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Biomimetic on-a-chip platforms for studying cancer metastasis

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Abstract

Cancer metastasis is a multi-step, secondary tumor formation that is responsible for the vast majority of deaths in cancer patients. Animal models have served as one of the major tools for studying metastatic diseases. However, these metastasis models inherently lack the ability to decouple many of the key parameters that might contribute to cancer progression, and therefore ultimately limit detailed, mechanistic investigation of metastasis. Recently, organ-on-a-chip model systems have been developed for various tissue types with the potential to recapitulate major components of metastasis. Here, we discuss recent advances in *in vitro* biomimetic on-a-chip models for cancer metastasis.

Introduction

Cancer metastasis is the dissemination of a primary tumor to distant sites in the body, often resulting in the formation of many secondary tumor masses. The dissemination process often involves multiple steps, including tumor angiogenesis (the ingrowth of new capillary vessels that feed the growing tumor), intravasation (the migration of cancer cells into the blood stream), extravasation (attachment and escape of cancer cells out of the blood vessels at a distant site), and colonization at a distal tissue [1]. Even after primary tumor resections or a series of chemotherapeutic treatments, cancer metastasis can be lethal and is responsible for 90% of deaths in cancer patients [2].

Animal models have served as one of the primary tools for studying cancer metastasis [3]. Through technologies such as green fluorescent protein (GFP) transgene expression [4] and the development of spontaneous [5], experimental [6], and transgenic mouse metastasis models [7], we have been able to not only track the cancer cells *in vivo* but also identify key genes for metastatic progression. Although these *in vivo* studies provide physiologically relevant perspectives on tumor pathology, the inability to isolate the many interacting parameters that probably participate in metastasis in animal models presents disadvantages in identifying clear mechanisms. Metastasis is a complex and dynamic process, influenced

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by the multiple local tissue microenvironments the cancer cells experience as they transit through the body and involving cross-communication amongst several cell types [8]. These multiple parameters are intimately coordinated, which make it hard to study how each parameter contributes to metastasis using traditional *in vivo* models. Therefore, there has been a growing interest in developing biomimetic *in vitro* systems that can (1) recapitulate the key parameters that affect tumor progression (e.g., oxygen tension, nutrient gradients, and tissue stiffness) and (2) provide the flexibility to decouple these parameters in experimental settings.

For tuning certain physical conditions (e.g., oxygen tension, nutrient gradients, and tissue stiffness), *in vitro* models can be advantageous over *in vivo* models to study effects of these parameters. For example, oxygen tension and nutrient gradients are determined by the tissue location in the body, the metabolic needs of its parenchyma, and the vascular features, such as vessel density, network, and permeability. Manipulating and monitoring such parameters are therefore challenging in the *in vivo* setting. By contrast, tools such as hypoxia chambers and chemical inducers of hypoxia inducible factor (HIF) allow easier modulation of oxygen tension in the system and/or hypoxia-related gene expression. Composition of the cell culture media can also be optimized to create desired nutrient gradients. Tissue stiffness of tumor extracellular matrix (ECM) modulates tumor cell migration, aggressiveness, proliferation, chemotherapeutic response, and dormancy [2]. Artificially manipulating the stiffness and the composition of the tumor ECM *in vivo* is challenging. By contrast, we can easily tune the matrix stiffness and composition *in vitro* by manipulating crosslinking density and available cell attachment moieties in *in vitro* scaffolds.

Such models have begun to focus on capturing various aspects of cancer cell migration, adhesion, and proliferation; and cancer cell interaction with other cells in two-dimensional (2D) and three-dimensional (3D) culture. Two-dimensional wound healing models provided gradient-independent migration of cancer cells [9]. Co-culture system in 2D/3D allowed study of cell-to-cell interaction and paracrine signaling [10]. Three-dimensional spheroid models showed tumor proliferation and survival [11]. While these simple *in vitro* models have helped identifying the basic machinery, recent advances in biomaterials and fabrication techniques [12] have led to the development of various organ-on-a-chip models [13] that provide more physiologically relevant platforms for modeling the tissue microenvironment, including proper gradients of cell types and their paracrine signals, matrices, vessel network, and flow [14]. For example, lung-on-a-chip and bone marrow-on-a-chip recapitulated many aspects of these organs' properties *in vitro* [15,16]. Given that lungs and bones are vulnerable target organs for metastasis, these organ-on-a-chip models may serve as *in vitro* platforms to study organ invasion. In addition, our group and others have developed *in vitro* blood vessel models to mechanistically study angiogenesis [17,18••,19,20-22]. With advancements and appropriate combination of these systems, we believe biomimetic on-a-chip models can become an imperative platform for studying tissue-specific cancer metastasis that can ultimately provide a better guidance for future clinical studies. In this review, we discuss advances in biomimetic on-a-chip models that have allowed us to begin to investigate key mechanisms of cancer metastasis (Figure 1).

