells themselves. It is known that cancers have highly heterogeneous populations and therefore future models need to reflect this key feature. Inclusion of patient derived spheroids or organoids would better reflect cancer heterogeneity and introduce clonal variations within cultures.

MPS are continually developing to better recapitulate the influence of the TME on the potential and mechanism of cancer metastasis. Here, models which can recapitulate multiple organ microenvironments, each composed of numerous cell types, are crucial, such as the microfluidic system developed by Ronaldson-Bouchard *et al* [171]. The system is composed of 4 organ tissue niches, representative of heart, bone, liver and skin, are connected via a vascular flow and separated by semi-permeable endothelial membrane and via the use of human induced pluripotent stem cells and stromal cells, a physiological ECM in each compartment was matured by 4–6 weeks. Models that integrate multiple organs such as this may help to identify mechanisms that isolated models may miss.

Many models presented in this review have been key in identifying underlying mechanisms for specific cancer cell behaviour. These key findings can be further utilised to explore new potential therapeutic targets which could then be further explored within these MPS. Therefore, whilst the overarching goal may be to create a single device capable of modelling the whole metastatic cascade, it is also important to utilise the technology we have, to continue to investigate the mechanisms behind specific cancer behaviours in order to continue to identify potential therapeutic targets.

In summary, with the current understanding and technology available, it is impossible to recapitulate the full complexities of the 3D TME, with all the biological, mechanical and chemical components, within a single model, that is reproducible, easy to use by trained researchers and with a high enough throughput to provide a reportable dataset. In addition, with such variation between not only different cancer types but within a subset of cancers, the best method to design models is currently via a reductionist approach

whilst technology and model development advances towards a more holistic MPS. An approach such as this utilises advantages from both natural and synthetic materials to create a model with specific applications and features whilst acknowledging the limitations of the model.

Data availability statement

No new data were created or analysed in this study.

Acknowledgments

C J would like to thank the EPSRC, centre for doctoral training in Advanced Biomedical Materials for PhD studentship funding (EP/S022201/1). F C also thanks the Royal Society for funding of a Royal Society Leverhulme Trust Senior Research Fellowship 2022 (SRF\R1\221053).

Author contributions

writing—original draft, C E J, supervision, N H G, W R E and F C; writing—review and editing, C E J, N H G, W R E and F C All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Funding

This research was funded by EPSRC, Grant Number: EP/S022201/1 and the Royal Society, Grant Number: SRF\R1\221053.

ORCID iDs

Caitlin E Jackson https://orcid.org/0000-0002-4971-144X
Nicola H Green https://orcid.org/0000-0001-5413-0642
Frederik Claeyssens https://orcid.org/0000-0002-1030-939X