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Models for tumor angiogenesis

Tumor angiogenesis is formation of new blood vessels in tumor stroma, originating from pre-existing blood vessels in the host [8]. Tumor blood vessels provide cancer cells with oxygen and nutrients, and more importantly, they serve as disseminating routes for metastasis [23]. Physiological angiogenesis includes angiogenic growth factor-induced receptor activation in endothelial cells (ECs), followed by basement membrane degradation by proteases secreted from the activated ECs. Proliferating ECs in the surrounding matrix form new sprouts, and these sprouts make loops to become a vascular lumen [23]. There have been on-a-chip models that recapitulate hallmarks of the angiogenesis, such as vascular sprouting [18••,24], network formation [20,21], perfused lumen formation [18••,19,21], and maturation [25,26]. For example, Nguyen *et al.* developed endothelial sprouting model system, which induced *in vivo*-like directed invasion of tip cells with filopodia-guided protrusions, apical–basal polarization of stalk cells, and lumen formation (Figure 2i) [18••]. Three dimensional vascular network formation was demonstrated in an *in vitro* vasculogenesis model by Alonzo *et al.* (Figure 2ii) [21]. They reported perfused human capillary networks, and claimed that soluble factors derived from normal human lung fibroblasts (NHLFs) are necessary to form a vascular network derived from endothelial colony forming cell-derived endothelial cells (ECFC-ECs) [21]. Functionally perfused neovessels are finally matured by coverage of pericytes and smooth muscle cells. Jeon *et al.* mixed mesenchymal stem cells (MSCs) and ECs in fibrin matrix, observed network formation under growth factor stimulation [25]. They tested vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1), and transforming growth factor beta 1 (TGF-β1) in the platform (Figure 2iii). Compared to VEGF alone, VEGF and TGF-βl generated a non-interconnected microvasculature; VEGF and Ang-1 promoted functional networks, showing α-SMA (alpha smooth muscle actin) positive cells [25]. The nature and function of the MSCs and α-SMA positive cells, and their mechanistic roles in vascular network formation and barrier function remain to be defined. Another recent paper by Kim *et al.* employed human placental pericytes, and showed pericyte coverage on their 3D microvascular network [26].

The examples above exhibit phenotypes of physiological angiogenesis. However, tumor angiogenesis is very different from normal angiogenesis: tumor vasculature is characterized by irregular sprouting, tortuous networks of capillaries, and leaky barrier properties [27]. Only *in vivo* intravital imaging, MRI, and *in vivo* image-based computer simulation have thus far reported these tumor-specific vasculatures [28-30]. Though underlying pathology could partially be explained by the overproduction of angiogenic growth factors, hypoxia, and abnormal ECM composition in the tumor microenvironment (TME) [27], detailed mechanisms have not been sufficiently well understood. Indeed, the process of generating angiogenesis-on-a-chip models provides the opportunity to understand the relative contributions and interactions of these factors required to recapitulate the unique characteristics of tumor angiogenesis. In this regard, providing relevant tumor angiogenesis models *in vitro* is a pivotal approach for understanding of the abnormality of tumor endothelium, tumor drug/particle delivery, tumor vascular mimicry, and hematogenous metastasis. However, modeling tumor angiogenesis is not a simple task. The *in vitro* tumor

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angiogenesis models described here have not yet been reported to exhibit abnormalities for tumor vessels. One reason may be due to the differences in how these structures form *in vitro* versus *in vivo*: *in vivo* tumor angiogenesis usually occurs when the tumor develops to a certain size and stage, whereas *in vitro* cancer cells and HUVECs are directly mixed within devices does not recapitulate the time sequence of tumor angiogenesis. While these differences may limit the ability of current approaches to model certain complex steps of tumor development, their ability to nominally mimic the presence of vasculature and organization of tumor cells within a 3D environment may allow the system to recapitulate some aspects of the *in vivo* processes better than traditional culture approaches. As such, additional investigation is needed.

Models for tumor intravasation

Tumor intravasation is characterized by the migration of cancer cells through the basal membrane into a blood or lymphatic vessels near the tumor stroma [31,32]. Tumor intravasation can be triggered by oxygen tension, chemotactic gradient, ECM condition, impaired endothelial barrier function, and epithelial to mesenchymal transition (EMT) in cancer cells, etc. [8,33,34]. Despite this general notion, it is unclear which element is predominant for intravasation in certain types of cancer and which mechanisms govern the process under a specific treatment such as anti-angiogenic therapy. Developing *in vitro* models for intravasation will enhance our understanding of tumor intravasation in many different contexts.

The model systems for tumor intravasation contain tumor and vascular compartments, examining tumor cell migration into the vasculature. Such platforms could be based on microfluidic systems [35], transwells [36•], and pre-vascularized tumor spheroids [37••]. For example, Zervantonakis *et al.* demonstrated macrophage-mediated tumor intravasation in their microfluidic system that allowed them to study tumor and endothelium interface in 3D. They unveiled that tumor necrosis factor alpha (TNF-α) secreted by macrophages directly impairs endothelial barrier function and enhanced breast tumor intravasation (Figure 3ii) [35]. Similarly, Roh-Johnson *et al.* reported that direct contact between a macrophage, an EC, and a tumor cell plays a role in tumor transendothelial migration. In their transwell system, they showed that a direct contact of macrophages to the tumor cell activates RhoA in the tumor cell and induces invadopodium formation (Figure 3iii) [36•]. Their extending study on human tumor samples validated that this presence of the three cell types could serve as a marker for breast cancer metastasis [38]. This is a good example of synergy gained by using a biomimetic on-a-chip model and traditional models to understand metastatic diseases.

A recent study by Ehsan *et al.* reported that pre-vascularized tumor spheroids *in vitro* showed endothelial sprouting mimicking tumor angiogenesis. In the platform, hypoxia enhanced intravasation via Slug-dependent EMT signaling in the cancer cells (Figure 3i) [37••]. Hypoxia may contribute to tumor intravasation in other ways. For example, hypoxia induces ECM remodeling by HIF-1 regulated synthesis of ECM-modifying enzymes, which promote degradation of basement membrane and alignment of collagen fibers to facilitate tumor invasion to blood vasculatures [34], Further *in vitro* studies, examining hypoxia-

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mediated changes in ECM composition, alignment, stiffness, and cytokine production by cancer and stromal cells will make a significant progress in our understanding of tumor intravasation.

In addition to blood vessels, intravasation via lymphatic vessels is also an important model to be further developed in *in vitro* on-a-chip models. Tumor lymphatic vessels are formed by tumor secreted lymphangiogenic growth factors and are central routes for most carcinoma metastasis [8,32]. Tumor lymphatic vessels recruit cancer cells via several chemokine axes [39-41], promote angiogenesis and tumor growth by expressing growth factors [41,42]. Tumor invasion into lymphatic vessels is relatively under-investigated compared to the tumor invasion into blood vessels, and therefore on-a-chip models studying the interaction of tumor-lymphatic system would be of great interest as well.

Models for tumor extravasation

Cancer cells entering blood stream are referred to as circulating tumor cells (CTCs) [43]. CTCs in the blood stream finally lodge at secondary organs, mediated by capillaries in the organs. After the CTCs adhere to the endothelium, they transmigrate through the endothelium, which is referred to as tumor extravasation [44]. Extravasated tumor cells must adapt themselves in the new organ microenvironment to survive and form micrometastases [1]. According to Steven Paget's 'seed and soil' hypothesis, each organ has its unique environment, so that different cancer types may prefer to seeding themselves in a certain type of organ microenvironments [45]. This hypothesis has been supported by clinical observations: for examples, prostate cancer prefers to metastasize to bone; head and neck cancer often results in metastases in cervical lymph nodes and salivary glands, but not the brain despite a spatial proximity [8]. Despite these observations, underlying mechanisms determining these organ-specific preferences are not well understood.