References

[1] Siegel R L, Miller K D and Jemal A 2020 Cancer statistics, 2020 *CA Cancer J. Clin.* **70** 7–30
[2] Bray F, Ferlay J, Soerjomataram I, Siegel R L, Torre L A and Jemal A 2018 Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries *CA Cancer J. Clin.* **68** 394–424
[3] Laudicella M, Walsh B, Burns E and Smith P C 2016 Cost of care for cancer patients in England: evidence from population-based patient-level data *Br. J. Cancer* **114** 1286–92
[4] Gomez-Cuadrado L, Tracey N, Ma R, Qian B and Brunton V G 2017 Mouse models of metastasis: progress and prospects *Dis. Model Mech.* **10** 1061–74
[5] Céspedes M V, Casanova I, Parreño M and Mangues R 2006 Mouse models in oncogenesis and cancer therapy *Clin. Transl. Oncol.* **8** 318–29
[6] Bos P D, Nguyen D X and Massagué J 2010 Modeling metastasis in the mouse *Curr. Opin. Pharmacol.* **10** 571–7
[7] Mak I W, Evaniew N and Ghert M 2014 Lost in translation: animal models and clinical trials in cancer treatment *Am. J. Trans. Res.* **6** 114–8
[8] Dagogo-Jack I and Shaw A T 2018 Tumour heterogeneity and resistance to cancer therapies *Nat. Rev. Clin. Oncol.* **15** 81–94
[9] Wu Z, Huang D, Wang J, Zhao Y, Sun W and Shen X 2024 Engineering heterogeneous tumor models for biomedical applications *Adv. Sci.* **11** 2304160
[10] Tsai And H F *et al* 2017 Tumour-on-a-chip: microfluidic models of tumour morphology, growth and microenvironment *J. R. Soc. Interface* **14** 20170137
[11] Sontheimer-Phelps A, Hassell B A and Ingber D E 2019 Modelling cancer in microfluidic human organs-on-chips *Nat. Rev. Cancer* **19** 65–81
[12] Liu X, Fang J, Huang S, Wu X, Xie X, Wang J, Liu F, Zhang M, Peng Z and Hu N 2021 Tumor-on-a-chip: from bioinspired design to biomedical application *Microsyst. Nanoeng.* **7** 1–23
[13] Seaman K, Sun Y and You L 2023 Recent advances in cancer-on-a-chip tissue models to dissect the tumour microenvironment *Med-X* **1** 1–28
[14] Malandrino A, Kamm R D and Moeendarbary E 2018 *In vitro* modeling of mechanics in cancer metastasis *ACS Biomater. Sci. Eng.* **4** 294–301
[15] Jeon J S, Bersini S, Gilardi M, Dubini G, Charest J L, Moretti M and Kamm R D 2015 Human 3D vascularized organotypic microfluidic assays to study breast cancer cell extravasation *Proc. Natl Acad. Sci. USA* **112** 214–9
[16] Sleeboom J J F *et al* 2018 Metastasis in context: modeling the tumor microenvironment with cancer-on-a-chip approaches *Dis. Model Mech.* **11** 3
[17] Jouybar M, de Winde C M, Wolf K, Friedl P, Mebius R E and den Toonder J M J 2023 Cancer-on-chip models for metastasis: importance of the tumor microenvironment *Trends Biotechnol.* **42** P431–48
[18] Zhang X *et al* 2022 Cancer-on-a-chip: models for studying metastasis *Cancers* **14** 648
[19] Knight E, Murray B, Carnachan R and Przyborski S 2011 Alvetex®: polystyrene scaffold technology for routine three dimensional cell culture *Methods in Molecular Biology* vol 695 (Humana Press) pp 323–40
[20] Asghar W, El Assal R, Shafiee H, Pitteri S, Paulmurugan R and Demirci U 2015 Engineering cancer microenvironments for *in vitro* 3-D tumor models *Mater. Today* **18** 539–53
[21] Leung C M *et al* 2022 A guide to the organ-on-a-chip *Nat. Rev. Method Primers* **2** 1–29
[22] Abdulghani S and Mitchell G R 2019 Biomaterials for in situ tissue regeneration: a review *Biomolecules* **9** 750
[23] Kapałczyńska M, Kolenda T, Przybyła W, Zajączkowska M, Teresiak A, Filas V, Ibbs M, Bliźniak R, Łuczewski Ł and Lamperska K 2018 2D and 3D cell cultures—a comparison of different types of cancer cell cultures *Arch. Med. Sci.* **14** 910
[24] O'Brien F J 2011 Biomaterials & scaffolds for tissue engineering *Mater. Today* **14** 88–95
[25] Chan B P and Leong K W 2008 Scaffolding in tissue engineering: general approaches and tissue-specific considerations *Eur. Spine J.* **17** 479
[26] Lodish H *et al* 2002 Section 23.1, Tumor cells and the onset of cancer *Molecular Cell Biology* 4th edn (Freeman, W. H.)
[27] Lodish H, Berk A, Zipursky S L, Matsudaira P, Baltimore D and Darnell J 2002 Mutations: types and causes *Molecular Cell Biology* 4th edn (W. H. Freeman)
[28] Evan G I and Vousden K H 2001 Proliferation, cell cycle and apoptosis in cancer *Nature* **411** 342–8