Organ-on-a-chip systems can mimic organ microenvironments *in vitro* by organizing organ parenchymal or stromal cells, and posing diverse biochemical and physical cues. The systems therefore potentially serve as pre-metastatic organ *in vitro* to unveil mechanisms of organ-specific metastasis. There have been organ-on-a-chip systems based on transwells [46•], capillary bed platforms [47••], and vascular channel microfluidic devices [48] to study organotypic metastasis.

A transwell approach has been used for modeling brain metastases. Tominaga *et al.* modeled blood–brain barrier (BBB) in transwells by employing brain ECs, brain pericytes and astrocytes (Figure 4ii) [46•]. This model showed that breast cancer cell derived extracellular vesicles (EVs) break down the BBB through the change in actin dynamic of the ECs *in vitro*, and the cancer cell derived EVs also promoted brain metastasis *in vivo* [46•].

Bone microenvironment was mimicked in a capillary bed platform to study breast cancer seeding to the bone (Figure 4i) [47••]. Bone is one of the most vulnerable organs for metastasis in breast or prostate cancer. The device developed by Jeon *et al.*, featured a fibrin gel compartment next to microfluidic channels, where ECs, osteoblasts, and bone-marrow derived MSCs formed perfusable capillary beds in a bone mimicking microenvironment. As

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a control group, they also prepared muscle mimicking microenvironment by using myoblast cell line, C2C12. Breast tumor cells exhibited better extravasation in the bone mimicking environment than in a muscle mimicking one; and the anti-metastatic mechanism in the muscle microenvironment involved secretion of adenosine by the muscle cells [47••].

Bersini *et al.* recapitulated bone metastasis using vascular channel microfluidic devices [48]. Their 3D device included an endothelial channel and surrounding collagen 1 matrix embedded with osteoblasts and MSCs. Tumor cells were introduced inside the endothelial channel, and the extravasation of tumor cells was observed. The system revealed the extravasation was mediated by the CXCL5–CXCR2 axis. These bone mimicking models need to be explored further in prostate cancer or other bone metastatic cancer to investigate whether there are general mechanisms or molecular targets for bone metastasis.

Beyond the examples above, recently developed organ-on- a-chip models need to be further explored for their utility in metastasis research. For example, on-a-chip models for bone marrow [16], lung [15], liver [49], and brain [50] could be combined with capillary bed or vascular channel models to serve as novel platforms for organ metastasis. These models could serve as *in vitro* pre-metastatic organ platforms, for example to screen anti-metastatic drug agents as well as to study disease mechanisms.

Conclusions

In vitro organ-on-a-chip models for metastasis have just started gaining attention, and there are numerous topics still to be explored using these models. Several challenges still need to be addressed for on-chip platforms before they can be widely adopted. Many of the current models utilize purified collagen and/or fibrin from non-human sources as their ECM, which often contains residual growth factors and undefined components. Not only do these ECM sources make it challenging to decouple and manipulate biochemical parameters of the cellular microenvironment (cell adhesion peptide density, for example), they also lack physiological fibrillar structure and stiffness that are observed in *in vivo* TME. Development of highly controllable biomimetic synthetic materials or better sources of *in vivo* matrix would therefore significantly enhance our ability to make the *in vitro* models physiologically relevant. In addition, as the on-chip platforms are becoming more and more complex, the point at which an organ mimicry is both ‘functional enough’ for clinical relevance and ‘simple enough’ for practical experimentation remains to be defined. Given that human cancer is enormously complex and variable even between patients (e.g., with different outcomes often observed in the same tumor types depending on disease status, location, and the patient’s age or genetic background), it is unreasonable to expect to model every facet of human cancer metastasis *in vitro*. Therefore, identifying the universal key aspects of pathology and deducing what is ‘enough’ for an *in vitro* on-chip model would help set feasible engineering goals and measures of success. Despite more than 100 year of history of molecular biology, animal studies, and genetics in cancer metastasis, defeating it has not yet been accomplished. Successful interconnection between the biomimetic organ-on-a-chip models and traditional models will promise better understanding and curing malignant metastatic diseases.