[29] Brown T A 2002 Chapter 14 Mutation, repair and recombination *Genomes* 2nd edn (Wiley-Liss)
[30] Bremnes R M, Dønnem T, Al-Saad S, Al-Shibli K, Andersen S, Sirera R, Camps C, Marinez I and Busund L-T 2011 The role of tumor stroma in cancer progression and prognosis: emphasis on carcinoma-associated fibroblasts and non-small cell lung cancer *J. Thoracic Oncol.* **6** 209–17
[31] Bhowmick N A, Neilson E G and Moses H L 2004 Stromal fibroblasts in cancer initiation and progression *Nature* **432** 332–7
[32] Gupta G P and Massagué J 2006 Cancer metastasis: building a framework *Cell* **127** 679–95
[33] Meng F, Meyer C M, Joung D, Vallera D A, McAlpine M C and Panoskaltsis-Mortari A 2019 3D bioprinted *in vitro* metastatic models via reconstruction of tumor microenvironments *Adv. Mater.* **31** 1806899
[34] Hapach L A, Mosier J A, Wang W and Reinhart-King C A 2019 Engineered models to parse apart the metastatic cascade *npj Precis. Oncol.* **3** 20
[35] da Silva-diz V, Lorenzo-Sanz L, Bernat-Peguera A, Lopez-Cerda M and Muñoz P 2018 Cancer cell plasticity: impact on tumor progression and therapy response *Semin. Cancer Biol.* **53** 48–58
[36] Greaves M and Maley C C 2012 Clonal evolution in cancer *Nature* **481** 306–13
[37] Bozic I, Antal T, Ohtsuki H, Carter H, Kim D, Chen S, Karchin R, Kinzler K W, Vogelstein B and Nowak M A 2010 Accumulation of driver and passenger mutations during tumor progression *Proc. Natl Acad. Sci. USA* **107** 18545–50
[38] Ayob A Z and Ramasamy T S 2018 Cancer stem cells as key drivers of tumour progression *J. Biomed. Sci.* **25** 1–18
[39] Meacham C E and Morrison S J 2013 Tumour heterogeneity and cancer cell plasticity *Nature* **501** 328–37
[40] Cabrera M C, Hollingsworth R E and Hurt E M 2015 Cancer stem cell plasticity and tumor hierarchy *World J. Stem. Cells* **7** 27–36
[41] Senapati S, Singh R K and Batra S K 2015 Tumor microenvironment: blood vascular system in cancer metastasis *Systems Biology of Cancer* ed S Thiagalingam (Cambridge University Press) pp 309–22
[42] Cooper G 2000 The development and causes of cancer *The Cell: A Molecular Approach* 2nd edn (Sinauer Associates)
[43] Bacac M and Stamenkovic I 2008 Metastatic cancer cell *Annu. Rev. Pathol.* **3** 221–47
[44] Liotta L A, Steeg P S and Stetler-Stevenson W G 1991 Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation *Cell* **64** 327–36
[45] Behrens J 1993 The role of cell adhesion molecules in cancer invasion and metastasis *Breast Cancer Res. Treat.* **24** 175–84
[46] Shankar J, Messenberg A, Chan J, Underhill T M, Foster L J and Nabi I R 2010 Pseudopodial actin dynamics control epithelial-mesenchymal transition in metastatic cancer cells *Cancer Res.* **70** 3780–90
[47] Thiery J P, Acloque H, Huang R Y J and Nieto M A 2009 Epithelial-mesenchymal transitions in development and disease *Cell* **139** 871–90
[48] Perl A-K, Wilgenbus P, Dahl U, Semb H and Christofori G 1998 A causal role for E-cadherin in the transition from adenoma to carcinoma *Nature* **392** 190–3
[49] Frixen U H, Behrens J, Sachs M, Eberle G, Voss B, Warda A, Löchner D and Birchmeier W 1991 E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells *J. Cell Biol.* **113** 173–85
[50] Sannino G, Marchetto A, Kirchner T and Grünewald T G P 2017 Epithelial-to-mesenchymal and mesenchymal-to-epithelial transition in mesenchymal tumors: a paradox in sarcomas? *Cancer Res.* **77** 4556–61
[51] Aiello N M *et al* 2018 EMT subtype influences epithelial plasticity and mode of cell migration developmental cell article EMT subtype influences epithelial plasticity and mode of cell migration *Dev. Cell* **45** 681–95.e4
[52] Brabletz T 2012 EMT and MET in metastasis: where are the cancer stem cells? *Cancer Cell* **22** 699–701
[53] Dedhar S and Saulnier R 1990 Alterations in integrin receptor expression on chemically transformed human cells: specific enhancement of laminin and collagen receptor complexes *J. Cell Biol.* **110** 481–9
[54] Yamaguchi H 2012 Pathological roles of invadopodia in cancer invasion and metastasis *Eur. J. Cell Biol.* **91** 902–7
[55] Bravo-Cordero J J, Hodgson L and Condeelis J 2012 Directed cell invasion and migration during metastasis *Curr. Opin. Cell Biol.* **24** 277–83
[56] Liotta L A, Tryggvason K, Garbisa S, Hart I, Foltz C M and Shafie S 1980 Metastatic potential correlates with enzymatic degradation of basement membrane collagen *Nature* **284** 67–68
[57] Wolf K and Friedl P 2009 Mapping proteolytic cancer cell-extracellular matrix interfaces *Clin. Exp. Metastasis* **26** 289–98
[58] Friedl P, Borgmann S and Bröcker E B 2001 Amoeboid leukocyte crawling through extracellular matrix: lessons from the Dictyostelium paradigm of cell movement *J. Leukocyte Biol.* **70** 491–509
[59] Friedl P and Wolf K 2010 Plasticity of cell migration: a multiscale tuning model *J. Cell Biol.* **188** 11–19
[60] Lämmermann T and Sixt M 2009 Mechanical modes of 'amoeboid' cell migration *Curr. Opin. Cell Biol.* **21** 636–44
[61] Charras G and Paluch E 2008 Blebs lead the way: how to migrate without lamellipodia *Nat. Rev. Mol. Cell Biol.* **9** 730–6
[62] Fackler O T and Grosse R 2008 Cell motility through plasma membrane blebbing *J. Cell Biol.* **181** 879–84
[63] Yoshida K and Soldati T 2006 Dissection of amoeboid movement into two mechanically distinct modes *J. Cell Sci.* **119** 3833–44
[64] Clark A G and Vignjevic D M 2015 Modes of cancer cell invasion and the role of the microenvironment *Curr. Opin. Cell Biol.* **36** 13–22
[65] Denais C M, Gilbert R M, Isermann P, McGregor A L, te Lindert M, Weigelin B, Davidson P M, Friedl P, Wolf K and Lammerding J 2016 Nuclear envelope rupture and repair during cancer cell migration *Science* **352** 353–8
[66] Friedl P and Wolf K 2003 Tumour-cell invasion and migration: diversity and escape mechanisms *Nat. Rev. Cancer* **3** 362–74
[67] Yamada K M and Sixt M 2019 Mechanisms of 3D cell migration *Nat. Rev. Mol. Cell Biol.* **20** 738–52
[68] Zamir E *et al* 2000 Dynamics and segregation of cell-matrix adhesions in cultured fibroblasts *Nat. Cell Biol.* **2** 191–6
[69] Ballestrem C, Hinz B, Imhof B A and Wehrle-Haller B 2001 Marching at the front and dragging behind: differential αVβ3-integrin turnover regulates focal adhesion behavior *J. Cell Biol.* **155** 1319–32
[70] Friedl P and Alexander S 2011 Cancer invasion and the microenvironment: plasticity and reciprocity *Cell* **147** 992–1009
[71] Patsialou A, Bravo-Cordero J J, Wang Y, Entenberg D, Liu H, Clarke M and Condeelis J S 2013 Intravital multiphoton imaging reveals multicellular streaming as a crucial component of in vivo cell migration in human breast tumors *Intravital* **2** e25294
[72] Khalil A A and Friedl P 2010 Determinants of leader cells in collective cell migration *Integr. Biol.* **2** 568
[73] Wong S Y and Hynes R O 2006 Lymphatic or hematogenous dissemination: how does a metastatic tumor cell decide? *Cell Cycle* **5** 812–7
[74] Chiang S P H, Cabrera R M and Segall J E 2016 Tumor cell intravasation *Am. J. Physiol. Cell Physiol.* **311** C1–4
[75] Tremblay P L, Huot J and Auger F A 2008 Mechanisms by which E-selectin regulates diapedesis of colon cancer cells under flow conditions *Cancer Res.* **68** 5167–76
[76] Silvestri V L, Henriet E, Linville R M, Wong A D, Searson P C and Ewald A J 2020 A tissue-engineered 3D