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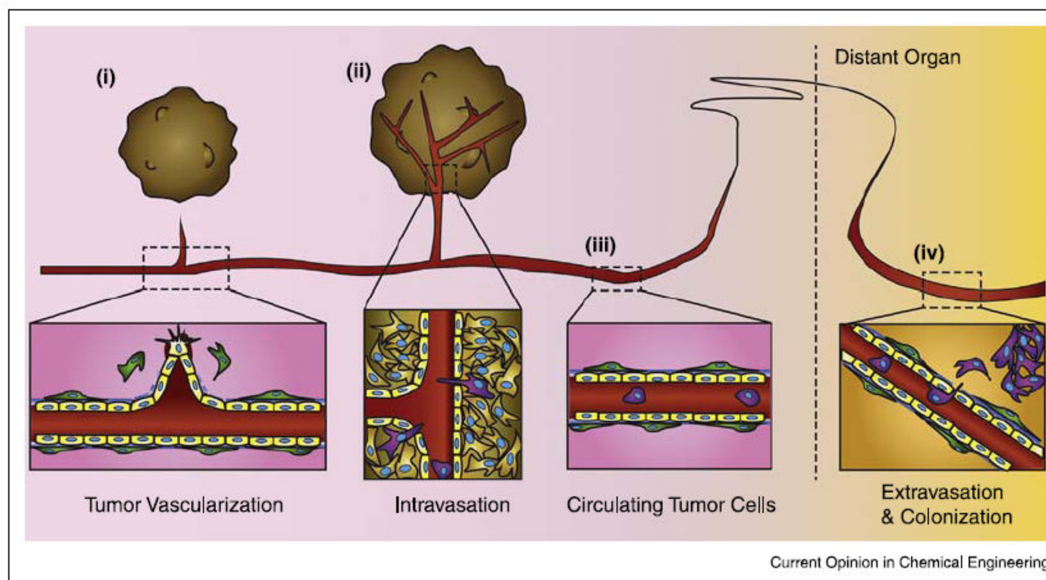

Figure 1.

Schematic of the metastic process. **(i)** Vascularization of growing tumor, Soluble growth factors and cytokines released from cancer cells activate endothelial cells (yellow), resulting in pericyte (green) detachment, basement membrane degradation, and endothelial sprouting. **(ii)** Intravasation of invasive cancer cells. Cancer cells expressing invasive phenotypes (purple) squeeze through the endothelial cells and enter the circulation. **(iii)** Circulating tumor cells (CTCs) traveling to a distant tissue. **(iv)** Extravasation of CTCs and colonization. Various factors induce CTCs to adhere to the vascular wall of a distant organ and enter its stroma. If the microenvironment is suitable for the cancer cells, they proliferate and colonize the organ, Figure not drawn to scale.

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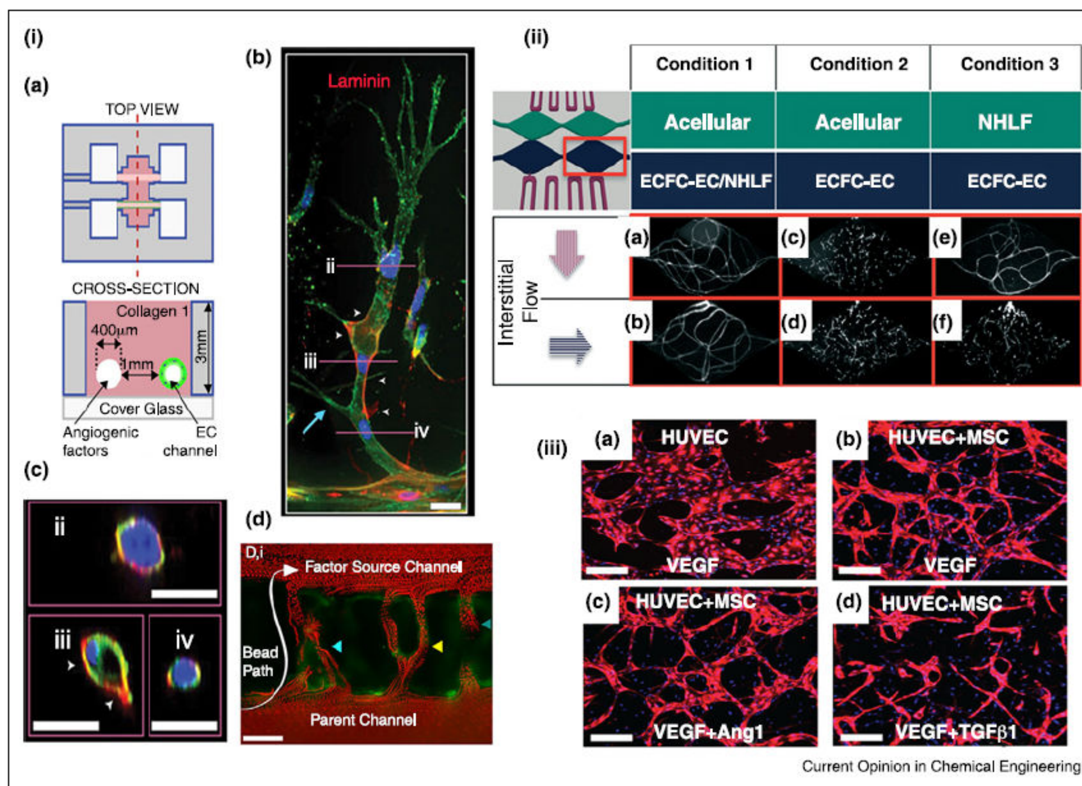

Figure 2.

3D models for angiogenesis. **(i)** Formation of endothelial sprouts and perfusable neovessels in a 3D microfluidic device. (a) Device schematic. Two parallel channels are in a 3D collagen matrix, One channel is coated with ECs and the other channel is perfused with angiogenic factors. (b) Mature sprouts stained for laminin (red), and (c) cross-section of sprouts. Scale bars are 25 μm. (d) Neovessels perfused with 3-μm red fluoescent beads. Scale bar is 100 μm. **(ii)** Human capillary network formation, confirmed by CD31 staining, depends on interstitial flow-driven communication between endothelial colony forming cell-derived endothelial cells (ECFC-ECs) and normal human lung fibrolast (NHLF). (a,b) Vessel networks developed when ECFC-Ecs and NHLFs were co-cultured regardless of the direction of the interstitial flow. (c,d) In the absence of NHLFs, ECFC-ECs failed to form vessel networks in any directional flow, (e) Vessel network is formed when the interstitial flow direction allows for ECFC-ECs to be exposed to NHLF soluble factors. (f) No significant vessel network formation occurred when the interstitial flow direction was arranged to restrict ECFC-ECs exposure to NHLF soluble factors. **(iii)** Generation of microvascular network in the presence of bone marrow derived MSCs and HUVEC under growth factor stimulation. Scale bars are 200 μm.