microvessel model reveals the dynamics of mosaic vessel formation in breast cancer *Cancer Res.* **80** 4288–301
[77] Linville R M, Maressa J, Guo Z, Chung T D, Farrell A, Jha R and Searson P C 2023 A tissue-engineered model of the blood-tumor barrier during metastatic breast cancer *Fluids Barriers CNS* **20** 1–17
[78] Jing B, Luo Y, Lin B, Li J, Wang Z A and Du Y 2019 Establishment and application of a dynamic tumor-vessel microsystem for studying different stages of tumor metastasis and evaluating anti-tumor drugs *RSC Adv.* **9** 17137–47
[79] Nagaraju S, Truong D, Mouneimne G and Nikkhah M 2018 Microfluidic tumor–vascular model to study breast cancer cell invasion and intravasation *Adv. Healthcare Mater.* **7** 1701257
[80] Joyce J A and Pollard J W 2009 Microenvironmental regulation of metastasis *Nat. Rev. Cancer* **9** 239–52
[81] Alitalo K and Carmeliet P 2002 Molecular mechanisms of lymphangiogenesis in health and disease *Cancer Cell* **1** 219–27
[82] Gasic G J, Gasic T B and Stewart C C 1968 Antimetastatic effects associated with platelet reduction *Proc. Natl Acad. Sci. USA* **61** 46–52
[83] Egan K, Cooke N and Kenny D 2014 Living in shear: platelets protect cancer cells from shear induced damage *Clin. Exp. Metastasis* **31** 697–704
[84] Szczerba B M *et al* 2019 Neutrophils escort circulating tumour cells to enable cell cycle progression *Nature* **566** 553–7
[85] Perea Paizal J, Au S H and Bakal C 2021 Squeezing through the microcirculation: survival adaptations of circulating tumour cells to seed metastasis *Br. J. Cancer* **124** 58
[86] Yang L *et al* 2020 DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25 *Nature* **583** 133–8
[87] Xia Y, Wan Y, Hao S, Nisic M, Harouaka R A, Chen Y, Zou X and Zheng S-Y 2018 Nucleus of circulating tumor cell determines its translocation through biomimetic microconstrictions and its physical enrichment by microfiltration *Small* **14** 1802899
[88] Yamauchi K *et al* 2005 Real-time in vivo dual-color imaging of intracapillary cancer cell and nucleus deformation and migration *Cancer Res.* **65** 4246–52
[89] Rianna C, Radmacher M, Kumar S and Discher D 2020 Direct evidence that tumor cells soften when navigating confined spaces *Mol. Biol. Cell* **31** 1726–34
[90] Cognart H A, Viovy J L and Villard C 2020 Fluid shear stress coupled with narrow constrictions induce cell type-dependent morphological and molecular changes in SK-BR-3 and MDA-MB-231 cells *Sci. Rep.* **10** 1–14
[91] Zhou L, Dicker D T, Matthew E, El-Deiry W S and Alpaugh R K 2017 Circulating tumor cells: silent predictors of metastasis *F1000Research* **6** 1445
[92] Wang L, Balasubramanian P, Chen A P, Kummar S, Evrard Y A and Kinders R J 2016 Promise and limits of the CellSearch platform for evaluating pharmacodynamics in circulating tumor cells *Semin. Oncol.* **43** 464–75
[93] Moussavi-Harami S F, Wisinski K B and Beebe D J 2014 Circulating tumor cells in metastatic breast cancer: a prognostic and predictive marker *J. Patient Centered Res. Rev.* **1** 85–92
[94] Allard W J, Matera J, Miller M C, Repollet M, Connelly M C, Rao C, Tibbe A G J, Uhr J W and Terstappen L W M M 2004 Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases *Clin. Cancer Res.* **10** 6897–904
[95] Rao C, Chianese D, Doyle G V, Miller M C, Russell T, Sanders R A and Terstappen L W M M 2005 Expression of epithelial cell adhesion molecule in carcinoma cells present in blood and primary and metastatic tumors *Int. J. Oncol.* **27** 49–57
[96] Dolfus C, Piton N, Toure E and Sabourin J-C 2015 Circulating tumor cell isolation: the assets of filtration methods with polycarbonate track-etched filters *Chin. J. Cancer Res.* **27** 479–87