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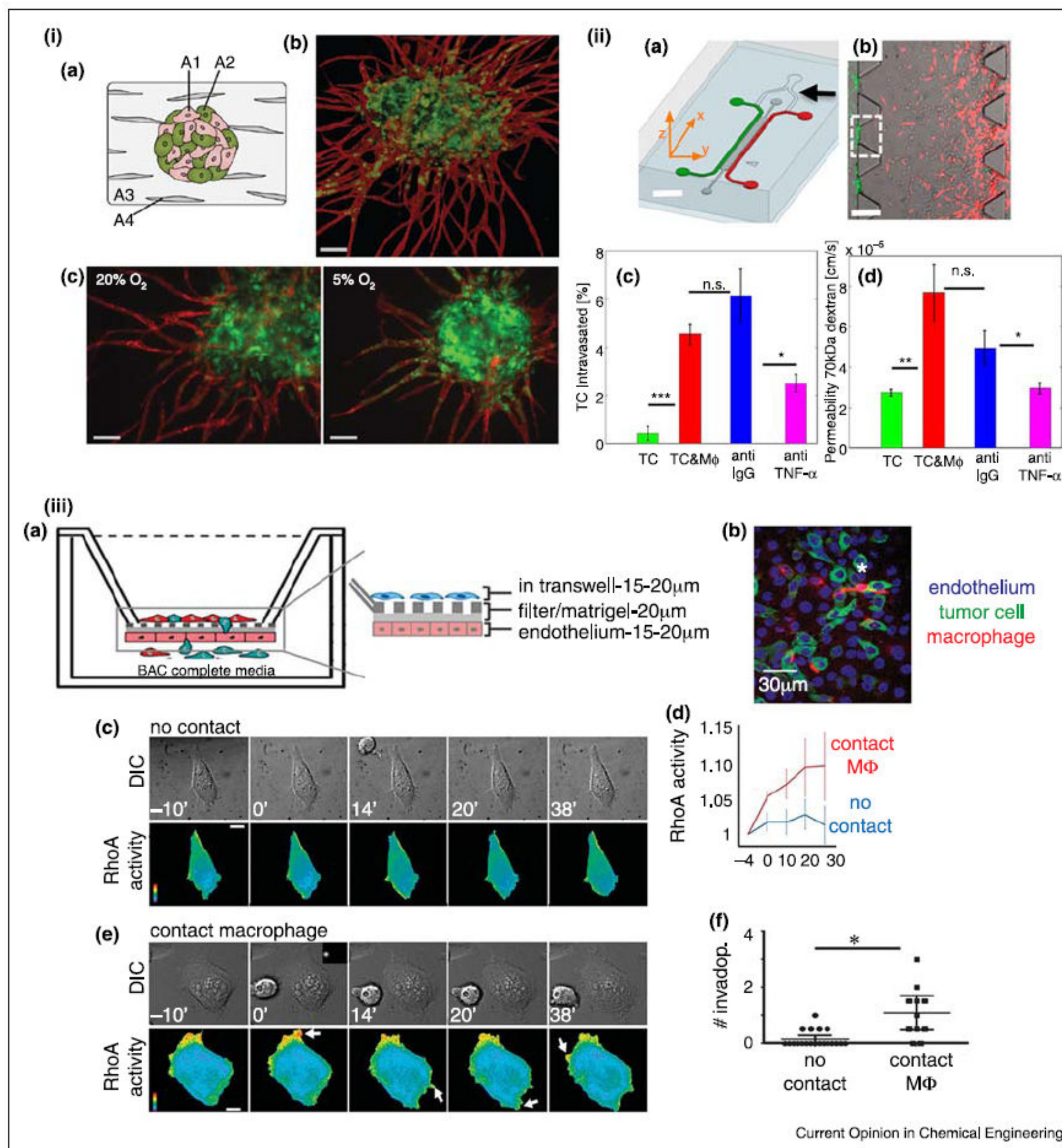

Figure 3.

Models for tumor intravasation. **(i)** Prevascularized tumor (PVT) spheroid model, (a) Schematic of the PVT spheroid model. EC (A1) and tumor cell (A2) spheroids were co-cultured with fibroblasts (A4) in 3D fibrin matrix (A3), (b) PVT spheroid shows radial EC sprouting (CD31, red) from the prevascularized tumor (EGFP-transfected SW620, colon) spheroid. Scale bar is 100 μm. (c) Decreased oxygen tension increases intravasation of the SW620 cells. Scale bar is 100 μm. **(ii)** Microfluidic tumor-vascular interface model, (a) Schematic of the device. EC channel (green), tumor channel (red). Scale bar is 2 mm. (b) Fibrosarcoma cells (HT1080, red) invade through the ECM toward EC (green). Scale bar is 300 μm. (c) Macrophages enable tumor intravasation though TNF-α signaling, (d) Enhanced tumor intravasation is endothelial permeability dependent. **(iii)** Transwell *in vitro* model for tumor intravasation. (a) Schematic of the transendothelial migration of cancer cells in the

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presence of macrophages. (b) A representative image of apical section of the transwell, (c) RhoA activity in cancer cells in the absence of direct contact of macrophages, (d) RhoA activity in cancer cells with or without direct contact of macrophage, (e) RhoA activity in cancer calls in direct contact of macrophages, (f) Tumor invasion with or without direct contact with macrophage. Scale bars (a,b) are 10 μm. (i) Reproduced from [37••] with permission from the Royal Society of Chemistry; (ii) reproduced from [34] with permission from the National Academy of Sciences; (iii) reproduced from [36•] with permission from the Nature Publishing Group.