[97] Mu Z *et al* 2016 Abstract P2-02-14: detection and characterization of CTCs isolated by ScreenCell® -Filtration in metastatic breast cancer *Cancer Res.* **76** P2-02-14
[98] Di Trapani M, Manaresi N and Medoro G 2018 DEPArray™ system: an automatic image-based sorter for isolation of pure circulating tumor cells *Cytometry* A **93** 1260–6
[99] Krebs M G *et al* 2012 Analysis of circulating tumor cells in patients with non-small cell lung cancer using epithelial marker-dependent and -independent approaches *J. Thoracic Oncol.* **7** 306–15
[100] Holmes D, Whyte G, Bailey J, Vergara-Irigaray N, Ekpenyong A, Guck J and Duke T 2014 Separation of blood cells with differing deformability using deterministic lateral displacement *Interface Focus.* **4** 20140011
[101] Chen Y, Li P, Huang P-H, Xie Y, Mai J D, Wang L, Nguyen N-T and Huang T J 2014 Rare cell isolation and analysis in microfluidics *Lab Chip* **14** 626–45
[102] Loutherback K, D'Silva J, Liu L, Wu A, Austin R H and Sturm J C 2012 Deterministic separation of cancer cells from blood at 10 ml/min *AIP Adv.* **2** 042107
[103] Au S H, Edd J, Stoddard A E, Wong K H K, Fachin F, Maheswaran S, Haber D A, Stott S L, Kapur R and Toner M 2017 Microfluidic isolation of circulating tumor cell clusters by size and asymmetry *Sci. Rep.* **7** 1–10
[104] Toh Y-C, Raja A, Yu H and van Noort D 2018 A 3D microfluidic model to recapitulate cancer cell migration and invasion *Bioengineering* **5** 29
[105] Chesnais F, Joel J, Hue J, Shakib S, Di Silvio L, Grigoriadis A E, Coward T and Veschini L 2023 Continuously perfusable, customisable, and matrix-free vasculature on a chip platform *Lab Chip* **23** 761–72
[106] Wu Y, Zhou Y, Paul R, Qin X, Islam K and Liu Y 2022 Adaptable microfluidic vessel-on-a-chip platform for investigating tumor metastatic transport in bloodstream *Anal. Chem.* **94** 12159–66
[107] Strilic B and Offermanns S 2017 Intravascular survival and extravasation of tumor cells *Cancer Cell* **32** 282–93
[108] Stegner D, Dütting S and Nieswandt B 2014 Mechanistic explanation for platelet contribution to cancer metastasis *Thrombosis Res.* **133** S149–57
[109] Crippa M, Bersini S, Gilardi M, Arrigoni C, Gamba S, Falanga A, Candrian C, Dubini G, Vanoni M and Moretti M 2021 A microphysiological early metastatic niche on a chip reveals how heterotypic cell interactions and inhibition of integrin subunit β3 impact breast cancer cell extravasation *Lab Chip* **21** 1061–72
[110] Chen M B, Whisler J A, Fröse J, Yu C, Shin Y and Kamm R D 2017 On-chip human microvasculature assay for visualization and quantification of tumor cell extravasation dynamics *Nat. Protocols* **12** 865–80
[111] Mei X *et al* 2019 Microfluidic platform for studying osteocyte mechanoregulation of breast cancer bone metastasis *Integr. Biol.* **11** 119–29
[112] van Zijl F, Krupitza G and Mikulits W 2011 Initial steps of metastasis: cell invasion and endothelial transmigration *Mutat. Res.* **728** 23–34
[113] Naidoo K and Pinder S E 2017 Micro- and macro-metastasis in the axillary lymph node: a review *Surgeon* **15** 76–82
[114] Hermanek P, Hutter R V P, Sobin L H and Wittekind C 1999 Classification of isolated tumor cells and micrometastasis *Cancer* **86** 2668–73
[115] Macedo F *et al* 2017 Bone metastases: an overview *Oncol. Rev.* **11** 321
[116] Scully O J, Bay B-H, Yip G and Yu Y 2012 Breast cancer metastasis *Cancer Genomics & Proteomics* **9** 311–320
[117] Minn A J, Kang Y, Serganova I, Gupta G P, Giri D D, Doubrovin M, Ponomarev V, Gerald W L, Blasberg R and Massagué J 2005 Distinct organ-specific metastatic