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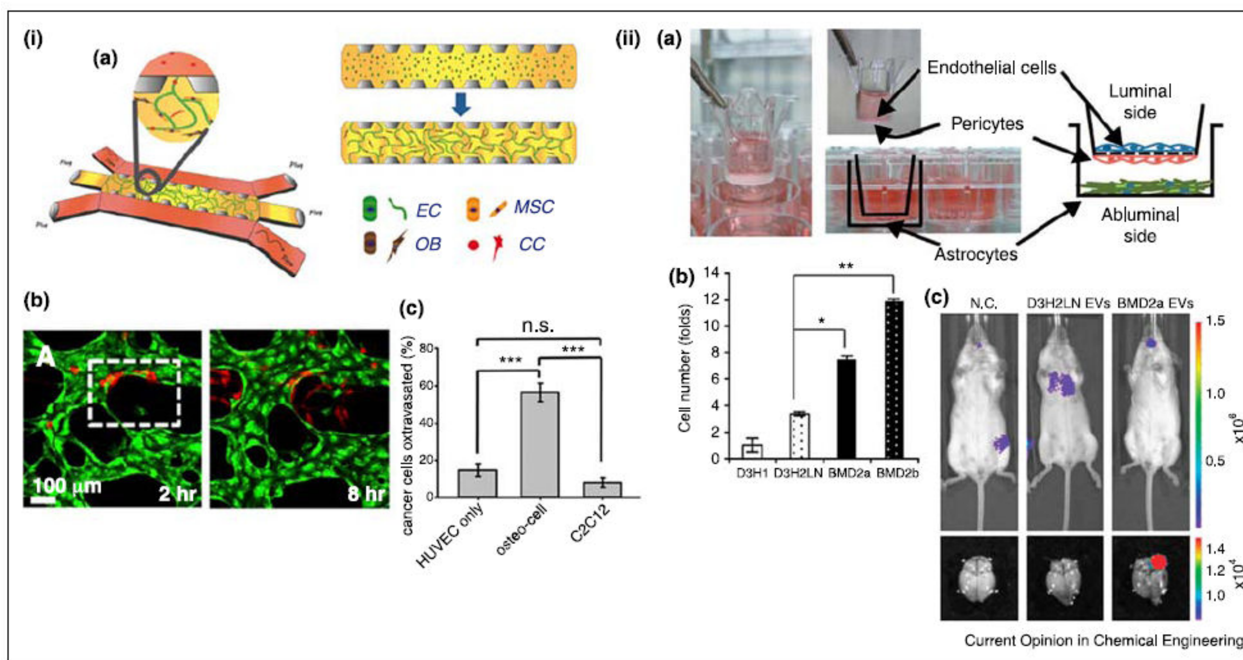

Figure 4.

Models for tumor extravasation. **(i)** Breast tumor extravasation in the bone-mimicking microenvironment (BMi). (a) Schematic of the microfluidic device. ECs, MSCs, and osteoblasts (OBs) were initially seeded in fibrin gel. After formation of vascularized BMi, cancer cells (CC) were added to the side channels. (b) Cancer cell (red) extravasation through HUVEC (green) network in the BMi. (c) Cancer cell extravasation enhanced by osteocells. Myoblasts, C2C12, were used as a control. **(ii)** Breast tumor extravasation in the brain microenvironment. (a) Schematic of the *in vitro* model of blood–brain barrier (BBB). (b) Tumor extravasation in the presence of extracellular vesicles (EVs) from different tumor cells (D3H1: primary tumor cells, D3H2LN: lymph node metastases, BMD2a and 2b: brain metastases). Brain metastases derived EVs enhanced breast tumor extravasation. (c) Bioluminescence images of D3H2LN and BMD2a derived EVs injected mice (negative control, N.C.). BMD2a-derived EVs promoted brain metastasis *in vivo*. (i) Reproduced from [47••] with permission from the National Academy of Sciences and (ii) reproduced from [46•] with permission from the Nature Publishing Group.