potential of individual breast cancer cells and primary tumors *J. Clin. Invest.* **115** 44–55
[118] August D A, Ottow R T and Sugarbaker P H 1984 Clinical perspective of human colorectal cancer metastasis *Cancer Metastasis Rev.* **3** 303–24
[119] Popper H H 2016 Progression and metastasis of lung cancer *Cancer Metastasis Rev.* **35** 75–91
[120] Riihimäki M, Hemminki A, Fallah M, Thomsen H, Sundquist K, Sundquist J and Hemminki K 2014 Metastatic sites and survival in lung cancer *Lung Cancer* **86** 78–84
[121] Bersini S, Jeon J S, Dubini G, Arrigoni C, Chung S, Charest J L, Moretti M and Kamm R D 2014 A microfluidic 3D *in vitro* model for specificity of breast cancer metastasis to bone *Biomaterials* **35** 2454–61
[122] Skardal A, Devarasetty M, Forsythe S, Atala A and Soker S 2016 A reductionist metastasis-on-a-chip platform for *in vitro* tumor progression modeling and drug screening *Biotechnol. Bioeng.* **113** 2020–32
[123] Xu Z, Li E, Guo Z, Yu R, Hao H, Xu Y, Sun Z, Li X, Lyu J and Wang Q 2016 Design and construction of a multi-organ microfluidic chip mimicking the in vivo microenvironment of lung cancer metastasis *ACS Appl. Mater. Interfaces* **8** 25840–7
[124] Mukhopadhyay R 2007 When PDMS isn't the best *Anal. Chem.* **79** 3249–53
[125] Raj M K and Chakraborty S 2020 PDMS microfluidics: a mini review *J. Appl. Polym. Sci.* **137** 48958
[126] Toepke M W and Beebe D J 2006 PDMS absorption of small molecules and consequences in microfluidic applications *Lab Chip* **6** 1484–6
[127] Firatligil-Yildirir B, Bati-Ayaz G, Tahmaz I, Bilgen M, Pesen-Okvur D and Yalcin-Ozuysal O 2021 On-chip determination of tissue-specific metastatic potential of breast cancer cells *Biotechnol. Bioeng.* **118** 3799–810
[128] Lu P, Weaver V M and Werb Z 2012 The extracellular matrix: a dynamic niche in cancer progression *J. Cell Biol.* **196** 395–406
[129] Egeblad M, Rasch M G and Weaver V M 2010 Dynamic interplay between the collagen scaffold and tumor evolution *Curr. Opin. Cell Biol.* **22** 697–706
[130] Shen S and Clairambault J 2020 Cell plasticity in cancer cell populations [version 1; peer review: 2 approved] *F1000Research* **9** 635
[131] Regier M C, Maccoux L J, Weinberger E M, Regehr K J, Berry S M, Beebe D J and Alarid E T 2016 Transitions from mono- to co- to tri-culture uniquely affect gene expression in breast cancer, stromal, and immune compartments *Biomed. Microdevices* **18** 70
[132] Bradney M J, Venis S M, Yang Y, Konieczny S F and Han B 2020 A biomimetic tumor model of heterogeneous invasion in pancreatic ductal adenocarcinoma *Small* **16** 1905500
[133] Gadde M *et al* 2020 *In vitro* vascularized tumor platform for modeling tumor-vasculature interactions of inflammatory breast cancer *Biotechnol. Bioeng.* **117** 3572–90
[134] Gadde M, Mehrabi-Dehdezi M, Debeb B G, Woodward W A and Rylander M N 2023 Influence of macrophages on vascular invasion of inflammatory breast cancer emboli measured using an *in vitro* microfluidic multi-cellular platform *Cancers* **15** 4883
[135] Junttila M R and De Sauvage F J 2013 Influence of tumour micro-environment heterogeneity on therapeutic response *Nature* **501** 346–54
[136] Grivennikov S I, Greten F R and Karin M 2010 Immunity, inflammation, and cancer *Cell* **140** 883–99
[137] Erdogan B *et al* 2017 Cancer-associated fibroblasts promote directional cancer cell migration by aligning fibronectin *J. Cell Biol.* **216** 3799–816
[138] Özbek S, Balasubramanian P G, Chiquet-Ehrismann R, Tucker R P and Adams J C 2010 The evolution of extracellular matrix *Mol. Biol. Cell* **21** 4300–5
[139] Agrawal A, Shahreza S, Javanmardi Y, Szita N and Moeendarbary E 2022 The tumour microenvironment modulates cancer cell intravasation *Organs-on-a-Chip* **4** 100024
[140] Smith I O, Liu X H, Smith L A and Ma P X 2009 Nanostructured polymer scaffolds for tissue engineering and regenerative medicine *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **1** 226–36
[141] Provenzano P P, Eliceiri K W, Campbell J M, Inman D R, White J G and Keely P J 2006 Collagen reorganization at the tumor-stromal interface facilitates local invasion *BMC Med.* **4** 38
[142] Nallanthighal S, Heiserman J P and Cheon D-J 2019 The role of the extracellular matrix in cancer stemness *Front. Cell Dev. Biol.* **7** 86
[143] Walker C, Mojares E and Del Río Hernández A 2018 Role of extracellular matrix in development and cancer progression *Int. J. Mol. Sci.* **19** 3028
[144] Henke E, Nandigama R and Ergün S 2020 Extracellular matrix in the tumor microenvironment and its impact on cancer therapy *Front. Mol. Biosci.* **6** 160
[145] Ramaswamy S, Ross K N, Lander E S and Golub T R 2002 A molecular signature of metastasis in primary solid tumors *Nat. Genet.* **33** 49–54
[146] Paszek M J *et al* 2005 Tensional homeostasis and the malignant phenotype *Cancer Cell* **8** 241–54
[147] Paszek M J and Weaver V M 2004 The tension mounts: mechanics meets morphogenesis and malignancy *J. Mammary Gland Biol. Neoplasia* **9** 325–42
[148] Pathak A and Kumar S 2012 Independent regulation of tumor cell migration by matrix stiffness and confinement *Proc. Natl Acad. Sci. USA* **109** 10334–9
[149] Strelez C *et al* 2021 Human colorectal cancer-on-chip model to study the microenvironmental influence on early metastatic spread *iScience* **24** 102509
[150] Yang G, Mahadik B, Choi J Y and Fisher J P 2020 Vascularization in tissue engineering: fundamentals and state-of-art *Prog. Biomed. Eng.* **2** 012002
[151] Sarker M, Chen X B and Schreyer D J 2015 Experimental approaches to vascularisation within tissue engineering constructs *J. Biomater. Sci. Polym. Ed.* **26** 683–734
[152] Saman H, Raza S S, Uddin S and Rasul K 2020 Inducing angiogenesis, a key step in cancer vascularization, and treatment approaches *Cancers* **12** 1172
[153] Ko J, Song J, Lee Y, Choi N and Kim H N 2024 Understanding organotropism in cancer metastasis using microphysiological systems *Lab Chip* **24** 1542–56
[154] Kameda Y, Chuaychob S, Tanaka M, Liu Y, Okada R, Fujimoto K, Nakamura T and Yokokawa R 2022 Three-dimensional tissue model in direct contact with an on-chip vascular bed enabled by removable membranes *Lab Chip* **22** 641–51
[155] SynVivo SynTumor 3D cancer model (available at: www.synvivobio.com/syntumor/#1588022204361-a177b155-1008) (Accessed 31 July 2023)
[156] Zervantonakis I K, Hughes-Alford S K, Charest J L, Condeelis J S, Gertler F B and Kamm R D 2012 Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function *Proc. Natl Acad. Sci. USA* **109** 13515–20
[157] Li Y, Zhao L and Li X-F 2021 Hypoxia and the tumor microenvironment *Technol. Cancer Res. Treat.* **20** 153303382110363
[158] Chen Z, Han F, Du Y, Shi H and Zhou W 2023 Hypoxic microenvironment in cancer: molecular mechanisms and therapeutic interventions *Signal Transduct Target Ther.* **8** 70
[159] Emami Nejad A *et al* 2021 The role of hypoxia in the tumor microenvironment and development of cancer stem cell: a novel approach to developing treatment *Cancer Cell Int.* **21** 1–26
[160] Song J, Miermont A, Lim C T and Kamm R D 2018 A 3D microvascular network model to study the impact of

hypoxia on the extravasation potential of breast cell lines *Sci. Rep.* **8** 1–11
[161] Jagannathan L, Cuddapah S and Costa M 2016 Oxidative stress under ambient and physiological oxygen tension in tissue culture *Curr. Pharmacol. Rep.* **2** 64–72
[162] Mas-Bargues C, Sanz-Ros J, Román-Domínguez A, Inglés M, Gimeno-Mallench L, El Alami M, Viña-Almunia J, Gambini J, Viña J and Borrás C 2019 Relevance of oxygen concentration in stem cell culture for regenerative medicine *Int. J. Mol. Sci.* **20** 1195
[163] Ma Y-H V, Middleton K, You L and Sun Y 2018 A review of microfluidic approaches for investigating cancer extravasation during metastasis *Microsyst. Nanoeng.* **4** 17104
[164] Hajal C, Ibrahim L, Serrano J C, Offeddu G S and Kamm R D 2021 The effects of luminal and trans-endothelial fluid flows on the extravasation and tissue invasion of tumor cells in a 3D *in vitro* microvascular platform *Biomaterials* **265** 120470
[165] Polacheck W J, German A E, Mammoto A, Ingber D E and Kamm R D 2014 Mechanotransduction of fluid stresses governs 3D cell migration *Proc. Natl Acad. Sci. USA* **111** 2447–52
[166] Huang Y L, Tung C K, Zheng A, Kim B J and Wu M 2015 Interstitial flows promote amoeboid over mesenchymal motility of breast cancer cells revealed by a three dimensional microfluidic model *Integr. Biol.* **7** 1402–11
[167] Han J J 2023 FDA Modernization Act 2.0 allows for alternatives to animal testing *Artif. Organs* **47** 449–50
[168] Marx U *et al* 2020 Biology-inspired microphysiological systems to advance patient benefit and animal welfare in drug development *Altex* **37** 365–94
[169] Lanz H L *et al* 2017 Therapy response testing of breast cancer in a 3D high-throughput perfused microfluidic platform *BMC Cancer* **17** 1–11
[170] Oliver C R *et al* 2019 A platform for artificial intelligence based identification of the extravasation potential of cancer cells into the brain metastatic niche *Lab Chip* **19** 1162–73
[171] Ronaldson-Bouchard K *et al* 2022 A multi-organ chip with matured tissue niches linked by vascular flow *Nat. Biomed. Eng.* **6** 351–71